The diversity of root nodule bacteria associated with *Lebeckia* species in South Africa

by

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Dissertation submitted in partial fulfillment of the requirements for the degree

of

Magister Scientiae

in the

Department of Microbiology and Plant Pathology

Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

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May 2008

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I declare that the thesis/dissertation, which I hereby submit for the degree Masters in Microbiology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ACKNOWLEDGEMENTS

PREFACE

Nitrogen-fixing diazotrophic root-nodule bacteria are of immense economic importance because of their symbiosis with leguminous plants. Diazotrophic bacteria infect the host legume root inducing the formation of nodules in which the bacteria (also termed rhizobia) replicate and synthesize the enzyme nitrogenase. This enzyme catalyzes the reduction of atmospheric dinitrogen to ammonia for subsequent use by the plant as a major source of nitrogen. Nitrogen is an essential element required by plants for growth and synthesis of protein and is usually the most limiting element in agricultural production as well as being the most expensive component of fertilizer.

The aim of my study was to determine the diversity and taxonomy of a specific group of root nodule bacteria associated with different species of *Lebeckia*. The genus *Lebeckia* Thunb. (Family *Leguminosae*, subfamily *Papilionoideae*, tribe *Crotalarieae*) comprises some 33 plant species. These plants are mainly indigenous to the southern and Western Cape regions of South Africa. They are divided into shrubby trifoliate-leaf species in the sections *Calobota, Stiza* and *Viborgioides* and suffrutescent unifoliate needle-leaf species in the section *Lebeckia*. Plants of this genus are adapted to harsh environmental conditions such as are found in the Karoo and Namaqualand. Several *Lebeckia* species are beneficial, such as *L. spinescens* and *L. multiflora*, which are valuable as pasture legumes and well grazed by animals especially in winter. All the species have ecological value because of their nitrogen-fixing symbiosis with rhizobia. To my knowledge, no attempts have been made in the past to investigate these microsymbionts of *Lebeckia*.

Root nodules were collected from *Lebeckia* species at a wide variety of localities in the western and southern Cape regions of South Africa. Indigenous rhizobia isolated from these nodules were investigated for their nodulation abilities on their respective host plants as well as on non-host promiscuous legumes, cowpea and siratro. The isolates were then characterized using random amplified DNA fingerprinting followed by DNA sequencing of selected isolates. Results presented in this study showed that the indigenous South African genus *Lebeckia* is nodulated by diverse rhizobia from both α- and β-*Proteobacteria*.

The first chapter contains a literature review of symbiotic nitrogen fixation that includes a general description of the biology, inoculant technology and the taxonomy of legumes and

their rhizobia. The genera within the tribe *Crotalarieae* (such as *Crotalaria*, *Lotononis*, *and Aspalathus*) were discussed with special reference to the genus *Lebeckia*. Technical methods used for the classification of rhizobia were also reviewed. Non-DNA-based methods such as host specificity, substrate utilization, antibiotic resistance, morphological characters and biochemical properties as well as DNA based fingerprinting methods (ARDRA, RFLP RAPD, and AFLP), DNA sequence information, analysis of whole genomes, DNA-DNA hybridization and polyphasic approaches were outlined.

The second chapter describes the isolation of 79 rhizobial isolates from the root nodules of 10 *Lebeckia* species. The isolates were purified and tested for nodulation and nitrogen fixation on cowpea and siratro as well as their host plants. All the isolates fixed nitrogen on their respective *Lebeckia* hosts, whereas 56% of the strains were effective on cowpea and 77% on siratro.

The third chapter describes initial comparison and screening of the isolates by SP-PCR fingerprinting analysis. DNA profiles showed that most of the isolates grouped according to host plant species rather than geographical location. Isolates selected from different clusters were subjected to partial 16S rDNA gene sequencing to confirm their taxonomic identity. This revealed that *Lebeckia* is nodulated by diverse genera of root nodule bacteria from both the *α-Proteobacteria* (*Bradyrhizobium*, *Mesorhizobium,* and *Sinorhizobium*) as well as the *β-Proteobacteria* (*Burkholderia*). The final chapter (Chapter 4) provides concluding remarks on my results and possible future avenues of research on the *Lebeckia* rhizobia.

CHAPTER 1

LITERATURE REVIEW

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1.1 INTRODUCTION

Nitrogen in the form of protein represents a significant part of human and animal diets. In 1994, individual protein consumption of the 5.3 billion people on earth averaged about 70 g of protein/day or 23 million tonnes of N/annum (Vance & Graham, 1995). Maintenance of this is estimated to require a doubling or tripling of crop production together with the use of large areas now considered marginal, as the earth's population doubles over the next 40 years (Vance & Graham, 1995). In terms of importance for plant growth and development, nitrogen acquisition and assimilation is second only to photosynthesis (Granli & Bøckman, 1994). Yet nitrogen is perhaps the single most important factor currently limiting plant yields (Graham & Vance, 2003) and is largely alleviated by application of commercial fertilizer. In 1994, world fertilizer application was estimated at 77 million tonnes/year (Granli & Bøckman, 1994).

Agricultural application of nitrogen fertilizer has great disadvantages as it results in the leaching of toxic nitrate into the soil and groundwater (Hardy & Eaglesham, 1995). These and other nitrogen-based toxic chemicals threaten human health because they may cause illnesses such as cancer (Bohlool *et al.,* 1992; Graham & Vance, 2003). From an environmental perspective, contamination with inorganic and organic nitrogen compounds can lead to eutrophication of surface water, while reactive gaseous nitrogen oxides are associated with ozone layer depletion (Bohlool *et al.,* 1992; Graham & Vance, 2003). Industrial nitrogen fertilizer production is also very expensive, not only in terms of money, but also in terms of requirements for non-renewable energy resources (Hardy & Eaglesham, 1995). To synthesize ammonia from nitrogen under high temperature and pressure conditions during the Haber-Bosch process involves extremely large inputs of fossil fuel energy. It is estimated that the cost of nitrogen fertilizer production is around $20-60 billion per year (Hardy & Eaglesham, 1995).

In contrast, biological nitrogen fixation (BNF) by so-called diazotrophic bacteria is sustainable, less polluting and significantly cheaper compared to industrial nitrogen fertilizer (Vance, 2001; Giller, 2001; Graham & Vance, 2003). Diazotrophs are known from most lineages of the Eubacteria (i.e. proteobacteria, cyanobacteria, actinobacteria spirochaetes, clostridiales and chlorobiales) and the Archaeal methanogens (Doyle & Luckow, 2003; Kneip *et al.,* 2007). They may be free-living in soil and water or form associations and symbioses with certain animals, protists, fungi and a variety of plants (e.g. Kneip *et al.,* 2007). It is in these associations, especially with plants, where diazotrophs contribute most to BNF and agriculture (Giller, 2001;

Graham & Vance, 2003). BNF accounts for about 65% of agricultural nitrogen (roughly 140 million metric tonnes/year) and is becoming increasingly important in crop productivity especially for sustainable systems, small scale operations and marginal land use (Granli & Bøckman, 1994; Hardy & Eaglesham, 1995).

Overall, the symbiosis between the so-called rhizobial *Proteobacteria* and leguminous plants is responsible for by far the greatest amount of BNF in agriculture and plays a central role in sustainable agricultural systems (Giller, 2001; Graham & Vance, 2003). As a result, grain and forage legumes account for 27% of the world's primary crop production with grain legumes alone contributing 33% of the dietary protein needs of humans (Graham & Vance, 2003). In South Africa, Strijdom and Wassermann (1984) estimated that 100 000 tonnes of nitrogen are fixed per year – a figure that has probably doubled or tripled in the present decade. They also estimated a potential for the addition of 12.5 million ha of pasture leading to a total of 16 million ha which could fix an estimated 1.4 million tonnes of nitrogen (Strijdom & Wassermann 1984). This could enrich the soil by more than 400 000 tonnes of nitrogen, an amount comparable to the total nitrogen fertilizer sold annually in the country (Strijdom, 1998).

Developing countries are faced with the dilemma of increasing food production due to escalating population numbers. The problem is compounded by the low nutrient status of many agricultural soils and the expense and often limited availability of nitrogen and other fertilizers. BNF is a comparatively cheap alternative that can contribute directly to a growing crop and can increase soil fertility. Technologies based on the use of nitrogen-fixing plants are ideal for use by farmers in developing countries who do not have the financial resources to buy fertilizers even when these are available. It is clear that BNF, with its ability to capture an inexhaustible resource must play a fundamental role in agriculture (Giller, 2001). For optimal exploitation of this process, knowledge of legumes and their microsymbiont partners is essential.

The aim of this literature study is to review literature pertaining to the legume-rhizobium symbiosis and the taxonomy of rhizobia, as well as to provide a detailed overview of methods used to classify rhizobia. The taxonomy of legumes, especially those in the tribe *Crotalarieae* and genus *Lebeckia*, is also emphasized.

1.2 THE LEGUME-RHIZOBIUM SYMBIOSIS

An important step in the legume-rhizobium symbiosis is the development of nodules. Nodules are lateral outgrowths of the roots (e.g peanut) and stems of legumes as a result of an infection of the root-hair (e.g soybean) or cracks in the roots by rhizobia. Rhizobia enter their host's roots through the root hair. The infection is preceded by deformationor curling of root hairs and the formation of an infection thread caused by the rhizobial strains (Somasegaran & Hoben 1994). Effective nodules can have various forms ranging from spherical to branched and globular, but all with a pink leghaemoglobin centre, while the ineffective nodules have white centres (Corby *et al*., 1983; Heichel & Vance, 1983; Sprent 2001). Once the bacteria penetrate the root hair, bacteroids differentiate in the nodules and synthesize the rhizobial nitrogenase which fixes atmospheric nitrogen for the plant (Somasegaren & Hoben, 1994; Dean *et al.,* 1993).

Legumes are the only plant family, with the notable exception of *Parasponia* (family *Ulmaceae*) capable of being nodulated by rhizobia in the bacterial family *Rhizobiaceae* (*Rhizobium, Sinorhizobium, Azorhizobium, Bradyrhizobium*) and those in the *Beta-Proteobacteria* genera *Burkholderia*, *Herbaspirillum* and *Cupriavidis*, as well as the methylotrophic *Methylobacterium* (Sawada *et al.,* 2003; Weir *et al*., 2004; Wolde-Meskel *et al*., 2005; Weir, 2006). The mechanism by which host and symbiont recognize one another involves an interchange of molecular signals during infection and nodule formation (Hungria & Stacey, 1997). Bacterial nodulation gene products stimulate the expression of plant genes that result in the formation of nodules. The nitrogen-fixation (*nif, fix*) and nodulation genes are located on the *sym* loci which may be encoded on plasmids in the fast-growing rhizobia or chromosomal islands in the slow-growing rhizobia. The nodulation genes control the formation and structure of Nod factors (NFs) that are the primary signalling molecules responsible for host infection and nodule formation (van Rhijn & Vanderleyden, 1995). The NF consists of a lipo-chitooligosaccharide molecule which is formed by the products of the structural nodulation genes (*nodABC*).

During the symbiosis, the main energy source for nitrogen-fixation is provided by the legume in the form of photosynthetically fixed carbon (Minchin *et al.*, 1986). The latter is then translocated into the nodules in the form of sucrose, which is used as reductant for the conversion of atmospheric nitrogen to ammonia by the bacterial nitrogenase. Numerous estimates have been made on the carbon cost of symbiotic nitrogen-fixation, most of which are in the range between 5 and 18 g of carbon per g of nitrogen fixed. In addition to driving the

nitrogenase reaction in bacteroids, up to 33% of total plant photosynthate may be required when nodule development and maintenance and transport costs are considered (Minchin *et al.*, 1986). The bulk of the ammonia is however transferred to the legume partner to meet its nutritional nitrogen needs for synthesizing essential compounds such as protein, nucleic acids and chlorophyll (Minchin *et al.*, 1986).

Within the legume family, there is a great deal of host specificity. For example, lucerne is only nodulated by *Rhizobium meliloti* whereas soybean is mainly nodulated by *Bradyrhizobium japonicum.* The overall structure of the NF is believed to influence the host range of a specific rhizobial strain (Carlson *et al.*, 1993; Van Rhijn & Vanderleyden, 1995; Lopez-Lara *et al.*, 1996; Spaink *et al.*, 1998; Perret *et al.*, 2000; Moulin *et al.,* 2004;). The final structure of the NF core is determined by a class of nodulation genes often referred to as host-specific nodulation (*hsn*). The products of these genes catalyze the modification of the NF core by for example, transferring fatty-acid and other moieties to it. Rhizobial species differ broadly in terms of the activity of and complement of *hsn* genes (Carlson *et al.*, 1993; Van Rhijn & Vanderleyden, 1995; Lopez-Lara *et al.*, 1996; Moulin *et al.*, 2004;).

The practical consequence of highly specific legume-rhizobium associations for agriculture is that legume crops must be inoculated, especially when planted in soils that do not contain native populations of infective rhizobia. Considerable genetic variation exists within each symbiotic partner and careful selection of an inoculant strain is essential to ensure maximum nitrogen-fixing effectiveness with the desired cultivar. Of course, this is more important for a crop such as soybean which is highly selective in its rhizobial requirements, than a less selective crop such as cowpea (Brockwell *et al.*, 1995). Commercial inoculants for legume crops usually consist of rhizobia in a peat carrier and quality control must ensure that adequate numbers of viable rhizobia are applied at planting. Inoculants are generally attached to seed with a sticker and care must be taken to avoid contact with seed-applied pesticides that are often toxic to the rhizobia. An alternative application of inoculant in water suspension is more effective especially when seeds are easily damaged (Brockwell *et al.*, 1995).

An essential consideration in the establishment of nitrogen-fixing systems is response to adversities of soil environment. Since plant productivity can be defined by a single limiting factor in the system, no amount of biologically or chemically fixed nitrogen will increase production if some other factor is limiting growth. Major considerations affecting efficiency of

the legume symbiosis include soil acidity and related aluminum and manganese toxicity, calcium and phosphorous deficiency, salinity, flooding and the amount of available nitrogen mineralized from organic sources (Bohlool *et al.*, 1992). In addition to reducing the amount of nitrogen derived from BNF, nitrate in soil is inhibitory to nodulation and nitrogen-fixation, thus high level applications of fertilizer nitrogen must be avoided. A frequent and major obstacle to the successful use of legume inoculants is competition from native soil rhizobia for nodulation. Under such circumstances, magnitude of the nitrogen-fixing response to inoculation is related to population indices of soil rhizobia as well as the nitrogen status of the soil (Thies *et al.*, 1991).

1.3 TAXONOMY AND EVOLUTION OF LEGUMES

Together with the families *Polygalaceae*, *Surianaceae* and *Quillajaceae*, legumes (family *Leguminosae* or *Fabaceae*) form part of the monophyletic order *Fabales* in the Eurosid Clade I of the rosid angiosperms (Soltis *et al*., 2000; Angiosperm Phylogeny Group, 2003). Based on DNA-based analyses, members of the *Fabaceae* are more closely related to one another than to other angiosperms (Doyle *et al.,* 2000; Kajita *et al*., 2001). According to Doyle *et al*. (2000), the monophyletic nature of this taxon is also supported by non-molecular traits such as petal imbrication where the abaxial pair are internal to the lateral petals in the bud; a single carpel in the flower; endothecium ribs less than six per cell; and rib spacing less than twice the width.

The *Fabaceae* comprise some 18000-19000 species and about 600-750 genera (Allen & Allen, 1981, Van Wyk, 1991; Sprent, 1995; Chen *et al*., 2003; Graham & Vance, 2003; Wojciechowski, 2003; Weir *et al*., 2004; Wolde-Meskel *et al*., 2005), although estimates of actual numbers vary considerably with different authors. Nevertheless, the family *Fabaceae* is the third largest family of flowering plants in the world (Corby *et al.,* 1983; Sprent, 1995; Doyle & Luckow, 2003) and includes herbs, trees and shrubs. They are widely distributed and adapted to a variety of environments, although herbaceous species and shrubs are mostly found in cool temperate climates while leguminous trees are abundant in subtropical/tropical regions (Van Wyk, 1991). Overall, the *Fabaceae* appears to be most diverse in seasonally dry tropical forests and temperate arid to semi-arid shrub lands, while they are relatively poorly represented in mesic temperate regions such as arctic and alpine areas, as well as under stories of cool temperate forests (Wojciechowski, 2003). This distribution is thought to be linked to the nitrogen-demanding metabolism associated with semi-arid and arid habitats, conditions in which the *Fabaceae* are able to produce leaves more efficiently than most other plant families (McKey, 1994).

In the floral fossil record, legumes are not represented among the rich mid-Cretaceous about 90 million years ago (mya) and remained relatively under-represented until about 54-35 mya ago (Taylor, 1990; Herendeen *et al.,* 1992). The results of DNA-based analyses suggest that legumes evolved around 60 mya early in the Tertiary period (Lavin *et al.,* 2005; Cronk *et al.,* 2006; Sprent, 2007). This contrasts with previous ideas that legumes had a Gondwanan origin during the late Cretaceous (65-145 mya) in Africa, from where they spread to the rest of the world (Corby *et al.,* 1983). Contemporary hypotheses for explaining the global distribution of legumes propose a northern hemisphere origin and migration to the rest of the world across land bridges and via weather related phenomena (Doyle & Luckow, 2003; Cronk *et al.,* 2006; Sprent, 2007).

Nodulation is currently speculated to have arisen approximately 58 mya (Sprent, 2007; Sprent & James, 2007). The process is thought to have been prompted by an abrupt 5-10ºC rise in temperature over a range of latitudes and increased atmospheric levels of methane, carbon dioxide and humidity. As a result, carbon cycling through terrestrial ecosystems was greatly enhanced leading to the appearance of a vast plant biodiversity during this period. Sprent (2007) suggests that the probable limitation of nitrogen for plant growth during this period triggered the development of nodulation. Consistent with this idea, the major groups of nodulating legumes also emerged during this time (Sprent, 2007; Sprent & James, 2007).

The family *Fabaceae* contains many the economically valuable crops. For example, numerous species are cultivated for food (e.g. beans, peas, peanuts, soybeans and lentils) and forage (e.g., clover or *Trifolium* spp. and *Medicago* spp. such as lucerne, while others are favoured as garden ornamental trees and shrubs (e.g. *Lebeckia sericea, Acacia* spp., *Mimosa* spp.). Legumes such as clover, soybean, lupin and alfalfa are also commonly used as green manure (Doyle, 1998; Graham & Vance, 2003; Doyle & Luckow, 2003). Some members of the family have medicinal properties such as soybeans that produce isoflavones that can reduce the risks of cancer and lower serum cholesterol (Graham & Vance, 2003). Legumes such as soybean can also be milled in to flour to make bread (Garcia *et al.,* 1998).

All legumes are characterized by five-petalled flowers with an ovary that ripens to form the distinctive legume pod (Doyle & Luckow, 2003). The pod develops from a simple carpel and opens along a seam on two sides. At maturity, the two sides may split apart to release seeds

attached to the seams. Compared to other plant families, legumes exhibit greater floral morphological variation that also extends to fruit type, ranging from tiny single-seeded forms to meter long woody pods. Based primarily on the morphology of flowering plants, legumes are divided into three groups or subfamilies *Caesalpiniaceae*, *Mimosaceae* and *Papilionaceae* (Sprent, 1995; Wojciechowski, 2003). The *Papilionaceae* have one large petal, two adjacent wing petals on the sides and two bottom petals that join together in a boat-like structure (keel). The flowers of the *Caesalpiniaceae* are zygomorphic and very variable with five equal petals. The *Mimosaceae* have small petals, frequently spicate with showy stamens (Heichel & Vance, 1983; Somasegaran & Hoben 1994).

Members of the *Caesalpiniaceae* are mainly tropical and include 162 genera and 1900 species classified in one of four tribes: *Cassieae*, *Caesalpinieae*, *Cercideae* and *Detarieae*. Of these, only the members of tribes *Cassieae* and *Caesalpinieae* (about 30% of the total number of species in the subfamily) can be nodulated (Allen & Allen, 1981; Corby *et al.,* 1983; Herendeen *et al.,* 2003). The *Mimosaceae* include 77 genera and around 3000 species, which mostly occur in subtropical and temperate regions and are classified in three tribes *Mimosaceae*, *Acacieae* and *Ingeae*. The majority of these woody legumes (96%) are nodulated, and a few have apparently lost their nodulation abilities (Allen & Allen, 1981; Corby *et al.,* 1983). The *Papilionaceae* is the largest subfamily with 476 genera and about 14000 species representing 32 tribes (i.e. *Swartzieae, Sophoreae, Dipterygeae, Dalbergieae, Abreae, Tephrisieae, Robinieae, Indigofereae, Desmodieae, Phaseoleae, Psoraleeae, Amrpheae, Sesbanieae, Aeschynomeneae, Adesmieae, Galegeae, Carmichaelieae, Hedysareae, Loteae, Coronilleae, Vicieae, Cicereae, Trifolieae, Brongniartieae, Mirbelieae, Bossiaeeae, Podalyrieae, Liparieae, Crotalarieae, Euchresteae, Thermopsideae* and *Genisteae*) (Corby *et al.*, 1983). The members of this subfamily generally represent tropical herbaceous species with around 98% involved in nodulation and the nitrogen-fixing symbiosis (Allen & Allen, 1981; Corby *et al.,* 1983).

1.3.1 The tribe Crotalarieae

The tribe *Crotalarieae* is characterized by herbaceous and shrubby legumes with 3-7 foliolate digitate leaves (Corby *et al.*, 1983). The stipule in *Crotalarieae* is extremely reduced in size while in some genera (e.g. *Lebeckia*) the stipule is completely absent (Le Roux & van Wyk, 2007). The tribes *Crotalarieae*, *Podalyrieae* and *Genisteae* are considered to be closely related and include numerous agriculturally important legumes (Corby *et al.*, 1983; Van Wyk & Schutte, 1995; Sprent, 2007). Based on DNA-based phylogenetic studies, Wojciechowskie (2003)

demonstrated that the tribe *Crotalarieae*, together with the tribes *Podalyrieae, Genisteae, Thermopsideae, Euchresteae, Sophoreae* and *Lipariaea* form part of the genistoid *sensu lato* clade which all share a recent common ancestor (Corby *et al.,* 1983; Crisp *et al.,* 2000; Wojciechowski, 2003; Sprent, 2007).

The tribe *Crotalarieae* includes 16 genera (i.e. *Lebeckia, Wiborgia, Rafnia, Aspalathus, Spartidium, Crotalaria, Bolusia, Lotononis, Buchenroedera, Pearsonia, Rothia, Robynsiophyton, Melolobium, Dichilus, Anarthrophyllum* and *Sellocharis*) (Corby *et al*., 1983). Of these, *Lebeckia*, *Lotononis* and *Crotalaria* are taxonomically very similar as several characters found in some groups of *Lotononis* are present in *Lebeckia* and *Crotalaria* (Van Wyk, 1991). They are mostly centered in Africa and adjoining areas (Corby *et al*., 1983; Van Wyk, 1991), with *Crotalaria* species distributed extensively in Africa as well as Asia, Australia and across the Atlantic to South America. *Lotononis* is centered in southern Africa with some species also occurring in Australia, the Mediterranean region and India. *Lebeckia* species are mainly indigenous to the western and southern regions of South Africa (Germishuizen & Meyer, 2003; Boatwright *et al* 2007; Le Roux & van Wyk, 2007). In terms of the nodulation abilities of this tribe, most of the indigenous species examined produce indeterminate nodules with apical meristems that form branches (Corby *et al.,* 1983; Sprent, 2001; Sprent, 2007). The *Crotalarieae* appear to be nodulated by a variety of root nodule bacteria. In addition to nodulation with members of the family *Rhizobiaceae*, certain species of the African genera *Cyclopia* and *Aspalathus* are nodulated by *Burkholderia* belonging to the *β-Proteobacteria*, while certain *Crotalaria* and *Lotononis* species develop effective nodules when infected with *Methylobacterium* belonging to the *α-Proteobacteria* (Sy *et al*., 2001; Jaftha *et al*., 2002; Sprent, 2007).

Like many other legumes, those in the tribe *Crotalarieae* produce a range of secondary compounds that may be toxic. For example, pyrolizidine alkaloids cause chronic poisoning of livestock and quinolizidine causes heart-related diseases, vomiting in humans and teratogenic effects in cattle (Van Wyk *et al.,* 2002). The presence or absence of secondary compounds also represents an important classification criterion for the tribe. Compounds like nutalline, lupanine and sparteine have been identified previously in *Lebeckia* (Van Wyk & Verdoorn, 1989a), while the alkaloid, quinolizidine is common in *Lebeckia cytisoides* (Van Wyk *et al*., 2002). In the tribe *Crotalarieae, Lebeckia* differs from other quinolizidine/macrocyclic pyrrolizidine alkaloid-bearing genera *Buchenroedera, Crotalaria* and *Lotononis* by the absence of α-pyrodone

alkaloids and esters of alkaloids. Van Wyk & Verdoorn (1989a, b) used this chemotaxonomic evidence to conclude that *Buchenroedera* and *Lotononis* are more closely related to *Crotalaria* than to *Lebeckia* despite morphological evidence. Members of the *Crotalarieae* also accumulate large amounts of hydroxylated lupanine-type alkaloids (*Lebeckianine*) in their seeds (Van Wyk & Verdoorn, 1989a, b; Van Wyk & Schutte, 1995).

Several species in this tribe are agriculturally important. For example, plants of the genus *Crotalaria* are used in green manures and intercropping (Giller, 2001), *Aspalathus linearis* is the source of the well-known rooibos tea beverage (Van Heerden *et al*., 2003) and *Lotononis bainesii* is a valuable forage legume (Yates *et al.,* 2007). The genus *Lebeckia* is of concern for this study and is presently not commercially exploited but has horticultural and forage potential (see below).

1.3.2 The genus Lebeckia

The genus *Lebeckia* Thunb. was last revised taxonomically by Harvey in 1862, following Bentham (1844). This taxonomic system is however currently being revised (Le Roux & Van Wyk, 2007; Boatwright *et al.*, 2007) and this study follows the B-E. van Wyk (personal communication) classification of *Lebeckia* species into the three sections *Calobota, Virborgiodes* and *Stiza* with trifoliate leaves and shrubby growth habits and the two sections *Lebeckia* and *Spira* with needle-shaped or unifoliolate leaves and suffrutescent or herbaceous growth habits. The shape and size of fruits appear to be the most useful character for distinguishing the species (Le Roux & van Wyk, 2007). The genus consists of some 33-35 species (listed in Table 1.4) that are mainly indigenous to the western and southern regions of South Africa (Germishuizen & Meyer, 2003; Boatwright *et al.,* 2007; Le Roux & van Wyk, 2007). Le Roux & Schelpe (1997) also mentioned that 35 species of *Lebeckia* are found in southern Africa with 15 species occurring in Namaqualand. The plants within this genus are tolerant to acidic soils and harsh conditions and also show a high tolerance towards cold temperature and frost. No studies of the symbiotic rhizobia of *Lebeckia* have been documented prior to the investigation presented in this dissertation.

The largest section is *Lebeckia* with 12 species (Le Roux & van Wyk., 2007). They have acicular needle-shaped leaves that are spirally arranged into branches. The reference species for this section is *Lebeckia sepiaria* (Le Roux & van Wyk., 2007). They are mainly perennial herbs of height between 0.1-0.7 m and grow mostly in the Northern Cape and Western Cape provinces.

The very rare *L. wrightii* (Germishuizen & Meyer, 2003; Le Roux & van Wyk, 2007) also occurs here and is the only species in the section *Spira*. Many annual species are found in Clanwilliam and Bushmanland in the east of Namaqualand (Le Roux & Schelpe, 1997).

According to the revision by Boatwright *et al.* (2007), three species (*L. cuspidosa*, *L. psiloloba* and *L. pungens*) are classified in the shrubby section *Stiza*. Their heights are around 0.5-2.5 m and they are abundant in the Northern Cape, Eastern Cape and near Bloemfontein in the Free State Province (Germishuizen & Meyer, 2003). Section *Stiza* is characterized by plants that are extremely thorny, with unifoliolate leaves (Boatwright *et al.*, 2007). Species in section *Calobota* are perennial shrubs with heights of 0.15-0.6 m. The section consists of about nine species occurring in the Northern Cape, Western Cape and Eastern Cape Provinces. Section *Viborgioides* contains perennial shrubs with heights of 0.2-2 m. They develop well in the Western Cape, Free State, Eastern Cape and Northern Cape (Germishuizen & Meyer, 2003).

Various *Lebeckia* spp. represent pasture and forage legumes which are well suited to moderate to heavy grazing conditions. Several species, especially *L. spinescens*, *L. multiflora* and *L. sericea*, are recognized as valuable forage plants in natural veldt. *Lebeckia spinescens* (the name means "becoming spinous") or sand ganna is found in dry clay or granite slopes from Namibia to Malmesbury in the western Cape and in drier Karoo areas to the Eastern Cape (Manning & Goldblatt, 1996; 1997) and in Namaqualand, Bushmanland, Great Karoo and the southern Cape (Le Roux *et al.*, 1994; Shearing & van Heerden, 1994) and can survive with relatively little annual rainfall (100-420 mm pa.). It produces bean-pod fruits that have high nutritional value and are very well grazed. Another well grazed species is *L. sericea* or "blou fluitjies" (Fig. 1.1d) that produces edible flowers and pods and grows in rocky places in Namaqualand and drier areas of the south Western Cape Province (Le Roux & Schelpe, 1997). Although *L. multiflora* or "fluitjiesbos" (Fig 1.1c) is not readily grazed by animals, it is valuable in times of drought when even the dry leaves, flowers and pods can be eaten. This species also can be utilized to stabilize old lands and unstable sand (Van Breda & Barnard, 1991).

1.4 THE TAXONOMY OF RHIZOBIA

All nodulating nitrogen-fixing bacteria are part of the *Proteobacteria* found in the domain Eubacteria. The *Proteobacteria* represents the second largest group of bacteria and consist of a diverse range of organisms including purple phototrophic, nitrifying and enteric bacteria as well

as symbiotic and free-living nitrogen-fixing bacteria (Jordan, 1984). All *Proteobacteri*a are Gram-negative and motile by means of flagella. Based on analyses of the highly conserved small subunit 16S ribosomal RNA (16S rRNA) gene sequence, the *Proteobacteria* are divided into the five groups α-, *β*-, *γ*-, δ- and ε-*Proteobacteria* (Jordan, 1984; Zakhia & de Lajudie, 2001). Nodulation and nitrogen fixation is restricted to the *α-Proteobacteria* and *β-Proteobacteria*. The terms *α*- and *β*-rhizobia were proposed to distinguish rhizobia of the *α-Proteobacteria* and *β-Proteobacteria* (Chen *et al*., 2003a).

At first, bacteria that formed nitrogen-fixing (diazotrophic) nodules on legumes were all assigned to the genus *Rhizobium* with different species being recognized on the basis of cross-inoculation nodulation specificity (Fred *et al*., 1932; Young, 1996). However, Fred *et al*. (1932) recognized that rhizobia could be differentiated into fast-growing and slow-growing groups on laboratory media. Later, Jordan (1984) used DNA composition and other information to divide rhizobia into the fast-growing genus *Rhizobium*, including species that nodulate legumes such as bean, clover and pea, and the slow-growing genus *Bradyrhizobium*, including rhizobia that nodulate legumes such as soybean and cowpea. In 1984, Bergey's Manual of Systematic Bacteriology classified the *Rhizobiaceae* into four genera: *Rhizobium, Bradyrhizobium, Agrobacterium* and *Phyllobacterium* (Jordan, 1984). Further phylogenetic studies based on sequence comparisons of the 16S rRNA gene led to classification of the rhizobia into four genera: *Rhizobium, Bradyrhizobium, Sinorhizobium* and *Mesorhizobium*, all of which lie in different branches of the α-*Proteobacteria* (Fig. 1.2), and later separation of the genus *Rhizobium* into six genera (Young *et al*., 2001).

Like most other bacteria, the classification of nitrogen-fixing bacteria (diazotrophs) is under constant review as new species are discovered and existing species are split or condensed into one (Laguerre *et al*., 1994). The most recent taxonomy of rhizobia consists of 62 species found in 12 genera of nodule-forming diazotrophic bacteria, nine of which are *α*-rhizobia (A*zorhizobium, Bradyrhizobium, Devosia, Mesorhizobium*, *Methylobacterium, Ochrobactrum, Phyllobacterium, Rhizobium* and *Sinorhizobium)* and three *β*-rhizobia (*Burkholderia, Cupriavidus and Herbaspirillum*) (Moulin *et al*., 2001; Sawada *et al.,* 2003; Weir *et al*., 2004; Wolde-Meskel *et al*., 2005; Weir, 2006). The currently recognized species of rhizobia are listed in Table 1.2, as well as on the phylogenetic trees (Figs. 1.2, 1.3), and are also discussed below.

1.4.1 *α*-Rhizobia

1.4.1.1 The genus *Rhizobium*

The genus *Rhizobium* is phylogenetically heterogeneous and consists of more than 20 accepted species (Zakhia & de Lajudie, 2001; Quan *et al.,* 2005; Willems, 2006; Weir, 2006). The name *Rhizobium* means 'root living' and was first recognized by Frank in 1889. *Rhizobium*, *Agrobacterium* and *Allorhizobium* are closely related (Willems, 2006) and the latter two genera were incorporated into the genus *Rhizobium* (Young *et al*., 2001). Their strains are characterized by fast growth on yeast extract mannitol medium and the production of acid in mineral salts-mannitol medium. The DNA G+C content of the genus is in the range 59-64 mol% (Jordan, 1984). The finding that the fast-growing *R. meliloti* shares major features with the non-nodulating genus *Agrobacterium*, led Young *et al*. (2001) to propose combination of the genera *Agrobacterium* and *Rhizobium* into a single genus *Rhizobium*. The former species of agrobacteria are now classified in to the genus *Rhizobium*, including *Ag. tumefaciens, Ag. radiobacter, Ag. rhizogenes, Ag. rubi* and *Ag. vitis* (Holmes & Roberts, 1981; Kwon *et al*., 2005). Many *Agrobacterium* species are pathogens responsible for tumour or gall-like symptoms on their hosts (Young *et al*., 2001) and are therefore not considered here in detail.

(a) *Rhizobium leguminosarum*

Rhizobium leguminosarum, *R. trifolii* and *R. phaseoli* are now regarded as biovars of a single species *R. leguminosarum* (Jordan, 1984), of which the type strain for this species nodulates members of the legume genus *Vicia*. The three biovars of *R. leguminosarum* include biovar *viciae* nodulating the tribe *Vicieae* (*Pisum, Vicia, Lathyrus* and *Lens*); biovar *trifolii* nodulating *Trifolium* species (clover) and biovar *phaseoli* nodulating *Phaseolus vulgaris* (common bean).

(b) *Rhizobium etli* and *Rhizobium galegae*

Rhizobium etli was proposed after genetic and phenotypic characters of isolates nodulating both alfalfa and beans indicated a group distinct from *R. leguminosarum* bv. *phaseoli* (Segovia *et al*., 1993). *R. etli* contains two biovars, *R. etli* bv. *phaseoli* and *R. etli* bv. *mimosae* (Wang *et al*., 1999a). The strains nodulate and fix nitrogen in association with *Phaseolus vulgaris* and other legumes such as *Mimosa affinis* (Brenner *et al.,* 2005). *Rhizobium galegae* is the symbiont of *Galega orientalis* and *G. officinalis*. It appears to be rather specific to these species (Brenner *et al.,* 2005; Young, 1996).

(c) *Rhizobium tropici*

Rhizobium tropici forms symbiotic association with *Phaseolus vulgaris* and *Leucaena* spp. The species is distinguished from other rhizobia by DNA-DNA reassociation, multilocus enzyme electrophoresis profiles and biochemical tests, and 16S rRNA sequence comparison (Martinez-Romero *et al*., 1991; Brenner *et al.,* 2005).

(d) *Rhizobium gallicum* and *R. giardinii*

The two species were identified by Amarger *et al*. (1997) using phenotypic differentiation and 16S rRNA sequence analysis and are relatively fast-growing. For *R. gallicum*, two biovars, bv. *gallicum* and bv. *phaseoli* were identified based on nodulation specificity. Biovar *gallicum* nodulates and fixes nitrogen in association with *Leucaena leucocephala* and *Phaseolus* spp. whereas biovar *phaseoli* nodulates *Phaseolus* spp. and *Macroptilium atropurpureum*. *R. giardinii* has two biovars, bv. *giardinii* and bv. *phaseoli*. Biovar *giardinii* is symbiotic on *Phaseolus* spp. other than *P. vulgaris*, as well as *L. leucocephala* and *M. atropurpureum*. *R. gallicum* bv. *phaseoli* nodulates *Phaseolus* spp. but is partially efficient in fixing nitrogen in association with *L. leucocephala* (Brenner *et al.,* 2005).

(e) *Rhizobium hainanense*

Unique isolates from the roots of trees, herbs and vines with diverse phenotypic and genotypic characteristics were distinguished as *R. hainanense*, using DNA hybridization studies, cluster analysis of phenotypic features and 16S rRNA sequences (Chen *et al*., 1997). This fast-growing bacterium was first identified from tropical legumes in Hainan, China. The species also stimulate nodulation on *Acacia sinicus*, *Desmodium gyroides*, *D. sinuatum* and other *Desmodium* species (Brenner *et al.,* 2005).

(f) *Rhizobium huautlense*

The comparative sequence analysis of 16S rRNA indicates that *R. huautlense* is closely related to *R. galegae* (Wang *et al*., 1998). The two species are differentiated according to multilocus enzyme electrophoresis and DNA-DNA reassociation. *R. huautlense* is a symbiont of *Sesbania herbacea, S. rostrata* and *Leucaena leucocephala*, in Mexico (Wang *et al.,* 1998), although the most common symbionts of *Sesbania* species are *Azorhizobium caulinodans* as discussed below (Wang *et al*., 1998).

(g) *Rhizobium mongolense*

Rhizobium mongolense shares 99.2% similarity in its 16S rRNA sequence with *R. gallicum* (Van Berkum *et al*., 1998). *R. mongolense* was identified as the symbiont of *Medicago ruthenica* from the United States and also nodulates *Medicago sativa* (alfalfa) and fixes nitrogen in *Phaseolus vulgaris* (Van Berkum *et al*., 1998). Its presence in the root nodules of these plants contradicts the accepted notion that *Sinorhizobium* species are the predominant symbionts of the *Medigaco* species (Le Roux, 2003; Brenner *et al.,* 2005)

(h) *Rhizobium sullae*

The rhizobia isolated from *Sulla* root nodules were previously referred to as *R. hedysari* and were shown to exhibit a high degree of host specificity. 16S rRNA gene phylogenies revealed that the *Sulla* isolates are related to *R. gallicum*, *R. mongolense* and *R. leguminosarum*. Based on the results of this study, the species *R. sullae* was proposed (Squartini *et al*., 2002).

(i) *Rhizobium undicola*

Rhizobium undicola represents the former *Allorhizobium undicola* (De Lajudie *et al.,* 1998b; Young *et al*., 2001). It nodulates *Neptunia natans* an aquatic plant, indigenous to waterlogged areas in Senegal and India (De Lajudie *et al.,* 1998b). Phylogenetically the closest neighbour of *R. undicola* is the plant pathogen *Ag. vitis* (Zakhia & de Lajudie, 2001).

(j) *Rhizobium loessense*

Rhizobium loessense was identified from the plant genera *Astragalus* and *Lespedeza* in the Loess Plateau, China (Wei *et al*., 2003). Diverse rhizobia have previously been described from *Astragalus* that includes *Mesorhizobium, Rhizobium* and *Sinorhizobium*. The species *R. loessense* was distinguished from relatives using phenotypic characteristics (Wei *et al*., 2003).

(k) *Rhizobium indigoferae*

Rhizobium indigoferae was isolated from *Indigofera* host plants in the Loess Plateau China. As with other bacteria, a polyphasic approach (SDS-PAGE, DNA-DNA reassociation, 16S rRNA sequencing) was used to characterize the species. Phylogenetically it appears to be closely related to *R. mongolense* and *R. yuanglingense* (Wei *et al.,* 2002).

(l) *Rhizobium yuanglingense*

The species was isolated and described by Tan *et al.* (2001). The isolates were collected in arid and semi arid regions in China from the wild legumes *Amphicarpaea trisperma*, *Coronilla varia* and *Gueldenstaedtia*. *R. yuanglingense* produces ineffective nodules on *Phaseolus vulgaris* though it does not nodulate *Galega orientalis* and *Leucaena leucocephala* (Tan *et al.*, 2001).

(m) *Rhizobium lusitanum*

Rhizobium lusitanum was isolated in Portugal from nodules of *Phaseolus vulgaris*, a promiscuous legume that forms symbiotic associations with most of the species within the *Rhizobiaceae* family. *R. lusitanum* is closely related to *R. rhizogenes* and *R. tropici* on the 16S rRNA phylogenetic trees. It induces effective nodules on *Phaseolus vulgaris*, *Macroptilium atropurpureum*, *Leucaena leucocephala* and ineffective nodules on *Medicago sativa* (Valverde *et al.*, 2006).

1.4.1.2 The genus *Bradyrhizobium*

Initially, the slow-growing *B. japonicum* was the only recognized species of the genus although all soybean symbionts and other slow-growing rhizobia were also placed in the genus (Jordan, 1984; Young & Haukka, 1996). The cells of members of this genus are non-spore forming and motile by one polar or subpolar flagellum. The colonies are circular, opaque, rarely translucent, white and convex becoming visible five or more days after inoculation. They produce an alkaline reaction in mineral salts medium containing mannitol or other carbohydrates (Jordan, 1984).

Among the rhizobia in the family *Rhizobiaceae*, *Bradyrhizobium* is phylogenetically distinct from the more closely related genera *Rhizobium, Mesorhizobium* and *Sinorhizobium* (Van Berkum & Eardley, 1998). The G+C content of the *Bradyrhizobium* genus is 57-63 mol %. The genus currently contains seven species; *B. japonicum*, *B. elkanii*, *B. liaoningense, B. yuanmingense, B. betae*, *B. canariense* and *B. denitrificans* (Jordan, 1984; Kuykendall *et al.*, 1992; Xu *et al.*, 1995; Yao *et al.,* 2002; Rivas *et al.*, 2004; Vinuesa *et al.,* 2005b; Van Berkum *et al.*, 2006). Within the above species, only *B. betae* is not associated with nodulation and nitrogen-fixation but rather with tumour-like deformations of the roots of *Beta vulgaris* (Rivas *et al.,* 2004). Undescribed strains of *Bradyrhizobium* are known as *Bradyrhizobium* spp., followed

by the name of the legume host in parentheses e.g. *Bradyrhizobium* strain (*Arachis*) (Van Rossum *et al.,* 1995; Young, 1996).

(a) *Bradyrhizobium japonicum* and *B. elkanii*

Both species will effectively nodulate soybean (*Glycine max*). Soybean *B. japonicum* is sensitive to acid (Vinuesa *et al.,* 2005b). Among *B. japonicum* strains two DNA homology groups were initially recognized on the basis of fatty acid analysis, antibiotic resistance and DNA-DNA hybridization (Jordan, 1984; Graham *et al*., 1991). Hollis *et al.* (1981) showed that strains belonging to the DNA homology group II of *B. japonicum*, showed 30% homology with the type strain of *B. japonicum*. They suggested that these strains represent a distinct species and subsequently *B. elkanii* was proposed by Kuykendall *et al.* (1992) for this subgroup of soybean bradyrhizobia, based on DNA homology, RFLP analysis, 16S rRNA sequence data and antibiotic resistance profiles (Vinuesa *et al.,* 2005b).

(b) *Bradyrhizobium liaoningense*

Bradyrhizobium liaoningense, proposed by Xu *et al.* (1995), was identified from soybean and the related *Glycine soja* using DNA-DNA hybridization, G+C analysis and partial 16S rRNA sequence analysis. Colonies of this species are 0.2-1.0 mm in diameter after 7-14 days growth on yeast mannitol agar, compared with 5-7 days for other *Bradyrhizobium* species (Xu *et al*., 1995).

(c) *Bradyrhizobium denitrificans*

Bradyrhizobium denitrificans represents the former species *Blastobacter denitrificans* (Hirsch & Muller, 1985; Van Berkum *et al*., 2006). It represents fresh water bacteria that nodulate the water plant *Aeschynomene indica* (Van Berkum & Eardley, 2002). The amino acid sequences of certain photosynthetic genes of *B. denitrificans* are identical to those of bradyrhizobial isolates with which they also share the ability to propagate by budding.

(d) *Bradyrhizobium canariense*

Bradyrhizobium canariense got its name from the fact that the species is found in the Canary islands nodulating endemic shrub legumes. Strains of *B. canariense* are highly acid-tolerant (Vinuesa *et al*., 2005b) and nodulate diverse species in the tribes *Genisteae* and *Loteae*. *B. canariense* also fixes nitrogen on the promiscuous legume *Macroptilium atropurpureum* and does not nodulate *Glycine max* (Vinuesa *et al*., 2005b). *B. canariense* can be distinguished from other *Bradyrhizobium* species by a combination of genotypic, physiological and ecological

characteristics, most appropriately by DNA-DNA hybridization. *B. canariense* strains form a monophyletic cluster that appears to be most closely related to *B. japonicum* (Vinuesa *et al.*, 2005b).

(e) *Bradyrhizobium yuanmingense*

Bradyrhizobium yuanmingense was isolated from the root nodules of *Lespedeza* species growing in China (Yao *et al.,* 2002). The word "*yuanmingense*" refers to the royal garden Yuanmingyuan in Beiljing, China, where the bacteria was isolated. Again, a polyphasic approach (DNA-DNA hybridization, 16S rRNA sequencing and nodulation tests with selected host legumes) was used to characterize *B. yuanmingense* (Yao *et al.*, 2002).

1.4.1.3 The genus *Sinorhizobium*

The genus *Sinorhizobium* was originally proposed by Chen *et al.* (1988) for the group of fast-growing rhizobia that nodulate lucerne, soybean and various *Medicago* spp. (Scholla & Elkan, 1984; Chen *et al.*, 1988). De Lajudie *et al.* (1994) emended the genus *Sinorhizobium*, and reclassified *R. meliloti* as *S. meliloti* and also transferred *R. fredii* to the *Sinorhizobium* genus. Due to various technicalities regarding the *International Code of Nomenclature of Bacteria*, *Sinorhizobium* is considered to be synonymous with the genus *Ensifer* (Martens *et al.,* 2007). *Sinorhizobium* includes 11 species, with *S. meliloti* as the type strain.

(a) *Sinorhizobium fredii* and *S. xingiangensis*

These two species can effectively nodulate soybean (*Glycine max, G. soja*), cowpea (*Vigna unguiculata*) and pigeon pea (*Cajanus cajan*). Two groups within *S. fredii* are recognized based on DNA-DNA hybridization and serology, i.e. chemovars *fredii* and *siensis* (Scholla & Elkan, 1984). Within the genus *Sinorhizobium*, 16S rRNA phylogeny is sufficient to distinguish between *S. fredii* and *S. meliloti* (see below) (Jarvis *et al.,* 1992). However, *S. fredii* and *S. xingiangensis* share 100% 16S rRNA similarity. The fast-growing *S. xinjiangensis* was associated with soybean nodules in Xinjiang, China (Chen *et al.,* 1988).

(b) *Sinorhizobium meliloti* and *S. medicae*

Sinorhizobium meliloti is a symbiotic partner of three legume genera: *Medicago*, *Melilotus* and *Trigonella* (Eardly *et al.*, 1990; Rome *et al.*, 1996; Young, 1996). This species could be distinguished from other members of this genus by electrophoretic protein profiles, DNA-DNA

hybridization and 16S rRNA sequences (De Lajudie *et al*., 1994). *S. medicae* also nodulates *Medicago* species, and is distinguishable from *S. meliloti* using DNA-DNA hybridization studies among others (Rome *et al.,* 1996).

(c) *Sinorhizobium saheli* and *S. terangae*

In 1994, the two species *S. saheli* and *S. terangae* were accepted as new species (De Lajudie *et al*., 1994). The species were obtained from different *Acacia* and *Sesbania* hosts in the Sahel area of Senegal. *S. saheli* strains are capable of nodulating *Sesbania* species found in the Sahel area and are also found in association with *Acacia, Leucaena* and *Neptunia* species (Boivin & Giraud, 1999). On the basis of 16S rRNA sequences phylogeny, the strains cluster with *S. meliloti* and *S. fredii* (Fig. 1.2).

(d) *Sinorhizobium arboris, S. kostiense* and *S. morelense*

These three species have the ability to induce the development of root nodules on *Acacia senegal* and *Prosopis chilenses* originating from Kenya and Sudan (Nick *et al*., 1999a). Phylogenetically *S. arboris* and *S. kostiense* group separately from *S. morelense* (Fig. 1.2) (Nick *et al*., 1999b). The latter are associated with the legume *Leucaena leucocephala*. They may be identified using RFLP analysis of 16S rRNA together with Southern hybridization of *nifH* and *nodDAB* (Wang *et al*., 1999c; Wang *et al*., 2002).

(e) *Sinorhizobium kummerowiae*

Sinorhizobium kummerowiae was isolated from the drought-tolerant perennial *Kummerowia stipulacea* in China (*Wei et al.*, 2002), which is used as green manure and for preventing soil erosion (Allen & Allen, 1981). 16S rRNA gene analysis indicated that the isolates from *K. stipulacea* are related to *S. fredii, S. xinjiangense, S. saheli* and *S. terangae*.

(f) *Sinorhizobium americanus*

Sinorhizobium americanus was isolated from the nodules of *Acacia acatlensis* in Mexico (Toledo *et al.,* 2003). The species differ from other *Sinorhizobium* species in terms of DNA-DNA hybridization, 16S rRNA sequences and nif*H* gene sequences.

1.4.1.4 The genus *Mesorhizobium*

This genus was proposed by Jarvis *et al.* (1997), and its members share the property of acid production with species of *Rhizobium* and *Sinorhizobiu*m (Van Berkum & Eardley, 1998). There

are 12 recognized species within the genus with *M. plurifarium* being the type strain (Young, 1996) (Table 1.2). The genus is intermediate in growth between the fast-growing *Agrobacterium-Rhizobium-Sinorhizobium* group and the slow-growing genera *Azorhizobium* and *Bradyrhizobium* (Jarvis *et al*., 1997). The name *Mesorhizobium* implies intermediate growth rate and phylogenetic position (Fig. 1.2). Most of the species within this genus were former members of the *Rhizobium* genus, including *Mesorhizobium loti (*formerly *R. loti), Mesorhizobium huakuii (*formerly *R. huakuii), Mesorhizobium ciceri (*formerly *R. ciceri), Mesorhizobium mediterraneum (*formerly *R. mediterraneum)* and *Mesorhizobium tianshanense (*formerly *R. tianshanense*) (Young, 1996; Jarvis *et al*., 1997). They were however separated from the fast-growing rhizobia by properties such as the location of symbiotic genes, 16S rRNA phylogeny (Fig.1.2) and DNA homology.

(a) *Mesorhizobium loti* and *M. huakuii*

Mesorhizobium loti form effective nodules on species of *Lotus*, *Lupinus* and *Anthyllis* (Young, 1996). The *Mesorhizobium* isolates from the nodules of the legume genus *Astragalus* revealed a distinct DNA homology group that carry their symbiotic genes on plasmids and not on chromosomes, as is the case with other *Mesorhizobium* (Chen *et al.,* 1991). The isolates were identified as *M. huakuii*.

(b) *Mesorhizobium ciceri* and *M. mediterraneum*

The chickpea symbionts are classified as *Mesorhizobium* species regardless of generation time and they constitute a separate branch closely related to but clearly different from *M. loti.* Two phylogenetic lineages (groups A and B) were identified, with the more homogenous group B representing *M. ciceri* (Nour *et al*., 1994). Group A, on the other hand displayed great heterogeneity (Nour *et al.,* 1994). Based on genomic studies conducted in 1995, it was shown not to be related to any other species, including *M. ciceri* (Nour *et al.,* 1995). Group A was named *M. mediterraneum* and differentiated from other mesorhizobia by 16S rRNA sequence, DNA-DNA hybridization and fatty acid analysis (Nour *et al.,* 1995).

(c) *Mesorhizobium tianshanense*

Chen *et al*., 1995, used numerical taxonomy to analyze a number of strains isolated from various leguminous plants in the arid saline environment in the Tianshan Mountains of Xinjiang China. A large number of the fast-growing isolates corresponded to *Rhizobium* species whereas moderate slow-growers were described as *M. tianshanense*. *M. tianshanense* nodulate *Glycine*

max, *Glycyrrhiza* spp. and *Caragona polourensins* and does not induce nodules on the promiscuous legumes *Macroptilium atropurpureum, Vigna unguiculata, Phaseolus vulgaris*, or *Pisum sativum* (Chen *et al*., 1995).

(d) *Mesorhizobium plurifarium*

Mesorhizobium plurifarium was isolated from the root nodules of tropical *Acacia* species from Senegal, and *Lotus* species (De Lajudie *et al.,* 1994). De Lajudie *et al.* (1998a) performed a taxonomic investigation using techniques such as REP-PCR, 16S rRNA sequencing and DNA-DNA hybridization, which led to the identification of the species *M. plurifarium* (De Lajudie *et al.,* 1998a). *M. plurifarium* showed higher similarities of their 16S rRNA sequences to *M. loti, M. haukii* and *M. mediterraneum. M. plurifarium* is not host specific, having been isolated from *Acacia* spp., *Prosopis* spp., *Leucaena* spp., *Lotus* spp., *Cicer* spp., *Medicago* spp., *Sesbania* spp., *Trifolium* spp., *Phaseolus* spp., *Glycine* spp. and related genera (De Lajudie *et al.,* 1998; Wang *et al*., 2004).

(e) *Mesorhizobium amorphae* and *M. chacoense*

Mesorhizobium amorphae is the rhizobial symbiont of the legume shrub *Amorpha fruticosa* (Wang *et al.,* 1999b). The legume is native to the south eastern United States and has various agricultural applications including soil erosion control, green manure and biological control (Wang *et al*., 1999b). The bacterium *M. chacoense* was described after determining the diversity of rhizobia associated with *Prosopis chilensis* occurring in diverse geographical regions in central Argentina (Velazquez *et al*., 2001).

(f) *Mesorhizobium albiziae*

Mesorhizobium albiziae nodulates *Albizia kalkora*, and also forms effective nodules on *A. julibrissin*, *Glycine max*, *Leucaena leucocephala*, *Phaseolus vulgaris* and several other legumes. The species was discovered in subtropical regions of China (Wang *et al*., 2007). It varies from previously identified mesorhizobia with unique genomic characteristics (Wang *et al*., 2007).

(g) *Mesorhizobium septentrionale* and *M. temperatum*

Mesorhizobium septentrionale and *M. temperatum* were isolated from *Astragalus adsurgens* growing in northern regions of China (Gao *et al*., 2004). The name *M. temperatum* refers to temperate regions in China where isolated. These two species differ significantly in terms of DNA-DNA homology and *nodA* sequences (Gao *et al*., 2004).

(h) *Mesorhizobium thiogangeticum*

This species was isolated from the root nodules of *Clitoria ternatea*, which is a thin herbaceous legume from the Gangetic plains of India. *M. thiogangeticum* is the first member of *Mesorhizobium* found to oxidize sulphur (Ghosh & Roy, 2006). Its 16S rRNA and *recA* sequences were similar to those of other *Mesorhizobium*, but were most similar to *M. loti, M. plurifarium, M. amorphae* and *M. chacoense* (Ghosh & Roy, 2006).

1.4.1.5 The genus *Azorhizobium*

Azorhizobium produce nitrogen-fixing nodules on the stem and roots of *Sesbania rostrata* (Dreyfus *et al*., 1988) and its symbiotic association are important in tropical agriculture (Rinaudo *et al.,* 1991). *Azorhizobium* also assimilates dinitrogen in the free-living state (Dreyfus *et al*., 1988). Following numeric analysis of phenotypic characters, DNA-DNA hybridization and protein comparison (Dreyfus *et al*., 1988), this genus was introduced to include rhizobia that have intermediate to fast growth rates. Phylogenetically, *Azorhizobium* is most closely related to *Xanthobacter* and *Bradyrhizobium*.

(a) *Azorhizobium caulinodans*

Azorhizobium caulinodans is the type species of the genus and nodulates and fix nitrogen only on *Sesbania rostrata* (Dreyfus *et al*., 1988; Rinaudo *et al*., 1991). The species is very unique in that it grows fast in the free-living state at the expense of dinitrogen as its sole source of nitrogen, which is an essential feature distinguishing it from other rhizobial genera. On *S. virgata*, *Az. caulinodans* does not complete nodule formation, as pseudonodules are produced (Dreyfus *et al*., 1988; De Souza Moreira *et al.,* 2006).

(b) *Azorhizobium doebereinerae*

This species was formerly named *Azorhizobium johannae* and is a microsymbiont of the woody plant *Sesbania virgata* native to Brazil (De Souza Moreira *et al.,* 2006). *Az. doebereinerae*, nodulates *Macroptilium atropurpureum, Phaseolus vulgaris*, but with reduced nitrogen fixation. It does, however, only produce pseudonodules on *S. rostrata*, (De Souza Moreira *et al.,* 2006). Overall, this species is genotypically and phenotypically very similar to *Az. caulinodans*.

1.4.1.6 The genus *Phyllobacterium*

The genus *Phyllobacterium* includes five nodulating species. Mantelin *et al.* (2006) described the four new species *P. bourgognense, P. infrigiyense, P. leguminum* and *P.brassicacearum*, which were originally isolated from root nodules of *Brassica napus* (in France), *Argyrolobium uniflora*, *Astragalus algerianus* and *Lathyrus numidicus* (in southern Tunisia), respectively. *P. trifolii* forms symbiotic associations with plants of the genus *Trifolium pratense* and *Lupinus albus* (Valverde *et al.*, 2005). Phylogenetically, members of this genus are most closely related to *Mesorhizobium* species (Young *et al.*, 2001).

1.4.1.7 The genus *Ochrobactrum*

The genus *Ochrobactrum* was first described by Holmes *et al*. (1988) from family *Brucellaceae*, of the *α-Proteobacteria* (Fig 1.2). The genus *Ochrobactrum* currently comprises 11 species, with *Ochrobactrum lupini* and *O. cytisi* as the first species found to possess rhizobial properties within the genus. *O. lupini* was originally isolated from *Lupinus albus* plant nodules in Argentina, and can also nodulate other *Lupinus* plants from several geographical origins (Trujillo *et al*., 2005). *O. cytisi* was isolated from the root nodules of *Cytisus scoparius* in Spain and is named after this host plant (Zurdo-pineiro *et al.,* 2007).

1.4.1.8 *Methylobacterium nodulans*

The genus *Methylobacterium* consists of a variety of pink pigmented facultative methylotrophic (PPFM) bacteria that utilize carbon as their energy source (Green, 1992). The only exception is *M. nodulans*, described as a facultative methylotropic unpigmented isolate from *Crotalaria* spp. that utilizes methanol and formate as sole carbon source (Sy *et al*., 2001; Jourand *et al*., 2004). In 2002, Jaftha *et al.* characterized the red-pigmented rhizobia from *Lotononis bainesii* in South Africa as *Methylobacterium* isolates with 16S rRNA sequences that were 98% similar to that of *M. nodulans.* Their common feature is that their original host plants *Crotalaria* and *Lotononis* belong to the same tribe. PPFM *Methylobacterium* isolates have been also isolated from water and leaf surfaces (Holland, 1997).

1.4.1.9 *Devosia neptuniae*

The genus *Devosia* was initiated by Nakagawa *et al* (1996) and belongs to the family *Hyphomicrobiaceae*. Six species are classified in the genus *Devosia, D. insulae, D. limi, D. neptuniae, D. riboflavina, D. soli* and *D. subaequoris* (Euzéby, 2008). *Devosia* spp. are mostly

soil and water bacteria (Nakagawa *et al* 1996). *D. neptuniae* is the first *Devosia* species found to fix nitrogen in symbiosis with plants (Rivas *et al*., 2003). It nodulates and fix nitrogen on *Neptunia natans,* an aquatic legume in India (Rivas *et al*., 2003), as opposed to the *N. natans* from Senegal in Western Africa that form symbiotic associations with *Rhizobium undicola* (De Lajudie *et al.*, 1998b; Young *et al*., 2001).

1.4.2 *β-Rhizobia*

It recently became evident that the ability to induce nodules on the roots (or stems) of plants is not restricted to the *α-Proteobacteria* (Chen *et al*., 2003; Chen *et al*., 2006; Barrett & Parker, 2006). It is now commonly accepted that some genera in the *β-Proteobacteria* are also able to nodulate and establish effective nitrogen-fixing symbioses with legumes. The terms *α-* and *β-*rhizobia were proposed to distinguish rhizobia of the *α-Proteobacteria* and *β-Proteobacteria* (Chen *et al*., 2003). Various legumes and environments remain to be explored and it is, therefore, highly likely that further characterization of rhizobia will reveal an even greater diversity (Chen *et al*., 2003).

1.4.2.1 The genus *Burkholderia*

Burkholderia species are common soil and rhizosphere inhabitants, whilst others are plant and human pathogens (Baldani *et al*., 2000; Eastrada-de los Santos *et al.,* 2001; Goris *et al*., 2004; Payne *et al.,* 2005). The genus was created in 1992 through the transfer of seven *Pseudomonas* species to this genus. The genus currently includes about 30-34 species (Coenye *et al*., 2001; Coenye & Vandamme, 2004; Payne *et al*., 2005; Perin *et al.,* 2006). A number of *Burkholderia* spp. can effectively nodulate legumes and have predominantly been found to nodulate mimosoid legumes (Moulin *et al*., 2001; Sy *et al*., 2001; Perin *et al.,* 2006). Currently, five *Burkholderia* spp. are known to nodulate legumes. These are *B. tuberum, B. phymatum, B. caribensis, B. mimosarum and B. nodosa*, while a number are only known to fix nitrogen (e.g. *B. unamae, B. vietnamiensis, B. tropica* and *B. kururiensis*) (Moulin *et al.,* 2001; Vandamme *et al.,* 2002; Chen *et al.,* 2003a; Reis *et al*., 2004; Caballero-Mellado *et al*., 2004; Chen *et al.,* 2006; Elliott *et al.,* 2007; Chen *et al.,* 2007). They are however not necessarily related to one another as they are scattered throughout the 16S rRNA phylogeny of *Burkholderia* (Fig. 1.3) (Coenye & Vandamme, 2004. The various symbiotic species of this genus are described below.

(a) *Burkholderia tuberum* **and** *Burkholderia phymatum*

The first *Burkholderia* species identified as *β*-rhizobia were *B. tuberum and B. phymatum* (Moulin *et al*., 2001; Coenye & Vandamme, 2004). *B. phymatum* was isolated from *Machaerium lunatum* in French Guiana and has been shown to nodulate and fix nitrogen on almost 30 *Mimosa* spp. (Sprent, 2007). *B. tuberum* was isolated from *Aspalathus carnosa* in South Africa, but recent studies indicate it also nodulates several *Cyclopia* spp. (Elliott *et al.,* 2007; Sprent, 2007). Both these species also nodulate the promiscuous legume *Macroptilium atropurpureum* (siratro) (Moulin *et al*. 2001). The identity of *B. tuberum* and *B. phymatum* were confirmed with 16S rRNA sequencing and polyphasic taxonomy (Vandamme *et al*., 2002).

(b) *Burkholderia caribensis*

This species effectively nodulates *Mimosa diplotricha* and *M. pudica* (Chen *et al*., 2003a) and was identified using a polyphasic approach. The name *caribensis* refers to the fact that it originates from the Caribbean Islands (i.e. Martinique in French West Indies) (Achouak *et al*., 1999). Bacterial strains of *B. caribensis* are also responsible for the formation of microaggregates in an island vertisol that consists of very high clay content (Achouak *et al*., 1999, Vandamme *et al.,* 2002).

(c) *Burkholderia mimosarum* **and** *B. nodosa*

Burkholderia mimosarum and *B. nodosa* were both isolated from *Mimosa* species. *B. mimosarum* was isolated from root nodules of *M. pigra* and *M. scabrella* from Taiwan and South America (Chen *et al*., 2006). *B. nodosa* was extracted from the nodules of woody legumes *M. bimucronata* and *M. scabrella* in Brazil (Chen *et al*., 2007). Based on 16S rRNA sequence results, these species are always found close to one another and also to the nitrogen-fixing *B. tropica* and *B. unamae.* Whole-cell protein analyses distinguished them readily from other *Burkholderia* species (Chen *et al*., 2006).

1.4.2.2 *Herbaspirillum lusitanum*

Herbaspirillum lusitanum was isolated in Portugal from the root nodules of *Phaseolus vulgaris* (Valverde *et al*., 2003). It is the only diazotrophic species within the genus *Herbaspirillum* with the ability to form beneficial symbiotic associations with *Oryza officicalis* (Fig. 1.2). The species may be distinguished from other *Herbaspirillum* species using phenotypic tests and antibiotic resistance to antibiotics (Valverde *et al*., 2003).

1.4.2.3 *Cupriavidus taiwanensis*

Many of the bacterial strains associated with *Mimosa* nodules were originally placed in the genus *Ralstonia* (Chen *et al*., 2003b). They induce the formation of indeterminate nodules on most *Mimosa* species within 15 days of inoculation. Yabuuchi *et al*., (1995) proposed that the genus *Ralstonia* incorporate the previous species, *Alcaligenes eutrophus*, *Pseudomonas solanacearum* and *Pseudomonas picketii*. The emendation of *Cupriavidus* was based on the work presented by Makkar and Casida (1987) with several modifications (Vandamme & Coenye, 2004). All the members of the former genus *Ralstonia* were reclassified as *Cupriavidus* (Vandamme & Coenye, 2004). The only rhizobial species of this genus is *C. taiwanensis* (Chen *et al.,* 2003b). It is an effective symbiont of *M. pigra* and *M. pudica* in Taiwan and the association induces improved plant growth when compared to other rhizobia (Barrett & Parker, 2006).

1.5 METHODS FOR CLASSIFYING RHIZOBIA

The ability to nodulate a legume is still the basic criterion for deciding whether or not a bacterial isolate belongs to the different rhizobial groups. Early attempts at classification were based on specificity amongst rhizobia for their respective host plants and led to the concept of cross-inoculation groups in which each group of legume host species could only be nodulated by a corresponding group of compatible rhizobial strains, while incompatible strains from another group would be incapable of nodulation. This system provided the basis for initial classification of species in the genus *Rhizobium*, although inconsistencies such as differences in host specificity of strains within species and overlapping nodulation ranges between groups later showed that this system was not practical. The fact that evolution of rhizobia predates and differs from that of their respective symbiotic genes explains why this system had little chance of success (Sprent, 2007). Nevertheless the cross-inoculation concept still has practical value as it provides the foundation for selecting suitable effective inoculant strains for particular legume crops.

Cross-inoculation was often used together with other differentiation methods for the identification of rhizobial strains. These included cultural characteristics such as type and rate of growth on Congo red yeast mannitol medium, as well as serological and bacteriophage cross-reactivity (Somasagaren & Hoben, 1994). Other characterization techniques include substrate

utilization, enzyme linked immunosorbent assay (ELISA), antibiotic resistance, plasmid profile analysis, multilocus enzyme electrophoresis and fatty acid analysis (Vandamme *et al*., 1996; Niemann *et al.,* 1997; Young & Haukka 1996). Bacterial differentiation based on phenotypic traits generally requires the use of live cultures. In contrast, analysis based on DNA has enabled dramatic advances in unravelling relationships amongst bacteria and other living organisms. Nevertheless, phenotypic traits are useful when used in combination with genotypic DNA analysis in polyphasic classification approaches described below in section 1.5.1 (Rossum *et al*., 1995). Table 1.1., lists the phenotypic and genotypic methods employed for microbial identification and classification.

A variety of DNA-based methods have emerged for the identification of rhizobia. These include genomic restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNAs (RAPDs) using random probes, rRNA analysis, DNA-DNA hybridization, Southern blot analysis of nitrogen-fixation (*nif*) and nodulation (*nod*) genes and polyacrylamide gel analysis of total proteins (Richardson *et al*., 1995; Mathis & McMillin, 1996; Thies *et al*., 2001). The main reason for this shift in focus is associated with the fact that DNA-based approaches are relatively easy to apply, robust and resolve even closely related species (Thies *et al*., 2001). Also, the identity of the rhizobia in specific soils can be determined without having to culture the rhizobia from nodulated legumes (Thies *et al*., 2001). In the following sections, I briefly discuss some of the main DNA-based and non-DNA-based methods used in the polyphasic taxonomy of rhizobia.

1.5.1 Non-DNA-based methods

Phenotypic methods or non-DNA based techniques usually require the use of live cultures and do not directly involve genetic material for the classification of rhizobia. These techniques mainly include morphological and biochemical characters. Morphology encompasses both cellular and colonial characteristics of bacteria, while biochemical features include traits such as growth temperature, optimum pH, substrate utilization, salt concentration and activities of various enzymes (Bergerson, 1980). Rhizobial strains also have been characterized phenotypically using host specificity, antibiotic resistance, plasmid profile analysis, multilocus enzyme electrophoresis (MLEE) and fatty acid analysis (Vandamme *et al*., 1996; Niemann *et al.,* 1997; Young & Haukka, 1998). Computerized numerical analysis of phenotypic data allows for the comparison of large numbers of phenotypic traits for large numbers of strains.

1.5.1.1 Substrate utilization and antibiotic resistance

Carbohydrate utilization properties are of taxonomic significance (Somasegaren & Hoben, 1994). Resistance to low levels of antibiotics is also used for rhizobial strain characterization and identification. To perform these tests, rhizobial cultures are inoculated in media containing various carbohydrates and antibiotics. Generally, species of *Rhizobium* have the ability to utilize a wider range of carbohydrates than *Bradyrhizobium* strains. The introduction of automated systems such BIOLOG and API provides a much more convenient approach for conducting numerous sole carbon source tests in a single microtitre plate. Typically, the utilization of carbon is detected by colour change in a tetrazolium indicator dye and automated spectrophotometers enable plates to be scanned, and data analyzed, which can then be compared to library databases of strains (Rinaudo *et al*., 1991; McInroy *et al*., 1999; Young, 1996; Sadowsky & Graham, 2004). The traditional application of these tests are however laborious, time-consuming and not always useful for identifying isolates to species level.

1.5.1.2 Morphological characters

Morphological characters are generally regarded as less reliable or more difficult to apply experimentally than serological differences (see below). Morphology does however often provide secondary "back-up" for strain and species diagnoses. Differences in colony type may also provide a direct means of strain recognition. For example, the unique red colony of rhizobial strains that nodulate *Lotononis* can easily be distinguished from other rhizobial cultures (Jaftha *et al.*, 2002). Overall, morphological traits are of little use in identifying strains within a species, but are of some value for recognizing strains from different species or genera (Bergerson, 1980).

1.5.1.3 Serological methods

Antisera are useful for identifying many strains of microorganisms. Exceptions arise when permanent and acquired sharing of antigenic similarities are encountered, for example, loss of antigenic specificity by strains of *Sinorhizobium meliloti* after prolonged culturing (Bergerson, 1980). Serological methods have been applied extensively in the genus *Rhizobium*. The antigens and antibodies may be detected by agglutination and immunodiffusion within the nitrogen-fixers. The agglutination test is sensitive, but more complex to use and does not give clear distinctions between reactions of antigenic identity compared to immunodiffussion. In gel immunodiffusion two wells are cut in an agar gel in a Petri dish and a bacterial suspension is placed in one well

and the antiserum in the other well (Somasegaren & Hoben 1994). The agglutination reaction is based on the aggregation of bacterial cells or particle antigens caused by antibody molecules binding to their surfaces and cross-linking them whereas immunodiffusion bands or lines in agar are formed by precipitation of soluble antigens and antibodies, when they meet after migration towards one another (Bergerson, 1980). Immunodiffusion is used for the detection of soluble antigens and for determining the identities of strains occupying nodules (Somasegaren & Hoben 1994).

1.5.1.4 Biochemical properties

The main biochemical properties adaptable for identification purposes are those involving fermentation or specific enzyme functions, whereby colonies or agar zones surrounding colonies show distinctive colour reactions after treatment with certain reagents. Activity of specific enzymes in colonies is typically detected by the appearance of clear halos around these colonies, against an opaque, coloured, or fluorescing background of the substrate over the agar surface. For example, to detect nucleases the agar surface is flooded with dilute hydrochloric acid to precipitate undegraded nucleic acid incorporated in the agar. Similar procedures are available for cellulose, urease and other degradative enzyme activities. These methods have been used for diazotrophic nitrogen-fixing bacteria, but are usually not useful for diagnosing rhizobial isolates to the species-level.

The determination of moles guanosine (G) and cytosine (C) in DNA is a standard criterion in bacterial species descriptions. Amongst prokaryotes, mole % G+C content ranges between 24-76%, while within a well-defined species the range is less than 3% and less than 10% in a genus (Vandamme *et al*., 1996). Those organisms with different base composition will have few DNA sequences in common and are likely to be distantly related. *Rhizobium* species usually have mole % G+C values in the order of 59-64 %, *Azorhizobium* 66-68%, *Bradyrhizobium* are intermediate with 61-65 l% (Graham *et al.,* 1991), whereas *Sinorhizobium* (De Lajudie *et al*., 1994) and *Mesorhizobium* (Jarvis *et al*., 1997) have mole % G+C values of 60.8-65.7% and 59-64% respectively.

1.5.2 DNA-based methods

Techniques employed before the advent of DNA-based methods were limited in a variety of ways. These included restrictions on the number of strains studied, the labour intensity of many

techniques e.g. protein profiles and the lack of convenient methods to characterize the nature of indigenous rhizobial populations (Thies *et al.,* 2001). Most selection protocols used in the past did not incorporate strain markers and hence there was a high probability of duplicating genotypes. The application of DNA-based methods are associated with a number of additional advantages, in that they often are less time consuming, provide stable information and reflect phylogenetic relationships. The application of these approaches, therefore, has led to significant advances in rhizobial identification and phylogenetic studies.

Each method enables a certain level of phylogenetic classification depending on the primers used and/or genomic regions targeted (Fig. 1.4). For example, analysis of the intergenic 16S-23S rRNA site enables differentiation at genus and species levels while other methods, as mentioned, are more efficient at species-strain levels. (Rademaker & de Bruijn, 1997). The choice of a molecular typing method therefore depends upon the needs, level of resolution and resources of the laboratory.

In this review, I distinguish between the information generated for whole genomes and that for specific loci. Although both of these approaches are associated with its own set of caveats, the application of both hold significant advantages for studying the diversity of rhizobial populations, and the classification of species and strains. For example, whole genome analyses methods allow the researcher to take into account phenomena such as horizontal gene transfer (HGT) and provide a more complete picture of the diversity within and among species. In the case of rhizobia it is crucially important to also consider HGT as it plays an important role in the evolution of their symbiotic/nodulation abilities and host range (Moulin *et al.,* 2004). However, a major disadvantage of these methods is that they often produce results that are not clear-cut, e.g. AFLPs and DNA-DNA hybridization often do not reveal exact species boundaries (Willems *et al.,* 2001). Although this is also true to some extent for specific loci, the analyses of specific loci are in many cases easier and less labour intensive. The analyses of specific loci also do not take into account the horizontal gene transfer phenomenon. As a result, most studies employ the genetic information for several specific loci (for genes encoding both house keeping functions and symbiotic properties), as well as a range of other data types required for a polyphasic approach (see below) (Parker *et al*.; 2002; Weir *et al*.; 2004; Stepkowski *et al*.; 2005, Vinuesa *et al*.; 2005b, Barrett & Parker, 2006)

1.5.2.1 Analyses of whole genomes using fingerprinting methods

(a) RFLP and Southern hybridization

DNA fingerprinting technology relied on RFLP analysis of total genomes for years before PCR was developed. This technique involves digestion of total genomic DNA extracted from a specific organism with restriction endonucleases, which produces a complex pattern of bands that are typically revealed by Southern analysis (Vandamme *et al*., 1996). This technique has been widely applied for studying bacteria as it provides information at the species, subspecies or strain level. The main drawback of this fingerprinting approach is that it is extremely time-consuming and laborious. As a result it is not used widely anymore and has in most cases been substituted with PCR-based methods.

(b) RAPD, ERIC and REP

In 1990, two papers appeared describing the RAPD technique (Williams *et al*., 1990; Welsh & McClelland, 1990) as a way to rapidly generate genomic fingerprints for a specific organism. This method involves PCR amplification of genomic DNA using short arbitrary primers with 9-10 bases (Doyle *et al*., 1993; Micheli & Bova, 1996). According to Mathis & McMillin, (1996), variations in DNA structure of *Bradyrizobium* strains are observed when using RAPD-PCR with GC-rich arbitrary primers (e.g. CRL7) (Mathis & McMillin, 1996; Botha *et al.,* 2004; Law *et al*., 2007). This approach is routinely used for the authentication of inoculant strains, identification of predominant nodulating strains from particular field sites, assessment of genetic diversity and relatedness amongst field populations of rhizobia (Richardson *et al.*, 1995; Schneider, & de Bruijn, 1996; Botha *et al.,* 2004; Law *et al*., 2007).

REP, ERIC and BOX PCR work on the same principle as RAPDs, but employ primers with sequences that are complementary to naturally occurring genomic DNA sequences (Rademaker & de Bruijn, 1997; Thies *et al.,* 2001). The resolving power of these methods vary greatly within and among certain groups of organisms and may also be dependent on the primers used (Thies *et al.,* 2001; Vinuesa *et al.,* 2005a; De Lajudie *et al*., 1998b) allowing for the identification of strains of a single species (Vinuesa *et al.,* 2005a). Overall, however, these methods are extremely powerful for among-strain discrimination (Schneider, & de Bruijn, 1996; Rademaker & de Bruijn, 1997; Thies *et al.,* 2001; Vinuesa *et al.,* 2005a; Martens *et al.,* 2008) and their major advantage lies in the fact that they take into account plasmids, nonessential genes and DNA.

(c) Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a PCR-based fingerprinting method that was developed by Vos *et al*. (1995). It involves amplification of restriction fragments from total genomic DNA digests, after which fragments are separated using denaturing polyacrylamide gel electrophoresis. Compared to other marker technologies such as RAPD and RFLP, AFLP provides greatly enhanced performance reproducibility, resolution and time efficiency. This technique can be used for the identification of highly related bacterial strains. It is useful as a rapid screening technique for large collections of bacterial isolates. This method also yields results that are comparable to DNA-DNA hybridization (see below) (Willems *et al.,* 2001).

1.5.2.2 Analyses of whole genomes using non-fingerprinting methods

(a) DNA-DNA hybridization

DNA-DNA hybridization forms the basis for the determination of genera and species of bacteria. It reflects the sequence similarity between two entire genomes (Wayne *et al*., 1987). The technique is based on the characteristic ability of DNA to hybridize, a process that depends on similarity of the two sets of nucleotide sequences. This allows for quantification of the degree of relatedness and is expressed as percent similarity or homology. Genetically closely related organisms will have more nucleotide sequences in common and therefore a higher degree of nucleotide binding will occur (Vandamme *et al*., 1996). Based on DNA-DNA hybridization profiles a group of strains with homology of 70% or more under optimal hybridization conditions belong to the same genetic species (Wayne *et al*., 1987).

The method has proved reliable and has been used by various researchers for rhizobial characterization (Mergaert *et al.,* 2002; Wei *et al.,* 2003; Turado *et al.,* 2005; Van Berkum *et al.,* 2006; Mantelin *et al.,* 2006; Chen *et al.,* 2006). The major drawback of this technique is that it is technically challenging, labour intensive, time-consuming, non uniform and the method varies with different laboratories. It also requires a large amount of superior quality DNA for the entire genome, which is not always easy to obtain (Willems *et al.,* 2001). Another drawback is that the results obtained are not always clear-cut. Hybridization values of 50% or less are less informative and therefore DNA-DNA hybridizations are not suitable for the estimation of genetic distances between distantly related species (Owen & Pitcher, 1983; Martens *et al*., 2008).

(b) Whole genome sequencing

It is becoming increasingly possible to determine the nucleotide sequence information for the entire genome of a specific bacterium. The process usually involves random fragmentation of total genomic DNA followed by cloning of the resulting fragments and sequencing using vector-specific primers and standard Sanger sequencing (Sanger *et al.*, 1977) procedures. The recent introduction of commercial sequencing-by-synthesis instruments (e.g. Roche GS GLX and Ilumina Solexa) (Braslavsky *et al.,* 2003; Margulies *et al.,* 2005), largely negates the need for generating clone libraries for sequencing, although these technologies are associated with various important drawbacks (Bentley, 2006). Nevertheless, whole genome sequences are available for a number of important rhizobia (see the website http://www.genomesonline.org). Such genomic sequence data may not yet be directly applicable for classification purposes, but their analyses provide valuable information regarding the evolution of rhizobia.

1.5.2.3 Analyses of specific genomic loci

(a) Amplified rDNA restriction analysis (ARDRA)

Amplified rRNA restriction analysis (ARDRA) targets the genes encoding 16S or 23S rRNA or parts of both genes with or without the intergenic spacer (IGS) region (Willems *et al.,* 2000). The specific region is amplified using universal primers located in the conserved regions and digested with one or more restriction enzymes. ARDRA is a rapid technique, less demanding than direct sequencing and can distinguish between closely related species (Vandamme *et al*., 1996). This method has been applied for the differentiation of many bacterial genera, including several species of rhizobia (e.g. *Rhizobium galegae*) (Terefework *et al*., 1998).

A detailed study by Gürtler & Stanisich (1996), showed that the IGS region was composed of highly conserved blocks of sequences while others were more variable. As a result, ARDRA analyses targeting the IGS allow for a higher level of discrimination, particularly between closely related species and have been used for identification purposes of a number of rhizobia species (Gürtler & Stanisich 1996; Willems *et al*., 2001). An IGS-based ARDRA analysis has also been demonstrated to be useful at the intraspecific level. For example, Laguerre *et al*., (1996) applied this method to differentiate the biovars of *Rhizobium leguminosarum*.

(b) RPO1 fingerprinting analyses

The RPO1 primer targets *nif* genes and can be used to identify specific strains of rhizobia (Richardson *et al*., 1995; Thies *et al*., 2001). This primer corresponds to a conserved reiterated element of the *Rhizobium leguminosarum* biovar *trifolii nifHDK* promoter. A directed sequence-specific RPO1 primer of 20 nucleotides in length was designed to differentiate a diverse collection of *R. meliloti, R. leguminosarum* biovars and a wide range of rhizobial and bradyrhizobial strains (Richardson *et al*., 1995). The RPO1 primer is capable of generating amplification profiles at annealing temperatures as high as 65ºC, compared to other primers. The temperature stability of RPO1 resulted in a much wider applicability for differentiation of rhizobia (Richardson *et al*., 1995; Botha *et al*., 2002; Botha *et al*., 2004) compared to methods using small 10-20-mer oligonucleotides (e.g. RAPDs) that would not anneal at these high temperatures (Richardson *et al*., 1995).

(c) DNA sequence information for ribosomal RNA genes and regions

Most previous studies used comparison of sequences of 16S rRNA genes to differentiate between species of rhizobia (Young *et al*., 1991; Jarvis *et al*., 1992; Eardly *et al*., 1992; Yanagi & Yamasato, 1993; Willems & Collins, 1993; Laguerre *et al*., 1994). This is largely due to the fact that rRNA molecules are functionally stable, since they play an important role in protein synthesis. They consist of both conserved regions, for comparing distantly related organisms and variable regions used for comparing or grouping more closely related organisms (Woese, 1987). These features make RNA gene (5S, 16S, and 23S) and intergenic sequences very good choices to compare organisms and to infer phylogenies. Partial and complete 16S rRNA sequence analysis is a rapid tool for characterization of strains, though the technique lacks the ability to distinguish very closely related individuals (Sullivan *et al*., 1996; Willems *et al*., 2001; Martens *et al.,* 2008), but has high resolving power for measuring the degree of relatedness between organisms above species level. Those organisms with genomic similarity of 70%, when assessed by DNA-DNA homology, will share more than 97% 16S rRNA gene sequence similarity (Stackebrandt & Goebel, 1994).

Phylogenetic analysis using the sequence of the conserved 16S rRNA gene is a common and widely applied tool when classifying bacteria. Both the 16S rRNA gene and to a lesser extent the 23S rRNA gene, have been used to identify new species with limited variability between strains of bacterial species (Barry *et al*., 1991). In most cases, however, this gene provides very little resolution at the interspecies level (Stackebrant & Goebel, 1994; Willems *et al.,* 2001;

Vinuesa *et al*., 2005a). Also, several studies have demonstrated the presence of chimeric 16S rRNA sequences in certain rhizobia and evidence for transfer of complete 16S sequences between unrelated rhizobia (Eardly *et al.,* 1996; Sullivan *et al.,* 1996; Van Berkum *et al.,* 2003). Problems associated with this gene are also encountered when it is used as the only phylogenetic marker as its phylogenies are extremely sensitive to unequal evolutionary rates (among taxa and among sites in the gene) and phylogenetic tree building artefacts (Nichols, 2001). Therefore, many researchers support the notion of basing taxonomic decisions on phylogenetic analyses of sequences for multiple loci (Nichols, 2001; Stepkowski *et al.,* 2003; van Berkum *et al.,* 2003; Vinuesa *et al.,* 2005a).

(d) DNA sequence information for other housekeeping gene sequences

A range of protein-coding housekeeping genes are used for studying the taxonomy of rhizobia. In addition to the ribosomal genes, other 'housekeeping' genes with basic cell functions such as genes encoding recombinase A (*recA*), glutamine synthetase isoform II (*glnII*), 70 kilodalton heat shock protein (*dnaK*), ATPase subunit D (*atpD*), etc. have been used to characterize rhizobia (Stepkowski *et al.,* 2005; Vinuesa *et al*., 2005a), as they have profound usefulness in inferring phylogenetic relationships (Thies *et al*., 2001; Parker *et al*., 2002; Vinuesa *et al*., 2005a; Martens *et al.,* 2008). In most cases these are applied for Multilocus sequence analysis (MLSA), an alternative method to 16S rRNA sequence analysis and DNA-DNA hybridization. The information from the comparison of multiple genes also gives a consistent overview of interorganism relationships at a wide range of taxonomic levels (Gervers *et al.,* 2005). MLSA has been shown to be superior to 16S rRNA gene sequencing analysis for *Sinorhizobium* species discrimination (Martens *et al.,* 2007).

(e) DNA sequence information for symbiotic (*sym*) loci

Rhizobia encode their symbiotic properties at the *sym* loci, which forms part of the accessory genome (Parker *et al.,* 2002). These include the nodulation genes (*nod*, *nol* and *noe*) and nitrogen-fixation (*nif*, *fix*) genes which confer the ability to nodulate and fix nitrogen in symbiosis with legume hosts, respectively. Sequencing of symbiotic genes has been used for the identification and association of rhizobia and it has mostly been found to reflect geographic or host relationships among isolates (Haukka *et al.,* 1998; Parker *et al.,* 2002). The comparison of these sequences to those of housekeeping or core genes therefore highlights the involvement of HGT in the evolution of the *sym* loci (Moulin *et al*., 2001; Moulin *et al.,* 2004; Sawada *et al*., 2003, Martens *et al.,* 2008). Phylogenetic trees inferred from these *sym* genes are usually

strongly incongruent with the rhizobial housekeeping loci, typically revealing relationships that are host- and geographic-based rather than based on taxonomic status (Dobert *et al*., 1994; Parker *et al.,* 2002; Stepkowski *et al.,* 2005; Vinuesa *et al.,* 2005a). As a result the sequences encoding *nif* and *nod* genes have been used to conclusively show that various regional rhizobial populations have been effectively isolated during evolutionary time, and has resulted in the appearance of locally adapted ecotypes or biovarieties (e.g. Parker *et al.,* 2002; Sawada *et al*., 2003; Stepkowski *et al.,* 2005; Vinuesa *et al.,* 2005a; Steenkamp *et al*., 2008).

1.5.3 Polyphasic approach

Most, if not all, current rhizobial classification procedures involve a polyphasic approach whereby different data and information on groups of isolates are integrated to generate a consensus type of taxonomy (Vandamme *et al.,* 1996; Reis *et al.,* 2004; Vinuesa *et al.,* 2005b). Polyphasic taxonomy results in reliable resolution of relationship among microorganisms (Vandamme *et al*., 1996; Martens *et al.,* 2008). Vinuesa *et al.,* (2005b) determine the adequacy of the combined phylogenetic and population genetic methods for the description of bacterial species, more especially *Bradyrhizobium* species. This approach gives scientifically sound information. Woese (1987) also stressed that a small number of characters is unreliable for defining taxa. The move to a polyphasic approach (using several characters) was made to ensure that only valid new species or genera are created. For the description of a new taxa all genotypic (DNA-DNA hybridization, etc.), phenotypic (substrate utilization, etc.) and phylogenetic (rRNA gene sequence analysis, etc.) information should therefore be combined (Stackebrandt & Goebel, 1994; Martens *et al.,* 2008). As a result rhizobial classifications should be based on phylogenetic and phenotypic data relating to the symbiotic, cultural, and morphological properties of the bacteria. Descriptions should also be based on data from a large number of strains from different geographical regions and hosts (Graham *et al*., 1991). In my MSc. study, a polyphasic approach was used to study the diversity of the rhizobia associated with the root nodules of *Lebeckia* species by using symbiotic characters (i.e. nodulation tests and nodule morphology), RPO1 and CRL7 fingerprint analyses and 16S rRNA-based phylogenetic analysis.

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1.7 TABLES

Table 1.1. Phenotypic and genotypic methods or characteristics, used for the identification and taxonomic characterization of rhizobia.

Methods or Characteristics	Reference
PHENOTYPIC	
Range of substrates usable as sources of carbon (sugars, sugar alcohols, complex carbohydrates, etc.)	Vandamme *et al*., 1996; McInroy *et al*., 1999
Range of substrates usable as sources of nitrogen (amino acids, urea, nitrate, etc.)	Bergersen, 1980
Resistance to specific antibiotics	Somasegaren and Hoben , 1994
Electrophoretic mobility of different cell enzymes	Bergersen, 1980
Tolerance to different stresses (salt, temperature, pH)	Bergersen, 1980
GENOTYPIC	
Pattern of banding of DNA restriction fragments (genomic-RFLP)	Vandamme *et al*., 1996
Pattern of banding using REP-PCR primers such as BOX or ERIC	Niemann *et al*., 1997; Vila *et al.,* 1996 ; Versalovic *et al.,* 1994
Degree of DNA hybridization	Vandamme *et al*., 1996 ; Wayne *et al*., 1987
16S rRNA sequence analysis	Young *et al*., 1991; Jarvis *et al*., 1992; Eardly *et al*., 1992
Sequencing symbiotic and house keeping genes	Barry *et al*., 1999 ; Vinuesa *et al*., 2005; Thies *et al*., 2001
Genome sequencing	Vandamme *et al*.,1996

Table 1.2. The current taxonomy[a], host and references of nitrogen-fixing root nodule bacteria within the α- and β-subdivision of *Proteobacteria*.

Division	Family	Genus	Species[b]	Legume Host	Reference
α-Proteobacteria	Rhizobiaceae	Rhizobium	R. leguminosarum bv viciae	Pisium spp., Vicia spp., Lathyrus spp., Lens spp.	Jordan, 1984
"	"	"	R. leguminosarum bv trifolii	Trifolium spp.	Jordan, 1984
"	"	"	R. leguminosarum bv phaseoli	Phaseolus vulgaris	Jordan, 1984
"	"	"	R. galegae bv officinalis	G. officinalis	Lindstrom, 1989; Radeva et al., 2001
"	"	"	R. galegae bv orientalis	G. orientalis	Lindstrom, 1989; Radeva et al., 2001
"	"	"	R. tropici	Leucaena spp., Phaseolus vulgaris	Martinez-Romero et al., 1991
"	"	"	R. etli bv phaseoli	Phaseolus vulgaris	Segovia et al., 1993
"	"	"	R. etli bv mimosae	P. vulgaris, Mimosa affinis Leucaena spp.	Wang et al., 1999a
"	"	"	R. gallicum bv gallicum	Macroptilium artroprpureum, P. vulgaris, Leucaena leucocephala	Amarger et al., 1997
"	"	"	R. gallicum bv phaseoli	Phaseolus spp., M. artopurpureum	Amarger et al., 1997
"	"	"	R. giardinii bv giardinii	L. leuococephala, M. artopurpureum,	Amarger et al., 1997
"	"	"	R. giardinii bv phaseoli	Phaseolus spp.	Amarger et al., 1997
"	"	"	R. hainanense	Desmodium sinuatum	Chen et al., 1997
"	"	"	R. huautlense	Sesbania herbacea, L. leucocephala	Wang et al, 1998
"	"	"	R. mongolense	Medicago ruthenica, Phaseolus vulgaris	Van Berkum et al., 1998
"	"	"	R. yanglingense	Gueldenstaedtia, Coronilla varia, Amphicarpaea triserma	Tan et al., 2001
"	"	"	R. indigoferae	Indigofera spp., Kummerowia stipulacea	Weir et al., 2002
"	"	"	R. cellulosilyticum	Populus alba	Garcia-Fraile et al., 2007
"	"	"	R. daejeonense	Medicago	Quan et al., 2005
"	"	"	R. loessense	Astragalus spp., Lespedeza spp.	Wei et al., 2003
"	"	"	R. lusitanum	Phaseolus vulgaris	Valverde et al., 2006
"	"	"	R. sullae	Hedysarum coronarium	Squartine et al., 2002;
"	"	"	R. tianshanense		Chen et al., 1995
"	"	"	R. undicola	Neptunia natans	Young et al., 2001; De Lajudie et al., 1998a
"	"	Sinorhizobium	S. fredii	Glycine max, Cajunus cajam G. soja, Vigna unguiculata	Scholla and Elka, 1984 ; Chen et al., 1988
"	"	"	S. meliloti	Medicago spp., Melilotus spp., Trigonella spp.	Jordan, 1984
"	"	"	S. xinjiangensis	Cajanus cajun, Gycine max, G. soja, Vigna unguiculata	Chen et al., 1988
"	"	"	S. kostiense	Acacia spp., Prosopis chilensis	Nick et al., 1999
"	"	"	S. saheli bv acaciae	Acacia spp.	de Lajudie et al., 1994; Boivin & Giraud, 1999
"	"	"	S. saheli bv sesbaniae	Sesbabia spp.	Boivin & Giraud, 1999; De Lajudie et al., 1994
"	"	"	S. terangae bv acaciae	Acacia spp.	De Lajudie et al., 1994; Lortert et al., 1996
"	"	"	S. terangae bv sesbaniae	Sesbania spp.	De Lajudie et al., 1994
"	"	"	S. arboris	Acacia spp., Prosopis chilensis	Nick et al., 1999

Table 1.2. Continued.

Division	Family	Genus	Species	Legume Host	Reference
"	"	"	*S. medicae*	*Medicago polymorpha, Medicago* spp.	Rome *et al.,* 1996
"	"	"	*S. morelense*	*Leucaena leucocephala*	Wang *et al.,*2002
"	"	"	*S. kumerowiae*	*Kumerowia stipulacea, Indigofera* spp.	Wei *et al.,* 2002
"	"	"	*S. americanum*	*Acacia* spp., *Phaseolus vulgaris, Leucaena leucocephala*	Toledo *et al.,* 2003
"	"	"	*S. arboris*	*Acacia* spp., *Prosopis* spp.	Nick *et al.,* 1999; Young, 2003
"	"	*Mesorhizobium*	*M. albiziae*	*Albizia kalkora*	Wang *et al.,* 2007
"	"	"	*M. loti*	*Lotus* spp., *Lupinus* spp., *Anthyllis vulneraria*	Jarvis *et al.,* 1982
"	"	"	*M. huakuii*	*Astragalus sinicus*	Chen *et al.,* 1991
"	"	"	*M. ciceri*	*Cicer arietinum*	Nour *et al.,* 1994
"	"	"	*M. mediterraneum*	*Cicer arietinum*	Nour *et al.,* 1995
"	"	"	*M. tianshaneanse*	*Glycine max, Glycyrrhiza, Caragona polourensins*	Chen *et al.,* 1995
"	"	"	*M. thiogangeticum*	*Clitoria ternatea*	Ghosh & Roy, 2006
"	"	"	*M. amorphae*	*Amorphae fruticosa*	Wang *et al.,* 1999b
"	"	"	*M. plurifarium*	*Acacia* spp., *Neptunia* spp., *Leucaena leucocephala*	De Lajudie *et al.,* 1998b
"	"	"	*M. septentrionale*	*Astragalus adsurgens*	Gao *et al.,* 2004
"	"	"	*M. temperatum*	*Astragalus adsurgens*	Gao *et al.,* 2004
"	"	*Azorhizobium*	*Az. caulinodans*	*Sesbania rostrata*	Dreyfus *et al.,* 1988
"	"	"	*Az. doebereinerae*	*Sesbania virgata*	Moreira *et al.,* 2006
"	*Bradyrhizobiaceae*	*Bradyrhizobium*	*B. japonicum*	*Glycine* spp., *Macroptilium artropurpureum*	Jordan, 1982
"	"	"	*B. elkanii*	*Glycine max*	Kuykendall *et al.,* 1992
"	"	"	*B. betae*	*Beta vulgaris*	Rivas *et al.,*2004
"	"	"	*B. canariense*	*Lotus* spp., *Lupinus luteus*	Vinuesa *et al.,* 2005
"	"	"	*B. lianingense*	*Glycine max, G. soja*	Xu *et al.,* 1995
"	"	"	*B. yuanmingense*	*Lespedeza cuneata*	Yao *et al.,* 2002
"	"	"	*Bradyrhizobium denitrificans*	*Aeschynomene indica*	van Berkum and Eardley, 2002
"	*Methylobacte-riaceae*	*Methylobacterium*	*Methylobacterium nodulans*	*Crotalaria* spp.	Sy *et al.,* 2001
"	*Hyphomicro-biaceae*	*Devosia*	*Devosia neptuniae*	*Neptunia natans*	Rivas *et al.,* 2003
"	*Brucellaceae*	*Ochrobactetrum*	*Ochrobactetrum lupini*	*Lupinus albus, Lupinus* spp.	Trujillo *et al.,* 2005
"	"	"	*Ochrobactetrum cytisi*	*Cytisus scoparius*	Zurdo-Zurdo-pineiro *et al.,* 2007
"	*Phyllobacteriaceae*	*Phyllobacterium*	*Phyllobacterium trifolii*	*Trifolium pretence, Lupinus albus*	Valverde *et al.,* 2003
β-Proteobacteria	*Burkholderiaceae*	*Burkholderia*	*Burkholderia tuberum*	*Aspalathus carnosa*	Moulin *et al.,* 2001; Vandamme *et al.,* 2002
"	"	"	*Burkholderia phymatum*	*Machaerium lunatum*	Vandamme *et al.,* 2002
"	"	"	*B. caribensis*	*Mimosa diplotricha, Mimosa pudica*	Vandamme *et al.,* 2002
"	"	"	*B.mimosarum*	*Mimosa pigra, M. scabrella*	Chen *et al.,* 2006
"	"	"	*B. nodosa*	*Mimosa bimucronata*	Chen *et al.,* 2006
"	"	*Cupriavidis*	*Cupriavidis taiwanensis*	*Mimosa pudica, Mimosa pigra*	Chen *et al.,* 2001; Vandamme & Coeye, 2004
"	*Oxalobac-teraceae*	*Herbaspirillum*	*Herbaspirillum lusitanum*	*Phaseolus vulgaris*	Valverde *et al.,* 2003

[a] Taxonomy as listed in Euzéby's (1997) "List of Prokaryotic names with standing in nomenclature" as updated March, 2008.
[b] Biovars are indicated as "bv" following the species name.

Table 1.3. The section, growth habit, and provincial distribution in South Africa of species in the *Lebeckia* genus (Modified from Germishuizen & Meyer, 2003).

Lebeckia Species	*Lebeckia* Section	Habit	Province
L. acanthoclada	*Calobota*	Shrubby	Northern Cape
L. bowieana	*Viborgioides*	Shrubby	Northern Cape
L. brevicarpa	*Lebeckia*	Suffrutescent	Western Cape
L. carnosa	*Lebeckia*	Suffrutescent	Northern Cape, Western Cape
L. cinerea	*Calobota*	Shrubby	Northern Cape, Western Cape
L. contaminata	*Lebeckia*	Suffrutescent	Western Cape
L. cytisoides	*Calobota*	Shrubby	Northern Cape, Western Cape
L. dinteri	*Calobota*	Shrubby	Northern Cape
L. fasciculata	*Viborgioides*	Shrubby	Western Cape
L. grandiflora	*Lebeckia*	Suffrutescent	Northern Cape, Western Cape
L. halenbergensis	*Calobota*	Shrubby	Northern Cape
L. inflata	*Calobota*	Shrubby	Western Cape
L. leipoldtiana	*Viborgioides*	Shrubby	Northern Cape, Western Cape
L. leptophylla	*Viborgioides*	Shrubby	Western Cape
L. linearifolia	*Calobota*	Shrubby	Northern Cape, Western Cape
L. longipes	*Lebeckia*	Suffrutescent	Western Cape
L. lotonoides	*Lebeckia*	Suffrutescent	Northern Cape, Western Cape
L. macowanii	*Lebeckia*	Suffrutescent	Western Cape
L. macrantha	*Stiza*	Shrubby	Northern Cape, Eastern Cape
L.melilotoides	*Calobota*	Shrubby	Northern Cape, Western Cape
L. meyerianna	*Lebeckia*	Suffrutescent	Western Cape
L. mucronata	*Viborgioides*	Shrubby	Western Cape, Eastern Cape
L. multiflora	*Calobota*	Shrubby	Northern Cape, Eastern Cape
L. obovata	*Calobota*	Shrubby	Northern Cape
L. pauciflora	*Lebeckia*	Suffrutescent	Western Cape
L. plukenetiana	*Lebeckia*	Suffrutescent	Western Cape
L. psiloloba	*Stiza*	Shrubby	Western Cape, Eastern Cape
L. pungens	*Stiza*	Shrubby	Western Cape
L. sepiaria	*Lebeckia*	Suffrutescent	Western Cape, Eastern Cape
L. sericea	*Calobota*	Shrubby	Northern Cape, Western Cape
L. sessilifolia	*Viborgioides*	Shrubby	Western Cape
L. simsiana	*Lebeckia*	Suffrutescent	Western Cape
L.spinescens	*Calobota*	Shrubby	Northern Cape, Western Cape
L. wrightii	*Lebeckia*	Suffrutescent	Western Cape

1.8 FIGURES
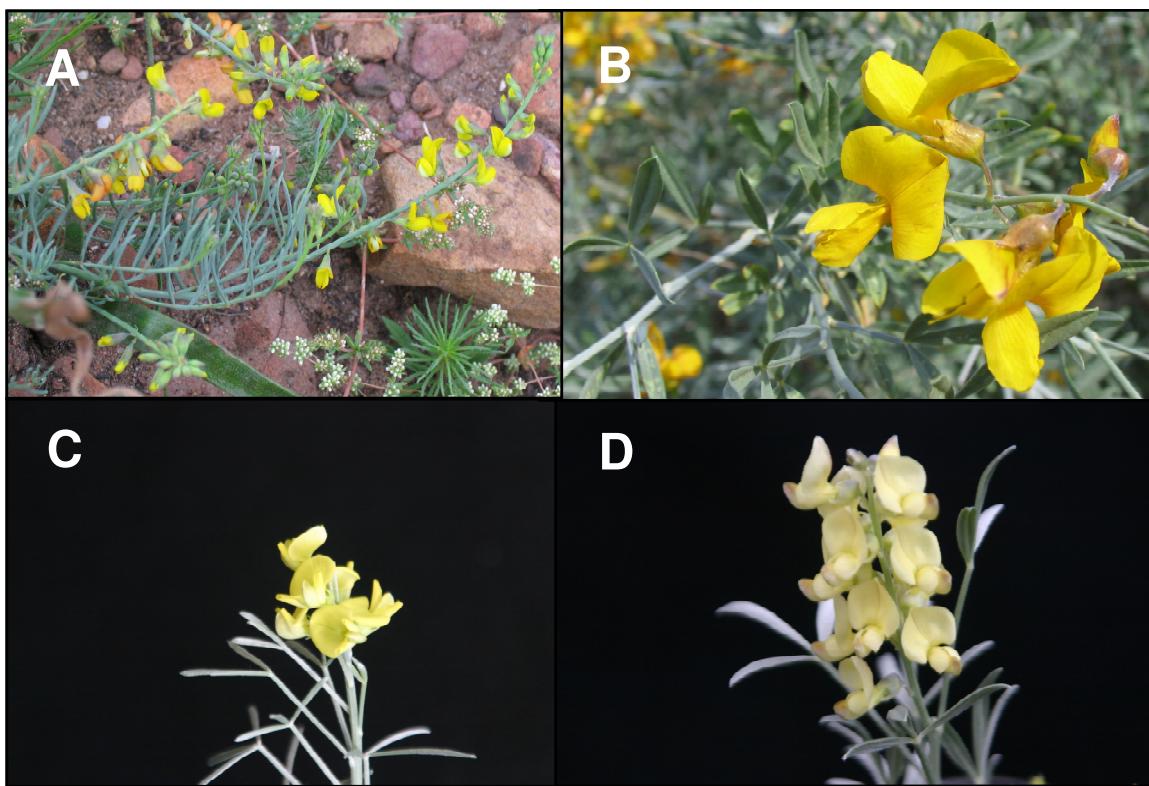
Figure 1.1. Examples of the spikes of yellow flowers characteristic of *Lebeckia* species found in the Western Cape. A: *L. plukenetiana,* a suffrutescent shrub with needle leaves *(Lebeckia-*suffrutescent). B: *L. cytisoides,* a shrub with trifoliate leaves *(Calabota-*shrubby); C: *L. multiflora,* shrub with pea-shaped flowers *(Calabota-*shrubby) and trifoliate leaves. D: *L. sericea,* shrub with trifoliate leaves *(Calabota-*shrubby), Photos courtesy of S. Boatwright (A, B) and K. Kasdorf (C, D).
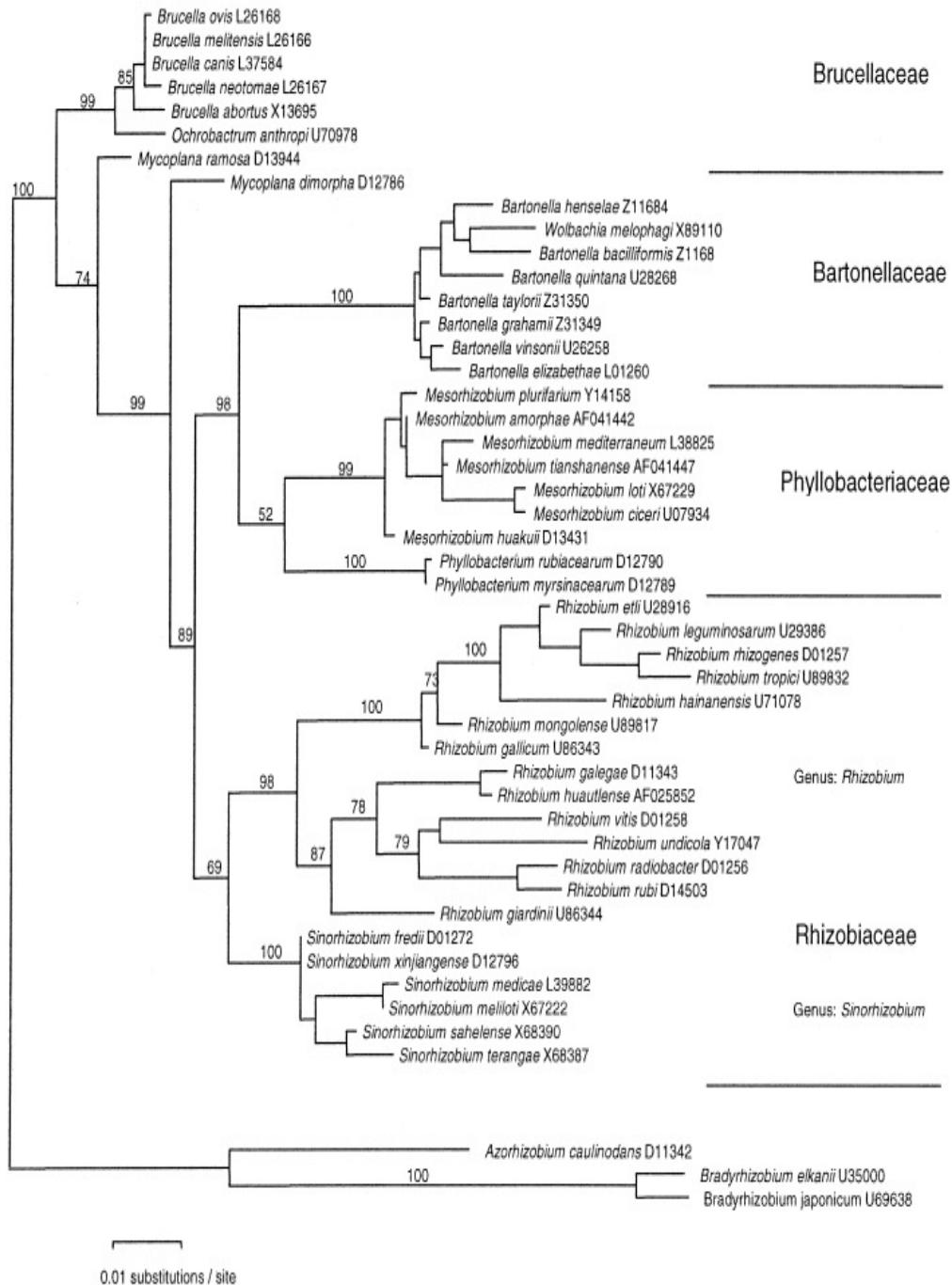
Figure 1.2. Neighbour-joining tree of *Rhizobiaceae* and relatives based on 16S rRNA sequences (Young *et al*., 2001).
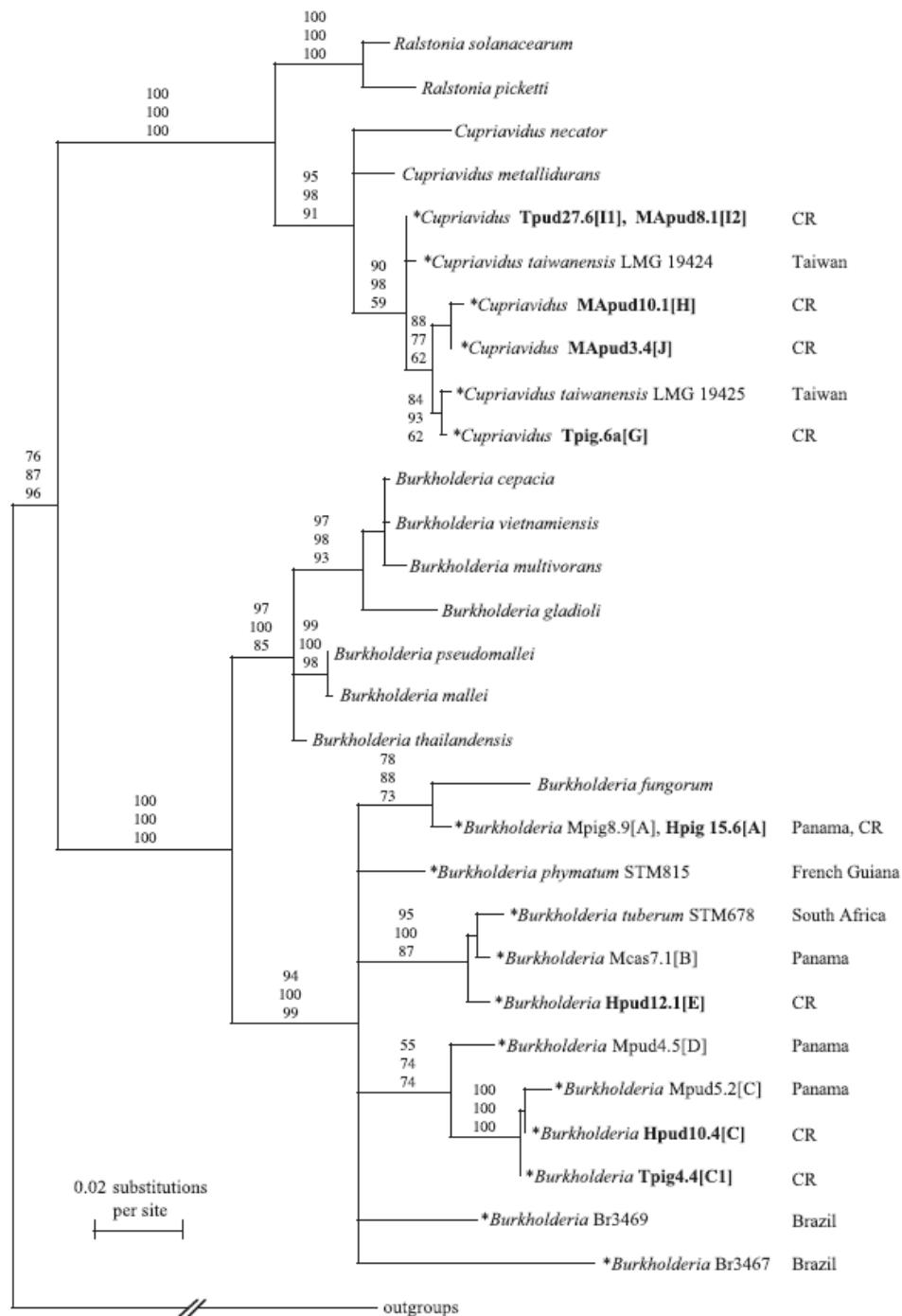
Figure 1.3. Maximum likelihood analysis of 16S rRNA genes of *Burkholderia*, *Cupriavidis* and other *β-Proteobacteria* (Barrett & Parker, 2006).

	Family	Genus	Species	Subspecies	Strain
DNA-DNA Hybridization		■	■	■	
ARDRA		■	■	■	
House-keeping gene sequences	■	■	■	■	■
16S rRNA sequences	■	■	■	■	■
ITS-PCR			■	■	
RFLP			■	■	■
Whole-cell protein profile			■	■	■
AFLP			■	■	■
RAPD			■	■	■
REP-PCR			■	■	■
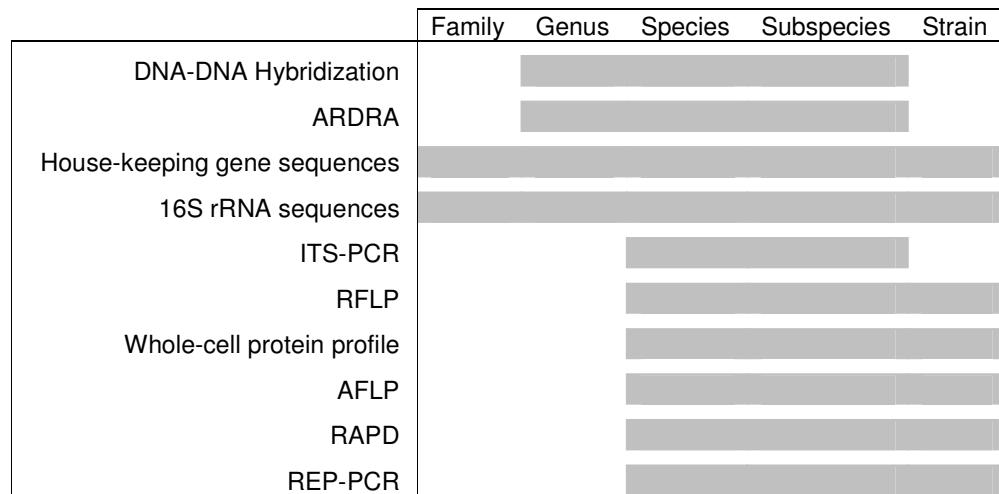
Figure 1.4. The relative taxonomic resolving power of various fingerprinting and sequencing techniques (modified from Rademaker & de Bruijn, 1997).

CHAPTER 2

ISOLATION OF RHIZOBIA FROM *LEBECKIA* SPECIES INDIGENOUS TO SOUTH AFRICA AND THEIR NODULATION PROPERTIES ON *LEBECKIA* AND THE PROMISCUOUS LEGUMES COWPEA AND SIRATRO

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2.1 ABSTRACT

The genus *Lebeckia* (family *Leguminosae*, tribe *Crotalarieae*) contains close to 35 shrubby and herbaceous legume species native to the Western Cape region of South Africa. Members of this genus typically have spikes of yellow pea-flowers and are divided into five sections based on growth habit and leaf shape. The shrubby trifoliate-leaf species are included in the sections *Viborgioides, Calobota* and *Stiza* and herbaceous needle-leaf species in the sections *Lebeckia* and *Spira*. Many *Lebeckia* species are recognized as valuable forage plants and, like most other legumes, *Lebeckia* species also form symbiotic relationships with rhizobial bacteria that fix atmospheric nitrogen in root structures called nodules. Any effort to exploit these plants for agricultural purposes would therefore require knowledge of the specificity of *Lebeckia* for their rhizobial symbionts. As a first step towards this goal, the nodulation specificity of the rhizobia associated with ten *Lebeckia* species representing three sections (*Calobota*, *Stiza* and *Lebeckia*) and the two growth habits were studied. A total of 79 Gram-negative rhizobia were isolated from these plants, after which their nodulation abilities were confirmed on their homologous hosts and the promiscuous legumes cowpea and siratro. These tests suggested some degree of specificity, as 56% of the strains were effective on cowpea and 77% on siratro, while all effectively nodulated their homologous *Lebeckia* hosts. This nodulation specificity was even more evident from the results of cross-inoculation studies where rhizobia isolated from shrubby *Lebeckia* species were not able to effectively nodulate suffrutescent *Lebeckia* species and *vice versa*. Root nodules were formed when rhizobia from *Lebeckia* species with a specific growth habit were inoculated onto other species with the same habit, but nodulation was most effective when rhizobia from a specific section were inoculated onto other members of that section. This is the first report on nitrogen fixation and nodulation by rhizobia of the genus *Lebeckia* and the results suggest that the apparent specificity between the two symbiotic partners will complicate commercial inoculation of these legumes.

Keywords: *Crotalarieae, Lebeckia*, cowpea, siratro, nodulation, nitrogen fixation

2.2 INTRODUCTION

Lebeckia species are evergreen shrubby or herbaceous (suffrutescent) legumes of the family *Fabaceae* and tribe *Crotalarieae*, which are mainly indigenous to the southern and Western Cape regions of South Africa (Germishuizen & Meyer 2003). The genus consists of about 33-36 species (Germishuizen & Meyer, 2003; Boatwright & van Wyk, 2007; Boatwright *et al.*, 2007; Le Roux & van Wyk, 2007; Le Roux *et al.*, 2007) and is presently undergoing taxonomic revision by researchers in the Botany Department, University of Johannesburg (van Wyk, personal communication; Le Roux & van Wyk, 2007; Boatwright *et al.*, 2007). Plants of this genus generally flower in spring and early summer and are characterized by spikes of yellow pea-flowers (Fig. 2.1). Based on morphological differences *Lebeckia* species are divided into the five sections *Calobota*, *Stiza*, *Lebeckia*, *Spira* and *Viborgioides* (Bentham, 1844; Harvey, 1862; Le Roux & van Wyk, 2007; Boatwright *et al.*, 2007; van Wyk, personal communication). Of these, shrubby trifoliate-leaf species are included in the sections *Calobota* and *Stiza* and *Viborgioides* (Bentham, 1844; Harvey, 1862) and suffrutescent needle-leaf species in the sections *Lebeckia* and *Spira* (Le Roux & van Wyk, 2007; van Wyk, personal communication).

Various *Lebeckia* species are recognized as valuable forage crops (Le Roux *et al.*, 1994; Shearing & van Heerden, 1994). In the veld, species such as *L. spinescens* and *L. multiflora* provide nutritious grazing, although other species such as *L. cytisoides* are toxic (Van Breda & Barnard, 1991; Le Roux *et al.*, 1994; Shearing & van Heerden, 1994). Due to the beautiful flowers of some species (e.g. *L. sericea*), they are also popular garden plants and suitable for commercial sale in garden nurseries. From an ecological point of view, all *Lebeckia* species are likely to also be of great value because of their nitrogen-fixing symbiosis with bacteria known as rhizobia (Corby *et al.,* 1983; Le Roux *et al.*, 1994) and consequent contribution to soil fertility and soil health maintenance (Le Roux *et al.*, 1994; Shearing & van Heerden, 1994). Any effort to exploit *Lebeckia* for agricultural and other purposes would however require knowledge of the level of specificity between the plant and rhizobial partners. Development of rhizobial inoculants will also involve selection of bacterial strains that are highly effective in their association with their compatible host.

As a first step to potentially developing inoculant strains for *Lebeckia,* the aim of this study was to determine the level of specificity between *Lebeckia* species and their symbiotic diazotrophic rhizobial partners. For this purpose, ten indigenous wild species of *Lebeckia* were selected from

the three sections *Calobota, Stiza* and *Lebeckia* for which seed or soil from collection sites for trapping of rhizobia were available. Five shrubby species (*L. cytisoides, L. multiflora, L. sericea, L. spinescens* and *L. pungens*) and five suffrutescent species (*L. ambigua, L. sepiaria, L. pauciflora, L. meyerianna* and *L. simsiana*) were used. Once rhizobia were obtained from the root nodules of these plants and their nodulation abilities were confirmed in nitrogen-free environments, their specificity towards other *Lebeckia* species and the promiscuously nodulating legumes siratro (*Macroptilium atropurpureum*, tribe *Phaseoleae*) and cowpea (*Vigna unguilata*, tribe *Phaseoleae*) were investigated.

2.3 MATERIALS AND METHODS

2.3.1 Collection of plant material and soil samples

Plant specimens, including flowers, leaves, seeds and root nodules were collected during the spring and summer seasons of 2004 to 2006 in diverse localities ranging from rocky Karoo plains and hills, sandy veld inland of Lambert's Bay and rugged mountain terrain of the Cedarberg and Namaqualand in South Africa. A map of the nine collection sites is shown in Fig. 2.2 and some locality details are provided in Table 2.1. The majority of the plants (*L. meyerianna, L. pauciflora, L. cytisoides, L. sepiaria, and L. multiflora*) were collected in the Western Cape at Clanwilliam, Cedarberg, Citrusdal and Lambert's Bay, while *Lebeckia sericea* was collected at Bitterfontein in the North Cape. The *L. ambigua* and *L. simsiana* specimens came from Modder River and Stellenbosch in the south of the Western Cape and *L. spinescens* and *L. pungens* in the east of the Western Cape at Beaufort West and Meiringspoort, respectively. Plant species were identified by Professor B.-E. van Wyk and co-workers in the Department of Botany, University of Johannesburg. At each site, soil was collected next to the roots of the plants at depths of 5-30 cm and later used in conjunction with the appropriate host species to trap rhizobia (see below). Nodules were carefully excised from roots and stored in vials containing silica gel desiccant. In some instances, small seedlings were potted in soil and transported to the greenhouse for growth and later examination for nodules. Soil pH was measured using 1:5 soil:H_2O mixtures, after shaking for 30 min and leaving to stand for 3 h at room temperature. Siratro and cowpea seed were obtained from the Agricultural Research Council-Range and Forage Institute (ARC-RFI) Seed Genebank, Pretoria, South Africa.

2.3.2 Seed germination, rhizobial trapping and plant growth conditions

Lebeckia seeds used for trapping experiments and nodulation tests were either collected in the field or kindly donated by Professor B.-E. van Wyk. These seeds were pretreated with concentrated sulphuric acid (H_2SO_4) for 20-30 min, washed 5 times with sterile deionised water (SDW) and imbibed in SDW for 3-4 h. Siratro and cowpea seeds were also prepared for germination. The siratro seed was treated with H_2SO_4 for 15 min and washed with SDW, after which the seed was surface sterilized in 3.5% (m/v) sodium hypochlorite for 5-10 min, followed by washing and imbibition as described for *Lebeckia* seeds. Cowpea seed was surface sterilized with 3.5% (m/v) sodium hypochloride for 10 min and washed and imbibed as before. After imbibition, seeds of *Lebeckia* were germinated in the dark under aseptic conditions for 3-5 d at

15°C on the surface of water agar (WA; 1.5%, m/v, Associated Chemical Enterprises, RSA). Cowpea and siratro seeds were germinated at 28°C on WA for 1 and 5 d, respectively.

For the trapping experiments and for testing nodulation and nitrogen-fixing effectiveness of the isolated bacteria, two seedlings of a specific *Lebeckia* species, or cowpea or siratro, were planted in Leonard jars containing quartz sand and nitrogen-free Hoagland solution (Somasegaren & Hoben, 1994). For the trapping experiments, the jars were then inoculated with a suspension of soil from a locality corresponding to that of the seed. The suspension was prepared by shaking 10 g soil in 100 ml water for 30 min. After sedimentation, the liquid supernatant was pipetted onto the seedlings. Alternatively, sand in the Leonard jar was scooped out and replaced with a plug of soil from the locality where the *Lebeckia* species was collected. For testing the nodulation and nitrogen-fixing effectiveness of the isolated bacteria (see below), seedlings were inoculated with inoculum prepared as described below and covered with a Petri dish. Once seedlings had reached appreciable height, the glass Petri dish cover of the Leonard jar was removed and sterile sand coated with paraffin wax sand added to cover the sandy part of the jar. Its purpose was to seal the sand in the Leonard jars after plants have emerged to reduce loss of moisture and prevent microbial contamination. The glasshouse was set at a 14 h day temperature of 28°C and a 10 h night temperature of 15°C. Plants were grown for 4-12 weeks before nodule harvest.

2.3.3 Isolation, characterization and maintenance of rhizobia

Nodulated roots were washed free of sand or soil and nodules carefully excised. For isolation of rhizobia, whole nodules were surface sterilized for 2-3 min in 3.5% (m/v) sodium hypochlorite. After washing in 5 changes of SDW, isolations were performed by squashing individual nodules in a drop of SDW with sterile forceps and streaking the extracts onto Yeast Mannitol Congo red (YM-CR) agar plates (Somasagaren & Hoben, 1994). Cultures were incubated at 28°C until there was sufficient bacterial growth to form colonies. The cultures were purified on YM-CR by single colony isolation and stored at -70°C using sterile 20% (v/v) glycerol as a cryoprotectant. All isolates (Table 2.2) are stored in a -70°C freezer at the South African *Rhizobium* Collection (SARC), Plant Protection Research Institute, Pretoria, South Africa. Two isolates previously isolated from *Lebeckia* species were obtained from the SARC and included in this study. The strain XHR1 (*L. simsiana*) was collected by C. Deschodt in 1979 and strain NK22 (*L. sericea*) was collected by J. J. le Roux in 2003.

All isolates were characterized using standard Gram stains (Preston & Morrell, 1962). This briefly entailed preparing a smear of a moderately dense suspension of bacteria in SDW on a clean slide and air drying it. After fixation with heat, 0.5% (m/v) crystal violet was applied to the smear for 1 min, after which it was rinsed with tap water. A few drops of iodine (0.5%, v/v) were then applied to the smear for 1 min, followed again by rinsing with tap water. To remove any of the non-absorbed dye, 95% ethanol was applied to the smear for 30 sec, after which several drops of 0.5% (m/v) Safranin O was applied to the smear for 10 sec. Following rinsing and drying, the slide was mounted in oil and observed using a Zeiss compound microscope at 1000X magnification. Cells which absorbed the crystal violet dye were purple and considered Gram positive, whereas those that were red were Gram negative. The alternative 3% KOH test to confirm whether bacterial cells are Gram negative or positive was also used (Halebian *et al*., 1981; Goszczynska *et al.,* 2000). A drop of 3% KOH was placed on a slide and mixed with a loopful of bacterial culture. The loop was lifted several times to check for formation of a mucoid thread. Bacterial strains that produce a mucoid thread in 3% KOH are Gram negative, whereas those that form a watery suspension are Gram positive (Bamarouf *et al*., 1996; Goszczynska *et al.,* 2000).

The morphology of colonies of individual rhizobial isolates on YM-CR medium was observed using a microscope with 100X magnification (Wild Heerbrugg, Switzerland). Relative colony growth rates were evaluated by measuring the diameter of the colonies at three days and six days after streaking. The isolates were regarded as fast-growing if they produced discernable colonies within 3 days and slow-growing when colonies were observed after 5 days.

2.3.4 Nodulation tests

To authenticate the nodulation and nitrogen-fixing abilities of the isolates used in this study (Table 2.1), all isolates were inoculated onto their homologous or compatible hosts. For this purpose inoculum was prepared by suspending two loopfulls of a fresh single colony culture from an YM-CR agar plate in 4 ml sterile distilled water (SDW) in a McCartney bottle. The suspensions were mixed by vortexing and 1 ml inoculated onto each seedling planted in a Leonard jar, after which seedlings were grown as described above. The isolated rhizobia were also inoculated onto the promiscuous legumes cowpea and siratro to test for host range specificity of nodulation and nitrogen fixation effectiveness. All nodulation experiments

contained at least four replicate Leonard jars per treatment, with two to four seedlings per jar. The plants from the four replicate Leonard jars were compared to verify the results.

To determine the symbiotic specificity between the various rhizobial isolates and their plant hosts, cross-inoculation experiments were performed using only those *Lebeckia* species for which sufficient seed was available. Three experimental sets of hosts and rhizobia were used. In the first set (Set 1), inoculum prepared with rhizobia isolated from shrubby hosts in either of the sections *Calobota* (*L. cytisoides*, *L. multiflora*, *L. sericea* and *L. spinescens*) and *Stiza* (*L. pungens*) were used to inoculate other species from either the same section or from the other sections. In the second experiment (Set 2), inoculum prepared with rhizobia isolated from the suffrutescent hosts in section *Lebeckia* was used to inoculate other species from this section (*L. meyerianna*, *L. ambigua* and *L. simsiana*). In the third experiment (Set 3), inoculum prepared with rhizobia isolated from both the suffrutescent and the shrubby *Lebeckia* sections were used to inoculate shrubby and suffrutescent *Lebeckia* species, respectively. These inoculations were also performed in the opposite direction, with inoculum prepared with rhizobia isolated from the shrubby and suffrutescent *Lebeckia* sections being used to inoculate suffrutescent and shrubby *Lebeckia* species, respectively.

All plants were grown under nitrogen-free conditions as described above, during which nitrogen-fixation was monitored in inoculated plants by comparing the greenness of their leaf colour with the yellow leaves of uninoculated control plants. In addition, harvested nodules were sliced in half with a scalpel and examined. A pink coloration, caused by the production of leghaemoglobin was indicative of an effective nitrogen-fixing symbiosis while a white colour signified an ineffective association (Somasagaren & Hoben, 1994; Sprent, 2007; Sprent & James, 2007). In all cases rhizobia were re-isolated from the root nodules induced and plated onto YM-CR, after which the colonies that emerged were compared for microscopic appearance and rate of growth to that of the original cultures used to inoculate test plants.

2.4 RESULTS

2.4.1 Rhizobia from South African Lebeckia species

In this study, 79 strains of rhizobia (Table 2.2) were isolated from the root nodules of ten *Lebeckia* species collected from different localities in South Africa (Table 2.1 and Fig. 2.2). The soil types at these collection sites ranged from sandy to sandy clay loam to stony clay loam that were mostly moderately acidic with pH 5.25-6.5 (Table 2.1). Two strains were extracted from *L. cytisoides* collected from Citrusdal, as well as one isolate from *L. sepiaria* also from the same locality (Table 2.2). *L. multiflora* was collected at Nortier Research Station, Lambert's Bay and four rhizobial strains were purified from root nodules. *L. sericea* was the most abundant of the *Lebeckia* species studied and was found in two localities, Bitterfontein and Kamiesberg. A total of eighteen isolates were extracted from *L. sericea*. *L. spinescens* was abundant at Bleak House farm, Beaufort West and sixteen rhizobial strains were obtained from this plant. Ten strains came from *L. pungens* collected at Meiringspoort. *L. ambigua* was collected from Modder River and nine rhizobial strains were obtained. *L. pauciflora* was collected in the Cedarberg and twelve strains were studied. *L. meyerianna* was collected at Clanwilliam with four isolates being purified from nodules. SARC records show that the two *L. simsiana* strains were collected from a Stellenbosch garden shrub.

2.4.2 Growth and colony characteristics of Lebeckia rhizobia

All the strains isolated from the *Lebeckia* root nodules were Gram negative rods, as they all appeared red upon staining with Safranin O. They also reacted positively to the 3% KOH test, with mucoid threads being observed in all. The colonies also did not absorb Congo red on YM, were pale yellow to white in colour and were generally low convex in shape with entire edges. All the rhizobia reisolated from the cowpea, siratro and *Lebeckia* nodules had colony characteristics similar to those of the original isolates from host plants when cultured on YM-CR.

In terms of growth rate on YM-CR agar, the bacteria isolated in this study varied substantially (Tables 2.2, 2.3). Of the *Lebeckia* spp. studied, three from section *Calobota* yielded intermediate growers and one fast grower, while the single species studied in section *Stiza* was nodulated by very slow growers. In addition, two of the five species from section *Lebeckia* yielded fast growers, two yielded slow growers, and *L. pauciflora* was nodulated by both fast and slow-growers (Tables 2.2, 2.3). Nineteen percent of all the strains studied were slow growers, while

30 % were intermediate growers reaching colony diameters of 1-2 mm within 5 to 6 d. The remaining 51% were fast-growers. Fifteen slow-growing strains from *L. pungens* (10 strains; Betal106a to Betal106j), *L. sepiaria* (WC12.1a), *L. pauciflora* (WC21.1k and WC21.1l) and *L. simsiana* (XHR1a and XHR1b) had colony diameters less than or equal to 1 mm at 6 to 8 d. In addition to the two slow-growing *L. pauciflora* strains mentioned above, isolates from *L. pauciflora* included 10 fast-growing strains (WC21.1a-j). The twenty four intermediate-growing strains included those isolated from *L. cytisoides* (2 strains; WC19.1b and WC19.1c), *L. multiflora* (4 strains; WC 23.1a to WC23.1d) and *L. sericea* (18 strains; WC28.1a to WC28.1j, WC33a to WC33h, and NK22). Approximately half of the *Lebeckia* rhizobia were fast growers (Table 2.2) and were isolated from four *Lebeckia* species, i.e. *L. spinescens* (16 strains; BH1LS to BW3S), *L. ambigua* (9 strains; WC5.4a to WC5.4i), *L. pauciflora* (10 strains; WC21.1a to WC21.1j) and *L. meyerianna* (4 strains; WC26.1c to WC26.1f). The fast-growing isolates from *L. spinescens* were watery mucoid with both large and small colonies and were 3 mm in diameter at 2-3 days (Table 2.2). The remaining 23 fast-growing strains from *L. ambigua*, *L. pauciflora* and *L. meyerianna* had an almost identical growth rate, with colonies developed at four days.

2.4.3 Nodulation properties of rhizobia from Lebeckia species

Representative bacterial strains originating from the root nodules of each species of *Lebeckia* were used to inoculate their corresponding hosts as well as cowpea and siratro. All were found to be effective on their homologous host plant, producing nitrogen-fixing nodules within a minimum of 6 weeks (Figs. 2.3 and 2.4, Table 2.3). When the root nodule isolates were tested for nitrogen fixation and nodulation on cowpea and siratro, many of the isolates were effective (i.e. stimulated production of green leaves and pink centered nodules indicating nitrogen fixation), whereas others had stunted growth and yellow leaves indicating ineffective nodulation or failure to nodulate. Overall nodulation of cowpea and siratro by the rhizobia from *Lebeckia* was similar, with little variation in nodulation compatibilities by certain strains. Siratro was, however, more promiscuous than cowpea when inoculated with *Lebeckia* rhizobia, as a higher percentage of the strains were effective on siratro (77%) than on cowpea (56%). No obvious correlation was observed between growth rate of the isolates and their ability to nodulate cowpea and siratro except that % effectiveness was sometimes lower amongst fast growers, such as the *L. spinescens* isolates (Table 2.3).

Some isolates appeared not to be able to nodulate the promiscuous hosts siratro and cowpea. For example, intermediate-growing isolates from *L. cytisoides* nodulated neither cowpea nor siratro, while the single isolate from *L. sepiaria* did not nodulate siratro but produced effective nodules on cowpea (Table 2.3). All four intermediate-growing strains from *L. multiflora* formed an effective symbiosis with siratro, while on cowpea only two were effective with the other two producing ineffective nodules. Eighteen intermediate-growing strains from *L. sericea,* nodulated cowpea and siratro although only 50% fixed nitrogen on cowpea and 66% on siratro (Table 2.3). Similarly, all 16 fast-growing strains from *L. spinescens* nodulated cowpea and siratro, but only 12% were effective on cowpea, whereas 75% were effective on siratro. In contrast, the fast-growing isolates from *L. pauciflora* and *L. meyerianna* were similar in effectiveness on cowpea and siratro plants, both obtaining 60-80% effective nodulation on these species. (Table 2.3). The two slow-growing strains from *L. pauciflora* produced effective nodules on both cowpea and siratro. The 10 slow-growing isolates from *L. pungens* were 80% effective on cowpea and 100% effective on siratro, while the two slow-growing *L. simsiana* isolates, as well as the two slow-growing *L pauciflora* isolates, were effective on both cowpea and siratro. All nine slow-growing *L. ambigua* rhizobia tested fixed nitrogen on siratro whereas only six of the nine strains were effective on cowpea (Table 2.3).

Inoculation of the rhizobia on to their homologous *Lebeckia* hosts resulted in the formation of indeterminate nodules (Fig. 2.4; Corby *et al.,* 1983). Some of these were elongate and flat finger-like or leg-like, while a few were branched, ovoid-cylindrical and laterally flat. The majority of the *Lebeckia* species, however, produced large fan-like nodules with separate lobe-shaped branches. The fan-shaped nodules were densely aggregated with 4-6 bulbous fingers and a single narrow connection to the root (Fig. 2.4). Inoculation of the *Lebeckia* rhizobia onto the promiscuous phaseoloid legumes produced spherical and clearly determinate nodules on cowpea (Fig 2.4; Corby *et al.,* 1983) and ovate to cylindrical indeterminate nodules on siratro (Fig. 2.4).

Representative isolates and plants from the two *Lebeckia* growth habits (shrubby trifoliate leaf and suffrutescent needle leaf) were used to perform cross-inoculation tests. In control experiments, the isolates were reinoculated on their respective homologous host plants to confirm their nodulation and nitrogen fixation abilities on their corresponding host (Table 2.3; Figs. 2.3, 2.4). Three sets of experiments were conducted, using only those plant species for which adequate seed was available. The first two sets of cross-inoculation tests (Sets 1 and 2;

Table 2.4) were performed among the plants and their respective rhizobia within each of the shrubby (sections *Stiza* and *Calobota*) or suffrutescent (section *Lebeckia*) growth habits. These cross-inoculations all resulted in the formation of root nodules. Inoculations performed with isolates from shrubby section *Calobota* plants onto species from section *Calobota* induced the production of effective nodules. The same was also true for inoculations performed with isolates from species of the suffrutescent section *Lebeckia* and host plants representing this section (Table 2.4). However, inoculations performed with isolates from shrubby section *Calobota* plants onto species of the shrubby section *Stiza*, and *vice versa*, induced production of ineffective nodules (Table 2.4). In a third set, suffrutescent section *Lebeckia* was inoculated with rhizobia from the shrubby section *Calobota*, and a reverse set of legumes from shrubby sections *Stiza* and *Calobota* inoculated with isolates from species in suffrutescent section *Lebeckia* (Set 3; Table 2.4). For this experimental set, no nodules were observed on the roots of the test plants (Table 2.4). The only exception was inoculation of *L. pungens* (Section *Stiza*; shrubby habit) with WC26.1e originating from *L. meyerianna* (Section *Lebeckia*; suffrutescent habit), which generated ineffective nodules (Table 2.4).

2.5 DISCUSSION

In this study, 79 isolates of rhizobial bacteria were obtained from the root nodules of ten species of *Lebeckia*. However, before these bacteria could be regarded as rhizobia, their nodulation properties had to be authenticated. Verification of the nodulation abilities of bacteria associated with root nodules of legumes represents an essential first step in characterizing the nitrogen-fixing symbiosis. The main reason for this is that the soil environment harbours diverse microorganisms and non-rhizobial contaminants are easily obtained during the isolation process. To ensure that all the isolates used in this study were authentic symbiotic root nodule bacteria of *Lebeckia*, the isolates were inoculated on their corresponding host plants. Each was found to produce effective nodules that stimulated shoot growth of the host. Examination of the cultural characteristics of the rhizobia re-isolated from these nodules also confirmed that they corresponded to those of the parental isolates (Tables 2.2, 2.3). These observations fulfilled Koch's postulates and indicated that the isolates were authentic root nodule bacteria of the *Lebeckia* species studied.

In this study, all the rhizobia isolated from the *Lebeckia* species examined were Gram-negative, a feature they share with all legume root nodule bacteria examined to date. These bacteria did, however, differ considerably from one another in terms of culture characteristics, growth rate and colony appearance (Table 2.3). Based on growth rate, the isolates examined were separated into a number of groups. These groupings corresponded to the slow- and medium-growing groupings that are, respectively, characteristic of bradyrhizobia and mesorhizobia, as well as the fast-growers found in some genera of root nodule bacteria especially *Rhizobium* and *Burkholderia* (Barrett & Parker, 2006). Where more than one isolate was obtained from a plant species, all had similar growth characteristics except for *L. pauciflora* from which both fast and slow growers were obtained (Tables 2.2, 2.3). These findings therefore confirm that the taxonomic diversity apparent in the genus *Lebeckia* (Le Roux & van Wyk, 2007), is also found amongst the symbiotic rhizobia present in root-nodules of this genus.

The majority of the isolates obtained from the root nodules of the examined *Lebeckia* species also nodulated cowpea and siratro (Table 2.3). Both are well known to be nodulated by bradyrhizobia (Thies *et al*., 1991; Mpepereki *et al*., 1996). Accordingly, slow-growing *Lebeckia* nodule isolates such as isolates XHR1 (*L. simsiana*), Betal (*L. pungens*) and WC21.1 (*L. pauciflora*) also nodulated and were effective on cowpea, and are therefore likely to be related to

other nitrogen-fixing cowpea bradyrhizobia. Nodulation of cowpea and siratro is, however, not limited to slow-growing bradyrhizobia. For example, Mpepereki *et al*. (1996) isolated fast-growing root nodule bacteria from cowpea in Zimbabwe, and Dakora *et al*. (2000) published results on fast-growing *Rhizobium* species from Ghana that effectively nodulate cowpea. Similar findings were obtained in the current study, with the intermediate-growing rhizobial strains from species *L. multiflora* and *L. sericea* and the fast-growing *Lebeckia* rhizobia from *L. spinescens, L. ambigua, L. pauciflora* and *L. meyerianna* all able to nodulate cowpea and siratro, often effectively (Table 2.3).

The association of rhizobia with their host legumes is often highly specific. For example, the South African legume *Lotononis bainesii* forms effective associations only with red-pigmented methylobacteria that cannot nodulate other legumes effectively (Jaftha *et al*., 2002; Le Roux, 2003). However, the ability of indigenous strains to effectively nodulate various or multiple agricultural legumes has important agronomic implications, mainly because it eliminates the need for commercial inoculant application (Mpepereki *et al*., 1996). Cowpea, for example, is native to Africa and is effectively nodulated by the indigenous rhizobial populations found in African soils (Mpepereki *et al.*, 1996; Law *et al*., 2007) as well as in soils of other lands, such as the island of Hawaii (Thies *et al*., 1991). The initial evaluation of the specificity of the rhizobia isolated from *Lebeckia* therefore involved inoculation studies of cowpea and siratro, two legumes that are well known for their nodulation compatibility with a wide range of fast and slow-growing rhizobia from other legume species (Thies *et al*., 1991; Mpepereki *et al*., 1996; Moulin *et al.,* 2001). Unexpectedly, both intermediate-growing *L. cytisoides* isolates failed to nodulate either legume although effectively nodulating their host plant, while the *L. sepiaria* isolate failed to nodulate siratro although effectively nodulating cowpea (Table 2.3). The other strains were all capable of nodulating either legume, although some degree of specificity was observed as nodulation did not always resulting in nitrogen fixation (Table 2.3). Generally, a higher percentage of *Lebeckia* rhizobia nodulated siratro than cowpea, suggesting that it is more promiscuous than cowpea. The root nodule bacteria of the *Lebeckia* species are therefore characterized by a variety of nodulation specificities on cowpea and siratro (Table 2.3).

To study nodulation specificity within the *Lebeckia* symbiotic system, cross-inoculation experiments were performed. However, due to the limited availability of seed supplies for most of the collected species, these tests concentrated only on certain cross-combinations between isolates from the major shrubby and suffrutescent groupings of *Lebeckia*. Only combinations of

rhizobia and legumes of the same sections within the shrubby and suffrutescent divisions (Experimental sets 1 and 2), resulted in effective nodulation in each instance (Table 2.4). This suggested that limited specificity might be encountered within each group. For example, cross inoculation between the plants and rhizobia from the sections *Stiza* and *Calobota* within the shrubby division resulted in ineffective nodulation. This suggested that the symbiotic nitrogen-fixing specificities of the two sections were quite different. Also, combinations of rhizobia and legumes between the shrubby and suffrutescent divisions (Experimental set 3) did not result in nodulation, except for one instance of ineffective nodulation recorded between an isolate from the suffrutescent *L. meyerianna* inoculated on the shrubby *L. multiflora* (Table 2.4). This suggested that considerable specificity existed between the two *Lebeckia* divisions. Similar trends have also been observed in other symbiotic systems. For example, rhizobia from *Lotononis angolensis* fix nitrogen on their host plant but do not nodulate other species within the *Listia* section of this genus (*L. bainesii, L. listii*), whereas reverse inoculation by *L. bainesii* and *L. listii* results in ineffective nodulation of *L. angolensis* (Yates *et al*., 2007). Taken together, the results of the cross-nodulations tests performed in this study seem to support the idea that nodulation specificity within a specific *Lebeckia* plant section is much less than that between sections, and that growth habit is an even greater determinant of specificity (Table 2.4). Clarification of this will, however require more exhaustive cross-inoculation tests once sufficient seed is available for this purpose. Further tests may also enable the selection of highly effective broad spectrum strains within each *Lebeckia* group that may prove useful as specific inoculants for species of this legume and be of commercial benefit.

The findings regarding nodule shape and nodulation presented in this study are in agreement with the taxonomic status of *Lebeckia* at higher order and sub generic levels. Nodule shape has long been recognized as an important taxonomic criterion in legume systematics (Sprent, 2002). Like other legumes in the tribe *Crotalarieae*, *Lebeckia* has indeterminate so-called "crotalarioid" nodules (Fig. 2.4) that are in most cases globular consisting of many lobes resulting in a fan-shape (Corby *et al.,* 1983; Sprent & James, 2007). The *Crotalarieae* share this property with other genistoid tribes (e.g. *Genisteae*, *Sophoreae* and *Podalyrieae*) with which it also shares a common ancestor (Lavin *et al.;* 2005). In contrast, inoculation of the *Lebeckia* rhizobia onto the phaseoloid legumes cowpea and siratro resulted in formation of typical "desmodioid" nodules (Sprent, 2002) that are a feature common among the millettioid crown clade (i.e. tribes *Phaseoleae*, *Desmodieae* and *Psoraleae*) (Lavin *et al.,* 2005). This illustrated the dominance of the host plant in determining nodule shape (Sprent, 2007). At the sub generic level, the results of

my cross-inoculation studies support the plant sections proposed for *Lebeckia* by van Wyk and co-workers (Le Roux & van Wyk, 2007). Rhizobia were only able to effectively nodulate other members within the same section, while rhizobia from one growth habit (shrubby trifoliate leaf) did not nodulate suffrutescent needle leaf plants in the other habit (Table 2.4). This supports the idea that the growth habit of *Lebeckia* is a useful taxonomic criterion for division of this genus into two genera (Boatwright and Van Wyk, personal communication).

This is the first report on the symbiotic properties of rhizobia of *Lebeckia* species indigenous to South Africa. The results showed that *Lebeckia* is nodulated by diverse rhizobia including fast-, slow- and intermediate-growers. Some of these may potentially be used as effective commercial inoculants if they can out-compete natural soil populations found in South Africa. As shown in this study, the symbiotic nitrogen-fixation effectiveness of indigenous rhizobia on siratro and cowpea were similar and may resemble the symbiotic capabilities of effective cowpea rhizobia found elsewhere in southern Africa (Law *et al.*, 2007). In some instances these indigenous bacteria are more effective on cowpea than the inoculant strain CB756 (Law *et al.,* 2007). However, many of the *Lebeckia* rhizobia included in this study appear to be specific to their homologous hosts and many did not effectively nodulate cowpea. As the *Lebeckia* species examined in this study were representative of the largest and most common sections in this genus, the use of *Lebeckia* rhizobia in broad spectrum inoculants for *Lebeckia* and other legumes is likely to be negligible considering the evidence presented in this study. Their potential usefulness may be restricted to nursery plantings only.

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2.7 TABLES

Table 2.1. Coordinates and soil properties for the collection sites of the ten *Lebeckia* species examined in this study.

Lebeckia species	Locality	Latitude	Longitude	pH	Soil type
L. cytisoides	Citrusdal	-32.2999	18.5990	5.25	Stony clay loam
L. multiflora	Lambert's bay	-32.0356	18.1896	6.50	Sandy
L. sericea	Bitterfontein	-30.5877	18.1466	6.50	Sandy loam
L. spinescens	Beaufort West	-32.1416	22.5519	6.50	Sandy
L. pungens	Meiringspoort	-33.1979	22.3246	6.00	Sandy loam
L. ambigua	Modder river	-33.2921	18.1936	6.70	Sandy
L. sepiaria	Citrusdal	-32.2999	18.5990	5.25	Stony clay loam
L. pauciflora	Cedarberg	-32.2185	19.0247	6.00	Sandy loam
L. meyerianna	Clanwilliam	-32.0187	18.4742	6.00	Sandy
L. simsiana	Stellenbosch	-33.9333	18.8500	6.00	Sandy

Table 2.2. Strains and geographic origin in South Africa of rhizobia isolated from ten *Lebeckia* species within three sections with either a shrubby or a suffrutescent habit.

Strain number	Growth rate[a]	Host species	*Lebeckia* Section	Habit	Geographic origin
BW1LSab	F	*L. spinescens*	*Calobota*	Shrubby	Beaufort West
BW2LSa	"	"	"	"	"
BW2LSb	"	"	"	"	"
BWS1iii	"	"	"	"	"
BW3LSc	"	"	"	"	"
BW3LSd	"	"	"	"	"
BHLSb	"	"	"	"	"
BH1LSa	"	"	"	"	"
BH1LSb	"	"	"	"	"
BH1LSc	"	"	"	"	"
BH2LSa	"	"	"	"	"
BH2LSb	"	"	"	"	"
BH2LSc	"	"	"	"	"
BH3LSa	"	"	"	"	"
BH3LSb	"	"	"	"	"
BH3LSc	"	"	"	"	"
WC19.1a	M	*L. cytisoides*	"	"	Citrusdal
WC19.1b	"	"	"	"	"
WC23.1a	M	*L. multiflora*	"	"	Lamberts bay
WC23.1b	"	"	"	"	"
WC23.1c	"	"	"	"	"
WC23.1d	"	"	"	"	"
NK22	M	*L. sericea*	"	"	Kamiesberg
WC28.1a	"	"	"	"	Bitterfontein
WC28.1b	"	"	"	"	"
WC28.1c	"	"	"	"	"
WC28.1e	"	"	"	"	"
WC28.1f	"	"	"	"	"
WC28.1g	"	"	"	"	"
WC28.1h	"	"	"	"	"
WC28.1i	"	"	"	"	"
WC28.1j	"	"	"	"	"
WC33a	"	"	"	"	"
WC33b	"	"	"	"	"
WC33c	"	"	"	"	"
WC33d	"	"	"	"	"
WC33e	"	"	"	"	"
WC33f	"	"	"	"	"
WC33g	"	"	"	"	"
WC33h	"	"	"	"	"
Betal106a	VS	*L. pungens*	*Stiza*	"	Meiringspoort
Betal106b	"	"	"	"	"
Betal106c	"	"	"	"	"
Betal106d	"	"	"	"	"
Betal106e	"	"	"	"	"
Betal106f	"	"	"	"	"
Betal106g	"	"	"	"	"
Betal106h	"	"	"	"	"
Betal106i	"	"	"	"	"
Betal106j	"	"	"	"	"
WC5.4a	F	*L. ambigua*	*Lebeckia*	Suffrutescent	Modder Rivier
WC5.4b	"	"	"	"	"
WC5.4c	"	"	"	"	"
WC5.4d	"	"	"	"	"
WC5.4e	"	"	"	"	"
WC5.4f	"	"	"	"	"
WC5.4g	"	"	"	"	"
WC5.4h	"	"	"	"	"
WC5.4i	"	"	"	"	"
WC12.1a	S	*L. sepiaria*	"	"	Citrusdal
WC21.1a	F	*L. pauciflora*	"	"	Cedarberg
WC21.1b	"	"	"	"	"
WC21.1c	"	"	"	"	"
WC21.1d	"	"	"	"	"
WC21.1e	"	"	"	"	"
WC21.1f	"	"	"	"	"
WC21.1g	"	"	"	"	"
WC21.1h	"	"	"	"	"
WC21.1i	"	"	"	"	"
WC21.1j	"	"	"	"	"
WC21.1k	S	"	"	"	"
WC21.1l	"	"	"	"	"
WC26.1c	F	*L. meyerianna*	"	"	Clanwilliam
WC26.1d	"	"	"	"	"
WC26.1e	"	"	"	"	"
WC26.1e1	"	"	"	"	"
WC26.1f	"	"	"	"	"
XHR1a	S	*L. simsiana*	"	"	Stellenbosch
XHR1b	"	"	"	"	"

[a] F, fast; M, intermediate; S, slow; VS, very slow

Table 2.3. Colony growth and nodulation characteristics of the rhizobia isolated from *Lebeckia* species used in this study.

Lebeckia species	*Lebeckia* section	Number of isolates	Growth rate [a]	Number of isolates that nodulated [b]		
				Lebeckia	Cowpea	Siratro
L. cytisoides	*Calobota*	2	Intermediate; 5-6d, 1mm	2E; 100%	N	N
L. multiflora	"	4	Intermediate; 5-6d, 1mm	4E; 100%	2E, 2I; 50%	4E; 100%
L. sericea	"	18	Intermediate; 5-6d, 1-2mm	18E; 100%	9E, 9I; 50%	12E, 6I; 66%
L. spinescens	"	16	Fast, 3d; ≥3mm	16E; 100%	2E, 14I, 12%	12E, 4I; 75%
L. pungens	*Stiza*	10	Very slow; 8d, ≤1mm	10E; 100%	8E, 2I; 80%	10E; 100%
L. ambigua	*Lebeckia*	9	Fast; 3-4d, 2mm	9E; 100%	6E, 3I; 67%	9E; 100%
L. sepiaria	"	1	Slow; 6d, 1mm	1E; 100%	1E; 100%	N
L. pauciflora	"	10	Fast; 3-4d, 2mm	10E; 100%	8E, 2I; 80%	6E, 4I; 60%
"	"	2	Slow; 6d, 1mm	2E; 100%	2E; 100%	2E; 100%
L. meyerianna	"	5	Fast; 3-4d, 2mm	5E; 100%	4E, 1I; 80%	4E, 1I; 80%
L. simsiana	"	2	Very slow; 8d, <1mm	2E; 100%	2E; 100%	2E; 100%

[a] Relative growth rates (very slow to fast) are indicated in terms of colony diameters (mm) reached after a specific number of days (d) incubation

[b] Columns show nodulated plant numbers and percentage effective nodulation of the respective rhizobia on their homologous hosts as well as cowpea and siratro. E, effective nodules; I, ineffective nodules; N, no nodulation

Table 2.4. Nodulation effectiveness of rhizobial strains isolated from shrubby and suffrutescent *Lebeckia* species after cross-inoculation on these species.

Cross-inoculation sets [a]	Strain	Original host	Inoculated species	Sections cross inoculated	Nodulation [b]
1: Shrubby	WC19.1b	*L. cytisoides*	*L. multiflora*	*Calobota → Calobota*	E
	WC19.1b	*L. cytisoides*	*L. sericea*	"	E
	WC23.1b	*L. multiflora*	*L. cytisoides*	"	E
	WC33b	*L. sericea*	*L. cytisoides*	"	E
	Betal106a	*L. pungens*	*L. spinescens*	*Stiza →Calobota*	I
	Betal106f	*L. pungens*	*L. spinescens*	"	I
	Betal106a	*L. pungens*	*L. sericea*	"	I
	Betal106f	*L. pungens*	*L. cytisoides*	"	I
	Betal106f	*L. pungens*	*L. multiflora*	"	I
	WC28.1f	*L. sericea*	*L. pungens*	*Calobota → Stiza*	I
	WC19.1c	*L. cytisoides*	*L. pungens*	"	I
	BH1LSc	*L. spinescens*	*L. pungens*	"	I
	BW3LSd	*L. spinescens*	*L. pungens*	"	I
	WC23.1b	*L. multiflora*	*L. pungens*	"	I
2: Suffrutescent	WC5.4d	*L. ambigua*	*L. meyerianna*	*Stiza → Lebeckia*	E
	WC5.4e	*L. ambigua*	*L. meyerianna*	"	E
	WC26.1d	*L. meyerianna*	*L. ambigua*	*Calobota→Lebeckia*	E
	WC26.1f	*L. meyerianna*	*L. ambigua*	"	E
	WC26.1f	*L. meyerianna*	*L. simsiana*	"	E
	WC26.1d	*L. meyerianna*	*L. simsiana*	"	E
	XHR1a	*L. simsiana*	*L. meyerianna*	"	E
	XHR1b	*L. simsiana*	*L. meyerianna*	"	E
3: Mixed	Betal106d	*L. pungens*	*L. meyerianna*	*Calobota → Lebeckia*	N
	Betal106a	*L. pungens*	*L. meyerianna*	"	N
	WC19.1b	*L. cytisoides*	*L. meyerianna*	"	N
	WC23.1b	*L. multiflora*	*L. ambigua*	"	N
	WC33a	*L. sericea*	*L. ambigua*	"	N
	WC26.1f	*L. meyerianna*	*L. pungens*	*Lebeckia → Stiza*	N
	WC26.1e	*L. meyerianna*	*L. pungens*	"	I
	WC26.1e	*L. meyerianna*	*L. cytisoides*	*Lebeckia → Calobota*	N
	WC26.1e	*L. meyerianna*	*L. spinescens*	"	N
	WC26.1c	*L. meyerianna*	*L. spinescens*	"	N
	WC26.1d	*L. meyerianna*	*L. cytisoides*	"	N
	WC26.1c	*L. meyerianna*	*L. cytisoides*	"	N
	WC5.4d	*L. ambigua*	*L. sericea*	"	N
	WC5.4d	*L. ambigua*	*L. multiflora*	"	N

[a] Set 1, Rhizobia isolated from shrubby *Lebeckia* species were used to inoculate other shrubby species. Set 2, Rhizobia from suffrutescent species were used to cross-inoculate other suffrutescent species. Set 3, Rhizobia from shrubby *Lebeckia* species were used to inoculate suffrutescent species and *vice versa*

[b] Nodulation by the respective rhizobia are indicated as follows. E, effective nodules; I, ineffective nodules; N, no nodulation

2.8 FIGURES
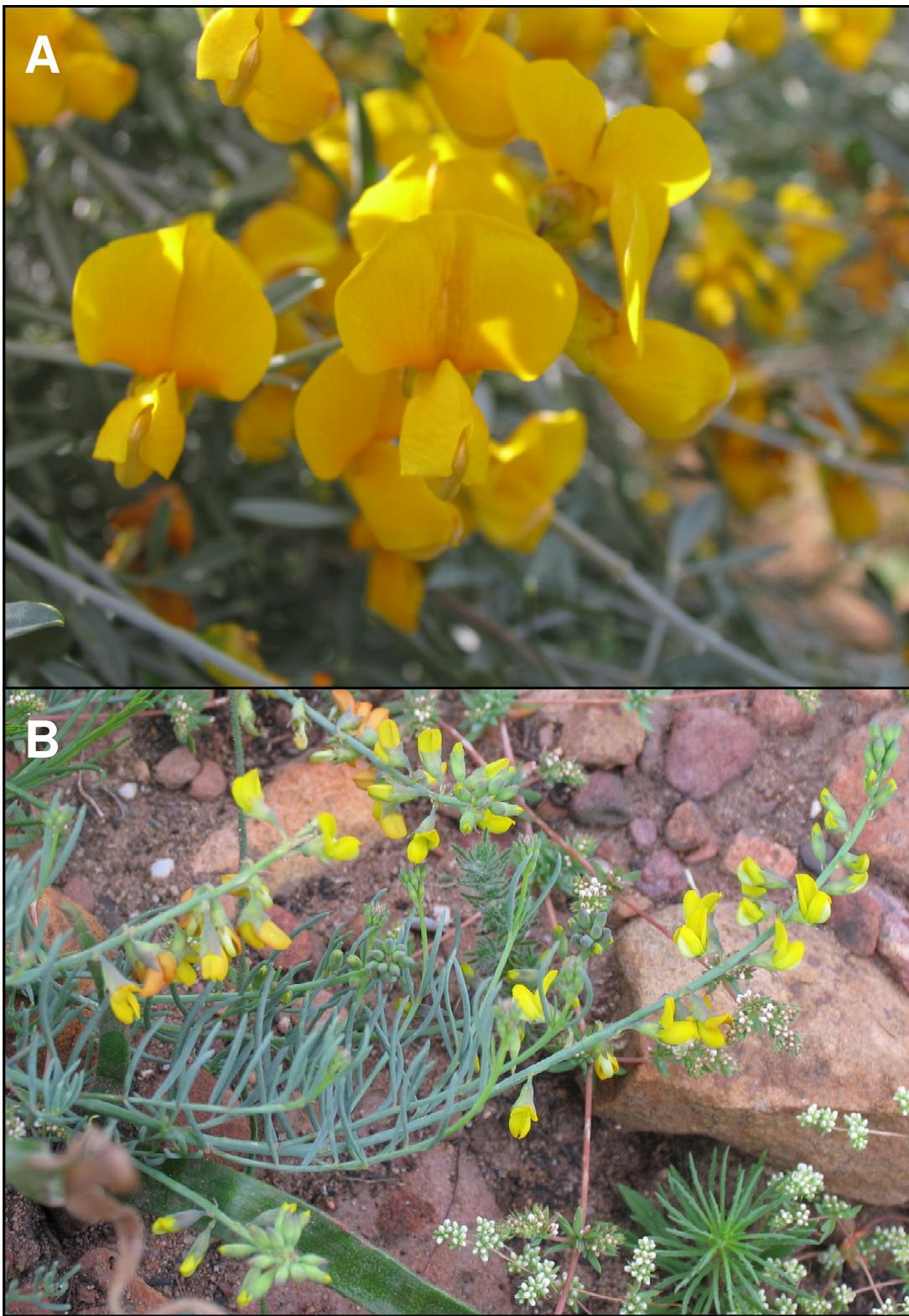
Figure 2.1. Flower spikes of the shrubby *Lebeckia cytisoides* (A) and the suffrutescent herbaceous *L. plukenetiana* (B) flowering during the spring season in the Western Cape. Photos courtesy of J. S. Boatwright
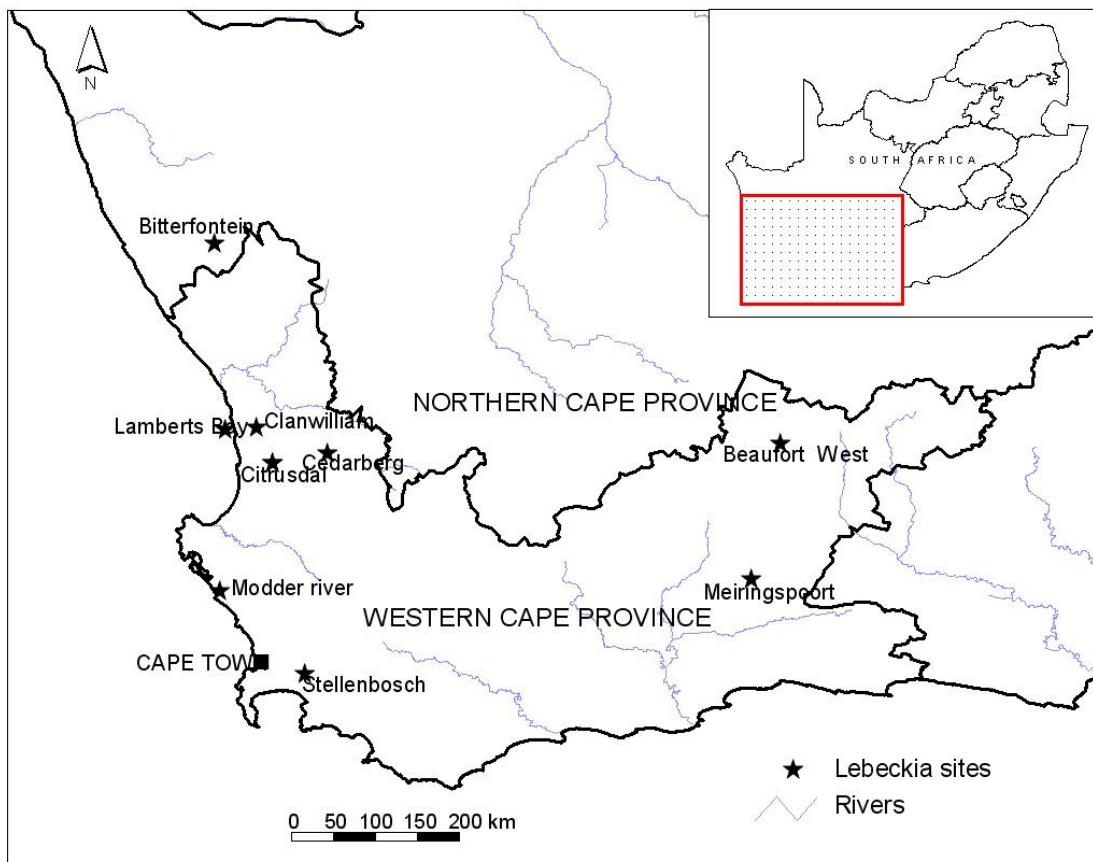
Figure 2.2. A map of South Africa showing the *Lebeckia* collection sites indicated with stars and rivers with stippled lines, in the Western and Northern Cape provinces.
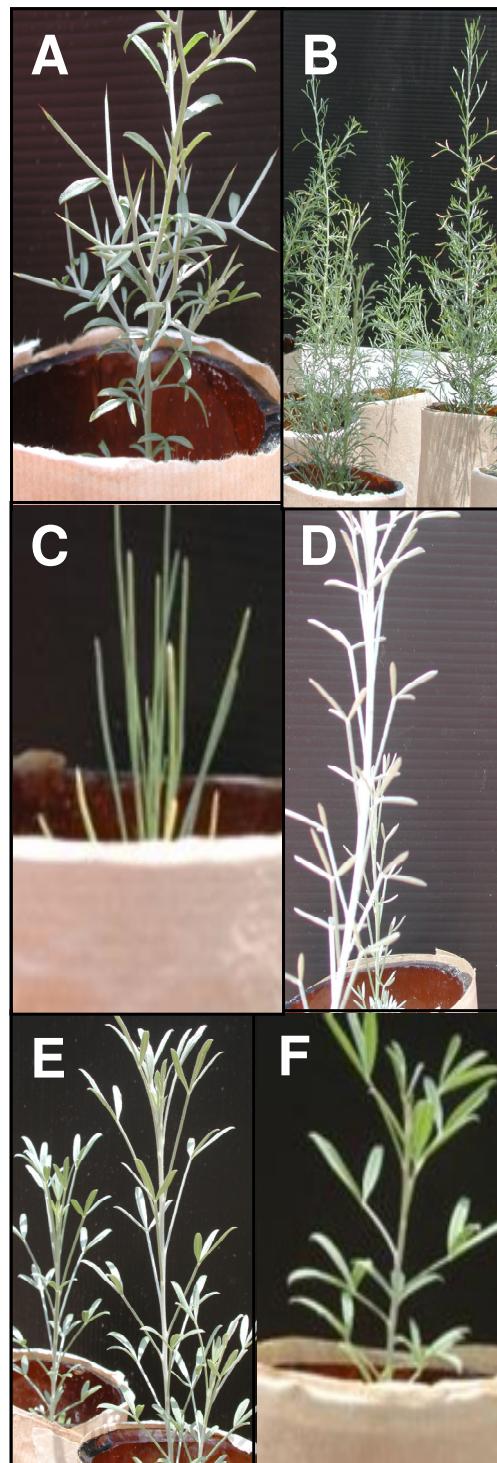
Figure 2.3. *L. ambigua* (C) with needle-shaped leaves (habit, suffrutescent) and the trifoliate-leaf species (habit, shrubby) *Lebeckia pungens* (A), *L. spinescens* (B), *L. multiflora* (D) *L. sericea* (E) and *L. cytisoides (F)* growing in nitrogen-free Leonard jars and effectively fixing nitrogen as indicated by the dark green colour of the leaves.
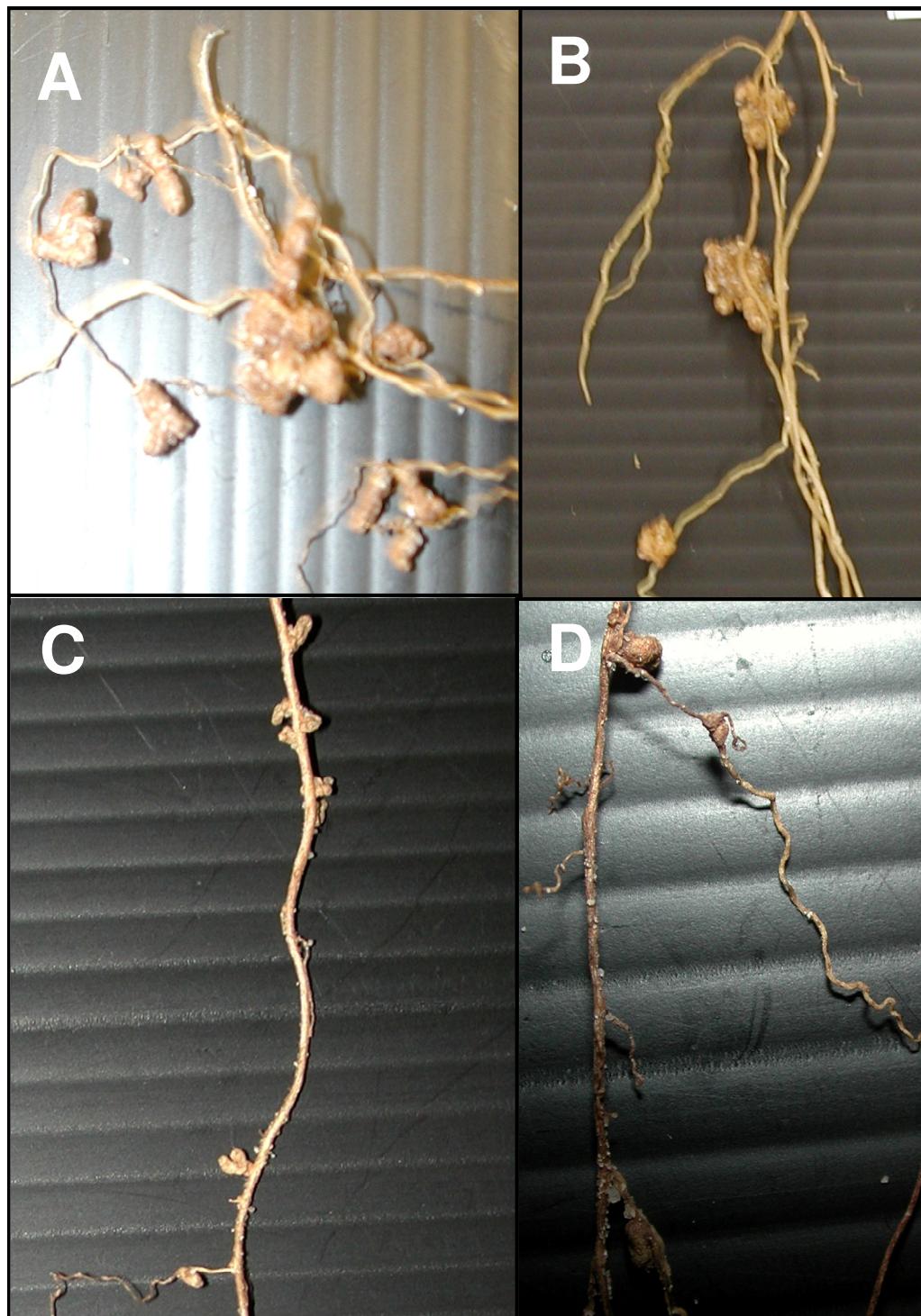
Figure 2.4. Examples of the branched, globular and elongated indeterminate nodules on the roots of *Lebeckia* species (A and B) and ovate to lobed indeterminate nodules on siratro roots (C) and spherical determinate nodules on cowpea roots (D).

CHAPTER 3

DNA FINGERPRINTING AND 16S rRNA GENE ANALYSIS OF RHIZOBIA ASSOCIATED WITH *LEBECKIA* SPECIES IN SOUTH AFRICA

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3.1 ABSTRACT

Diversity among 79 nodule isolates from shrubby and suffrutescent *Lebeckia* was assessed using random amplified DNA fingerprints. Cluster analyses indicated that isolates from the same species of *Lebeckia* generally grouped together in individual clusters with ≥65 % similarity. The gene encoding the 16S ribosomal RNA subunit was sequenced in 39 isolates representing the various clusters. The resulting sequences were compared to those in public domain nucleotide databases and subjected to phylogenetic analyses. The respective 16S sequences were most closely related to members of the *α*-proteobacterial genera *Bradyrhizobium*, *Sinorhizobium* and *Mesorhizobium*, as well as the *β*-proteobacterial genus, *Burkholderia*. These results indicate that indigenous South African *Lebeckia* species are nodulated by diverse rhizobia.

Keywords: *Lebeckia*, diversity, 16S rRNA, *Bradyrhizobium*, *Sinorhizobium Mesorhizobium* and *Burkholderia*.

3.2 INTRODUCTION

The nitrogen-fixing symbiotic bacteria of several legume genera within the tribe *Crotalarieae* have been studied taxonomically (Sy *et al*., 2001; Jaftha *et al*., 2002). These include agriculturally and economically important species of *Crotalaria*, *Lotononis* and *Aspalathus* (Sy *et al*., 2001; Moulin *et al.,* 2001; Jaftha *et al*., 2002; Le Roux*,* 2003; Yates *et al.,* 2007). Plants of the genus *Crotalaria* are used for green manures and intercropping (Giller, 2001), *Lotononis bainesii* is a valuable forage legume (Yates *et al.,* 2007) and *Aspalathus linearis* is the source of the well-known rooibos tea beverage (van Heerden *et al*., 2003). Dagutat (1995) carried out a systematic investigation into the identity of root nodule bacteria or rhizobia associated with a multiplicity of legumes found in South Africa, but neither she nor several later workers included *Lebeckia* spp. in their studies (Dagutat, 1995; Jaftha *et al*., 2002; Le Roux, 2003; Lindique, 2005).

Previously, 79 isolates of root nodule bacteria (rhizobia) from 10 species of *Lebeckia* were isolated and characterised in terms of their culture and nodulation properties (Chapter 2 of this dissertation). This was to complement a taxonomic revision of *Lebeckia* being undertaken at the University of Johannesburg, Johannesburg (Le Roux & van Wyk, 2007; van Wyk, personal communication). The results of this previous study indicated that *Lebeckia* is nodulated by diverse rhizobia including fast-, slow- and intermediate-growers. Some of these may potentially be used as effective inoculants for improving nitrogen fixation in commercial legumes. Inoculation studies also indicated that only combinations of rhizobia and legumes from the same sections within the shrubby and suffrutescent divisions of *Lebeckia* resulted in effective nodulation in each instance, suggesting that limited specificity might be encountered within each group. For example, cross-inoculation between the plants and rhizobia from the sections *Stiza* and *Calobota* within the shrubby division resulted in ineffective nodulation, suggesting that the symbiotic specificities of the two sections were quite different. Also, combinations of rhizobia and legumes between the shrubby and suffrutescent divisions generally did not result in nodulation, suggesting that considerable nodulation specificity existed between the two *Lebeckia* divisions.

The aim of the present study was to determine the taxonomic identities of the rhizobia obtained from the *Lebeckia* species found at nine localities in South Africa (Chapter 2 of this dissertation). For this

purpose, relatedness among the isolates was initially screened using specific primer (SP) PCR fingerprinting (Law *et al.*, 2007). From the resulting clusters of like isolates, representatives were chosen for further study. The gene encoding the 16S ribosomal RNA (16S rRNA) was sequenced for all representative isolates, followed by phylogenetic analyses to determine their taxonomic identities.

3.3 MATERIALS AND METHODS

3.3.1 Bacterial strains and DNA extraction

The rhizobia used in this study included 76 isolates from the root nodules of 10 *Lebeckia* plant species (Chapter 2 of this dissertation; note three *L. pungens* isolates were not analysed). For the SP-PCR, rhizobial type strain cultures were also included (Table 3.1). For DNA extraction, isolates were inoculated into Tryptone Yeast (TY) broth as it reduced the slime or gum formation by cultures as compared to growth in Yeast Mannitol (YM) broth. For this purpose, two loop-fulls of a fresh Yeast Mannitol Congo red (YM-CR) agar (Somasagaren & Hoben, 1994) culture were inoculated into 0.5 % tryptone yeast (TY) (Somasagaren & Hoben, 1994) broth in an Erlenmeyer flask. The cultures were incubated on an orbital or a reciprocal shaker at 28°C until there was sufficient growth (3-5d, approximately mid-log growth phase).

Whole cell DNA was extracted from the 76 rhizobial isolates and type strains, using the protocol described by Wilson (1989). The bacteria in 20 ml broth culture were harvested by centrifugation at 112 x *g* and 5°C for 10-15 min. The pelleted cells were then washed by suspension in 10mM Tris-HCL (pH 8) wash buffer and centrifuged as before. The washed pellet was then suspended in 2ml CTAB (N-cetyl-N, N, N-trimethyl ammonium bromide) extraction buffer (CTAB, 5% w/v; Tris-HCl, 150mM; EDTA, 40mM; NaCl, 2M; pH 8) and incubated overnight at 4°C in the presence of 100 μl, freshly prepared 0.5% lysozyme solution (Roche Molecular Biochemicals, Switzerland). To the lysing solution, 100 μl 5M NaCl and 200 μl 10% sodium dodecyl sulphate (w/v) were added and incubated for 20 min at 65-80°C. This was followed by the addition of 80 μl CTAB extraction buffer and mixing by inverting three times every 5 min, followed by centrifugation for 5 min at 10 000 x *g* at 25°C. The DNA in the aqueous phase was then precipitated by the addition of 0.6 volumes isopropanol. The precipitated DNA was pelleted by centrifugation at 10 000 x *g*, washed with 70% ethanol, air-dried and dissolved in 50 μl TE buffer (Tris-HCl, 10mM; 1mM, EDTA; pH 8). Isolates for which this extraction method failed to yield DNA were treated with the Promega Wizard Genomic DNA Purification Kit (Promega, Madison, USA) according to the manufacturer's instructions. The concentration of the extracted DNAs was determined using a NanoDrop spectrophotometer (NanoDrop Technologies, USA). The purified DNA was stored at -20°C until use.

3.3.2 Specific primer PCR fingerprinting procedures

The *nif* gene derived primer RPO1 (Richardson *et al*., 1995) and arbitrary sequence primer CRL7 (Mathis and McMillin, 1996) (Table 3.2) were used to fingerprint the 76 *Lebeckia* rhizobia and the type strains (Tables 3.4). All PCR amplifications were conducted in a total reaction volume of 10 μl containing 5 ng/μl DNA, 1.5 mM $MgCl_2$, 800 μM dNTPs, 1 μM primer, and 0.025 U/μl Taq polymerase and its reaction buffers (Promega, Madison, USA) . The RPO1 PCR cycling conditions included an initial denaturation at 94°C for 3 min followed by 5 cycles of denaturation at 94°C for 50 sec, annealing at 55°C for 1 sec and extension at 72°C for 1 sec, followed by another 25 cycles each consisting of denaturation at 94°C for 45 sec, annealing at 60°C for 1 sec and extension at 72°C for 1 sec. The thermal cycling profile for primer CRL7 consisted of 35 cycles of denaturation at 94°C for 50 sec, annealing at 35°C for 2 min and extension at 72°C for 2 min, followed by a final extension at 72°C for 7 min. PCRs were carried out in a Gene Amp-PCR system 2700 (Applied Biosystems, California USA). Amplified DNA was separated by agarose gel 2.0% (w/v) electrophoresis in the presence of TAE buffer (Tris-HCl, 40 mM; NaOAc, 20 mM; EDTA,1 mM; pH8.5) at 667 V/cm (Sambrook & Russell, 2001). The resulting DNA fingerprints were stained in the gels using ethidium bromide (Botha *et al*., 2004), visualized using ultraviolet light and recorded using a CCD camera. Fingerprints were scored for the presence and absence of bands and subjected to cluster analysis using the combined data for RPO1 and CRL7. The resulting data were analysed using Unweighted Pair Group Method Arithmetic Mean (UPGMA) (Sokal & Michener, 1958) under the Pearson correlation and Dice band based algorithms (Bionumerics Version 4.0 software, Applied-Maths, St. Martin-Latem, Belgium).

3.3.3 16S rRNA gene sequencing and phylogenetic analyses

Thirty nine isolates (Table 3.3) were selected as representatives for the various SP-PCR clusters. For these isolates, a portion of the 16S rRNA gene was amplified using the primers 16f 27 and 16r 1485 (Table 3.2; Lane, 1991). PCR amplification was carried out in 50 μl reaction volumes using the same reaction components and concentrations as described for the SP-PCR, except that 0.5μM of each primer was used. PCR was carried out in an Eppendorf Mastercycler Gradient apparatus (Merck chemicals, Johannesburg, SA) using the following thermal profiles: an initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30

sec and extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min (Laguerre *et al.*, 1994). The PCR products were separated by electrophoresis in 1% (w/v) agarose gels, TAE buffer and stained as before. The resulting PCR products were purified by precipitation with polyethylene glycol (PEG) (Steenkamp *et al.,* 2006) and sequenced in both directions using the original PCR primers and the ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) and the 3730 DNA Analyzer (Applied Biosystems).

All raw sequence files were inspected and corrected, where necessary, using Chromas Lite 2.0 (Technelysium) and BioEdit version 5.0.9 (Hall, 1999). All 16S rRNA sequences were also compared to those in GenBank (National Centre for Biotechnology Information; http://www.ncbi.nlm.nih.gov/) using *blastn* (Altschul et al., 1997. The 16S rDNA sequences for the relevant rhizobial type strains or reference strains were also obtained from GenBank and included together with the *Lebeckia* rhizobial sequences in multiple alignments. These alignments were constructed using MAFFT version 5.85 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) with the L-INS-i option effective (Katoh *et al.*, 2002). To determine the appropriate evolutionary models for phylogenetic analyses, Modeltest 3.7 (Posada & Crandall, 1998) and PAUP* version 10b (Swofford, 2002) were used. Phylogenies were inferred using maximum likelihood (ML) and neighbor joining (NJ) distance analyses. Bootstrap analyses were based on 1000 replicates and the same best-fit parameters used to infer the individual phylogenies.

3.4. RESULTS

3.4.1 SP-PCR fingerprint analysis

Cluster analysis of the SP-PCR fingerprints generated with primers RPO1 and CRL7 separated the 76 *Lebeckia* rhizobia into ten major clusters or lineages (C1-C10) at the ≥60% similarity level (Fig. 3.1). Cluster C1 contained the fast-growing *Sinorhizobium meliloti* (LMG6133) and intermediate-growing *Mesorhizobium plurifarium* (LMG11892) type strains, the intermediate-growing *L. cytosoides* strains WC19.1b and WC19.1c from Citrusdal and all the intermediate-growing *L. sericea* strains from Bitterfontein and the Kamiesberg, as well as a single fast-growing isolate from *L. ambigua* at Modder River. The Cluster C1 isolates shared ≥65% similarity. Cluster C2 was made up of two fast-growing Clanwilliam isolates from *L. meyerianna* and the slow-growing *B. japonicum* type strain LMG6138, which clustered at ≥65% similarity. The RPO1 profile of LMG6138 appeared different, however, to that of the apparently related fast-growing strains in cluster C2 suggesting its presence in this cluster was an experimental artifact (Fig. 3.1). Cluster C3 was composed of strains sharing ≥60% similarity that were isolated from quite widely separated localities and various species including the fast-growing isolates of *L. ambigua* (Modder River)*,* the slow-growing *L. sepiaria* isolate WC121a (Citrusdal),the intermediate-growing *L. multiflora* isolate WC23.1a (Lambert's Bay) and five fast-growing isolates from *L. meyerianna* (Clanwilliam). Cluster C4 included two intermediate-growing Lambert's Bay isolates from *L. multiflora* (WC23.1a and b) that grouped at 72% similarity with the fast-growing *R. tropici* type strain LMG9503. Intermediate-growing isolate WC23.1d (*L. multiflora*) formed a single cluster C5 that joined cluster C4 at ≥60% similarity. Clusters C4 and C5 were linked to C3 at a similarity of about 55% (Fig. 3.1). Cluster C6 contained the two very slow-growing isolates from *L. simsiana* obtained from Stellenbosch, which grouped at 70% similarity with the slow-growing *B. elkanii* type strain LMG6134. Cluster C7 contained all the fast-growing isolates from *L. spinescens* collected at Beaufort West, which grouped together at a high similarity (≥76%), and were related to the fast-growing *R. etli* and *R. leguminosarum* type strains, LMG6134 and LMG8820, respectively. Cluster C7 was separated from cluster C9 by a single slow-growing strain (Betal106f) isolated from *L. pungens* (Meiringspoort), which grouped with low similarity (52%) to the *L. spinescens* cluster C7 strains. Cluster C9 contained fast-growing isolates from *L. pauciflora* collected in the Cedarberg, which shared ≥70% similarity. Cluster C10, at similarities ≥75%, included the remaining six very slow-growing strains from *L. pungens* isolated

from Meiringspoort, as well as the two slow-growing Cedarberg *L. pauciflora* isolates WC21.1l and WC21.k, which had identical profiles, and an intermediate-growing *L. sericea* isolate WC33b1 from Bitterfontein. The presence of intermediate WC33b1 amongst fast growers in C10 may have been an artifact from the CRL7 fingerprint of this strain (Fig 3.1), as the RPO1 profile of this isolate was virtually identical to that of WC33a amongst the other intermediate *L. sericea* isolates in cluster C1 (Fig. 3.1). From the ten clusters, 39 isolates were selected for further taxonomic analysis (Fig. 3.1).

3.4.2 16S rRNA gene sequencing and phylogenetic analysis

Nearly full length (1200-1500 base pair [bp]) nucleotide sequences were obtained for the16S rRNA gene from each of the 39 isolates associated with the 10 *Lebeckia* species. Comparison of these sequences to those in GenBank, using *blastn,* indicated that 30 represented members of the bacterial division α-*Proteobacteria*, while nine isolates had homology to *Burkholderia*, a genus in the division *β-Proteobacteria* (Table 3.3). Amongst the *α-Proteobacteria*, all of the fast-growing isolates from *L. spinescens* showed similar identity to *Sinorhizobium* (which is fast-growing). Thirteen out of the fourteen intermediate-growth rate isolates from *L. cytisoides*, *L. multiflora* and *L. sericea* had 16S rRNA sequences similar to those of *Mesorhizobium* species, an exception being *L. sericea* strain NK22 which was assigned to *Bradyrhizobium* (Table 3.3). On the other hand, the slow-growing *L. sepiaria* strain WC12.1a was assigned to *Mesorhizobium*, whereas the other slow-growing isolates had 16S RNA sequences similar to *Bradyrhizobium* species including all three from *L. pungens*, one of the four *L. pauciflora* isolates and the *L. simsiana* isolate examined (Table 3.3). Among the *β-Proteobacteria*, three isolates from *L. ambigua*, the remaining three from *L. pauciflora* and the three *L. meyerianna* isolates all represented *Burkholderia* species (Table 3.3).

To determine the possible taxonomic identities for the representative rhizobia included in this study, phylogenetic analyses were performed. Two separate aligned datasets were constructed for the α- and *β-Proteobacteria*, which included the 16S rRNA sequences for the *Lebeckia* rhizobia as well as those for the relevant type strains obtained from GenBank. Maximum likelihood (ML) and neighbour joining (NJ) analyses of the *α*-proteobacterial rhizobia generated trees with similar topologies (Fig. 3.2). The isolates prefixed WC12.1a (*L. sepiaria*), WC19.1b, c (*L. cytisoides*), WC23.1b, c, d (*L. multiflora*), WC28.1b, c, e, g, h, i (*L. sericea*), WC33b, c, e, h (*L. sericea*), were clearly related to the different *Mesorhizobium* type strains. The *Lebeckia* isolates on the *α-*

Proteobacteria phylogenetic tree that grouped with *Mesorhizobium* formed two clusters. One cluster, consisting of four of the seven strains isolated from *L. sericea*, formed a sister taxon to the type species *M. loti*, *M. ciceri* and *M. tianshanense*, while being more distant to the species *M. mediterraneum* and *M. temperatum* (Fig. 3.2). In contrast, the other cluster contained 10 isolates that were quite distinct from the mesorhizobial type strains shown in Fig. 3.2. The isolates originating from *L. spinescens* at Beaufort West, with prefixes BH or BW, formed a clade very closely related to the *Sinorhizobium* type strains, with an apparent closer relationship to *S. arboris*, *S. medicae* and *S. meliloti* (Fig 3.2). Five slow-growing isolates and an intermediate isolate NK22, represented *Bradyrhizobium* species. Of these, four isolates grouped with *Br. elkanii* including *L. pauciflora* WC21.1l, (Cedarberg) and *L. pungens* isolates Betal106a, Betal106d, Betal106f (Meiringspoort). The single *L. simsiana* isolate XHR1a was most closely related to *Br. betae*, with the *L. sericea* isolate NK22 at its base (Table 3.2).

Within the *β*-proteobacterial phylogeny, two clades of *Lebeckia* rhizobia were identified (Fig. 3.3). Seven *Lebeckia* rhizobia formed a clade that clustered with the *Burkholderia tuberum* strain STM 678 originally isolated from the root nodules of *Aspalathus carnosa*. The two remaining isolates (WC5.4e and WC5.4d) from *L. ambigua* had identical 16S rRNA sequences and formed a separate cluster with *Bu. fungoram* strain LMG16225 (Fig. 3.3). The Bu. tuberum group comprised isolates with somewhat dispersed relatedness, including Clanwilliam isolates WC26.1d, WC26.1e and WC26.1f from L. meyeriana and Cedarberg isolate WC21.1j from *L. pauciflora*. They grouped apart from the other two *L. pauciflora* isolates WC21.1b and WC21.1i, and both sets were quite distant from the isolate WC5.4c from Modder River, which appeared closely related to *L. ambigua* (Fig. 3.3).

3.5 DISCUSSION

The taxonomic relationship between the symbiotic rhizobia associated with the indigenous genus *Lebeckia* species was studied using strains isolated from the root nodules of 10 *Lebeckia* species. The host plants sampled included species from both the shrubby trifoliate-leaf and suffrutescent needle-leaf types into which this genus is divided (Boatwright *et al.*, 2007; Chapters 1 and 2 of this dissertation). The strains were initially differentiated using SP-PCR fingerprinting, from which 39 representative isolates were selected for further study. Phylogenetic analyses using 16S rRNA sequence information revealed that the *Lebeckia* species is nodulated by diverse rhizobia representing members of the *α-* and *β-Proteobacteria*. This is the first report concerning the taxonomic identities of the rhizobia associated with the root nodules of this unique legume genus, indigenous to South Africa.

In previous studies, SP-PCR was primarily used to differentiate related strains within a defined species e.g. *Bradyrhizobium lupini* (Botha *et al.*, 2002) and cowpea-nodulating bradyrhizobia (Law *et al.*, 2007). In the present study, it provided a convenient means to screen the variety of isolates obtained from different *Lebeckia* species and to select representatives for further taxonomic identification. The results of this fingerprinting analysis (Fig. 3.1) supported previous observations of the different cultural and nodulation characteristics of these isolates (Chapter 2 of this dissertation) and suggested that many isolates had dissimilar taxonomic identities. Clusters revealed by the combined RPO1 and CRL7 fingerprints generally had excellent correlation with the host plants from which the strains were isolated, notably clusters C1 (*L sericea)*, C7 (*L spinescens*) and C9 (*L pauciflora*) and also strain growth rates and collection sites. Within the major clusters, including those containing isolates from several hosts, host-specific sub-clusters could be differentiated at more stringent (≥75%) similarity values. For example, *L. pungens*, *L. sericea* and *L. pauciflora* subgroups could be clearly differentiated within cluster C10 (Fig 3.1) and *L. meyerianna* and *L. ambigua* subgroups were also distinct in cluster C3. In ecological studies, previous authors observed that isolates have high sampling site endemicity (Vinuesa *et al.,* 2005a; Law *et al.,* 2007). However, in the present study, most isolates were from individual species sampled at a single collection site, thus suggesting that SP-PCR differentiation among the isolates was probably more species-based (Fig. 3.1). However, there was evidence of locality endemicity in cluster C1, in which

L. sericea isolates grouped according to their origin at the two sites WC28 and WC33 within the Bitterfontein locality from which they were sampled (Fig. 3.1).

Among the rhizobia selected for 16S rRNA-based studies, each of the ten SP-PCR clusters were represented. In several instances, they included a range of isolates from sub-groups belonging to a particular host species, such as the nine *L. spinescens* isolates selected from different sub-groups in cluster C7 (Fig. 3.1). Initial comparisons of the 16S rRNA gene sequences to those in GenBank revealed that the selected *Lebeckia* isolates probably represented both *α*- and *β*-proteobacterial species (Table 3.3). The latter observation was unusual as *β*-proteobacterial rhizobia were only recently identified (Moulin *et al*., 2001; Chen *et al*., 2003) and seem to be more prominent among the mimosoid legumes (Elliott et al., 2007; Sprent, 2007). The generic relationships revealed by the *blastn* analyses did, however, match observations of the culture properties of the *Lebeckia* isolates discussed in Chapter 2 of this dissertation. Based on their 16S rRNA sequences, most of the fast-growing strains were identified as *Sinorhizobium*, the intermediate-growing strains as *Mesorhizobium* and the slow-growers as *Bradyrhizobium* (Table 3.3, Fig 3.2). Similarly, several others in the fast to intermediate category were found to represent *Burkholderia* species in the *β-Proteobacteria* (Table 3.3, Fig. 3.3). The colonies for the *Burkholderia* isolates also appeared white and wrinkled (Chapter 2 of this dissertation) as previously reported for bacteria in this genus (Francis *et al*., 2004; Howard and Inglis, 2003).

Based on 16S rRNA sequence analysis (Fig. 3.2), the *Lebeckia* rhizobia included in the *Mesorhizobium* and *Sinorhizobium* clades probably represent novel, as yet undescribed, species. Four *Mesorhizobium* strains clustered together in a clade that appeared related to *M. tianshanense, M. loti* and *M. ciceri* type strains (Jarvis *et al*., 1982; Jarvis *et al*., 1997), although *M. loti* nodulates species such as *Leucaena, Lupinus* and *Anthyllis* that do not occur naturally in South Africa (Young, 1996) and *M. ciceri* specifically nodulates chickpea (Nour *et al*., 1994). In contrast, the other *Lebeckia* mesorhizobia formed a second clade separate from all the *Mesorhizobium* type species, suggesting that this clade may contain novel species of this genus (Fig. 3.2). It is difficult to say if the two *Lebeckia* clades might be host-related. The four *M. ciceri-M. loti* related isolates (WC28.1g, WC33b, WC33c, WC33h) were all from *L. sericea* in the shrubby *Calobota* section of *Lebeckia* (Chapter 2 of this dissertation), but mesorhizobia in the second quite tightly knit clade included

isolates (WC28.1f, WC19.1b, WC12.1a, WC23.1d, WC33e) from three species in section *Calobota* (*L. sericea, L. cytisoides* and *L. multiflora*) as well as one strain from the suffrutescent section *Lebeckia* (*L. sepiaria* isolate WC12.1a).

Within the *Sinorhizobium* clade, the bacteria isolated from the root nodules of *L. spinescens* were closely related to one another and did not appear to be near relatives of any other species in this genus (Fig. 3.2). It is interesting to note, however, that the *Lebeckia* sinorhizobia were most closely associated with a clade that included *S. arboris*, and *S. medicae* and *S. meliloti*. *S. arboris* is an African fast-growing species isolated from the leguminous trees, *Acacia senegal* and *Prosopis chilensis* found in Sub-Saharan Africa (Sudan and Kenya) (Nick *et al*, 1999; Wolde-Meskel *et al*., 2005), whereas *S. medicae* and *S. meliloti* were isolated from *Medicago* spp. of eastern Mediterranean origin (Rome *et al*., 1996).

For classification of species in the genus *Bradyrhizobium*, 16S rRNA gene sequences are of little value as this region is too conserved for differentiation (Vinuesa *et al*., 2005a, b). The results of this study (Fig. 3.2), did however, indicate that two of the *Lebeckia* rhizobia from *L. sericea* and *L. simsiana*, respectively, grouped with *Br. betae*, a species that does not nodulate legumes having been isolated from *Beta vulgaris* root tumours (Rivas *et al.*, 2004). The four remaining *Bradyrhizobium* isolates from the shrubby *L. pungens* (Meiringspoort) and the suffrutescent species *L. pauciflora* (Cedarberg) appeared to be closely related to *Br. elkanii* (Fig. 3.2; Table 3.3). *Br. elkanii* was originally isolated from *Glycine max*. Determining whether these isolates respectively represent *Br. betae* and *Br. elkanii,* will require the analyses of multiple additional housekeeping loci as demonstrated by Vinuesa *et al*. (2005a, b).

The *Lebeckia* strains identified as *Burkholderia* were isolated only from the nodules of suffrutescent needle-leaf species within section *Lebeckia*. Among these, two 16S rRNA-based clades were evident (Fig. 3.3). In one clade, two evidently identical strains isolated from *L. ambigua* (Modder river) were closely related to *Bu. fungorum*, forming a distinct cluster (Fig. 3.3). *Bu. fungorum* has previously been isolated from the environment, the white-rot fungus *Phanerochaete chrysosporium* and animal and human tissue samples (Coenye *et al*., 2001). This species has not been reported to nodulate legumes and its nitrogen-fixing ability has not been studied. The second *Lebeckia-*

Burkholderia clade appeared to be quite heterogeneous, with the third isolate from *L. ambigua* grouping very closely with *Bu*. *tuberum* (Fig. 3.3). This *Burkholderia* species was originally isolated from *Aspalathus carnosa* in the Western Cape (Moulin, *et al*., 2001) and has also been shown to nodulate various *Cyclopia* species and siratro (Elliot *et al*., 2007; Moulin, *et al*., 2001; Chen *et al.,* 2003). Further investigation is required, however, to confirm whether this *Lebeckia* strain (WC5.4c) indeed represents *Bu. tuberum.* The remaining *Burkholderia* isolates, originating from *L. meyeriana* (Clanwilliam) and *L. pauciflora* (Cedarberg), were in separate clusters from WC5.4c (Fig. 3.3). Although more research is required for complete taxonomic characterization of these *Lebeckia* rhizobia, the results of this study suggests the likely occurrence of *Burkholderia* species capable of nodulation and nitrogen fixation in regions where suffrutescent species of *Lebeckia* are found. *Burkholderia* species were previously isolated from *Aspalathus* and *Cyclopia* spp. growing in the western and southern Cape regions of South Africa (Moulin *et al.,* 2001, Kock, 2004). A number of *Burkholderia* species have been identified that are capable of nodulating legumes (Moulin *et al*., 2001; Chen *et al*., 2003; Perin *et al*., 2006). The isolation, in the present study, of *Burkholderia* from suffrutescent needle-leaf *Lebeckia* spp. thus complements the observation that nodulation of legumes by this genus is fairly widespread and diverse (Sprent, 2007).

Although it is well established that the taxonomy of the bacterial microsymbiont is unrelated to that of the host legume, rhizobia were initially classified according to cross-inoculation specificity (Somasegaren & Hoben, 1994). Although this system later proved unworkable (Thies *et al*., 2001), the existence of nodulation specificity must have dictated which rhizobia became associated with which legumes, thus resulting in certain groups of legumes sharing common symbiotic bacteria (Allen & Allen,1981; Brenner *et al.,* 2005). This is clearly illustrated by the fact that *Mimosa* species spread over different continents prefer nodulation by specific *Burkholderia* species (Elliott *et al*., 2007; Sprent, 2007), while 'genistoid' legumes such as *Lupinus* species indigenous to Europe and North and South America are nodulated by specific *Bradyrhizobium* species (Stepkowski *et al*., 2005). This apparent link between legume endemicity and rhizobial identity also seems to extend to other African genistoid genera (Crisp *et al.*, 2000). For example, certain species of the 'genistoid' tribe *Podalyrieae* (e.g. *Cyclopia* species) and *Crotalarieae* (e.g. *Aspalathus carnosa*) are nodulated specifically by *Burkholderia* species (Kock 2005; Sprent, 2007). However, many African 'genistoid' legumes appear to be nodulated by diverse rhizobia, including *Crotalarieae* species. For example,

Crotalaria podocarpa and species in the *Listia* section of the genus *Lotononis* are specifically nodulated by methylobacteria (Le Roux, 2003; Jaftha *et al.*, 2002; Samba *et al.*, 1999; 2002; Yates *et al.*, 2007), while *Aspalathus linearis* (the Rooibos tea legume) is nodulated by slow-growing *Bradyrhizobium* species (Staphorst & Strijdom, 1975; Deschodt & Strijdom, 1976; Dagutat, 1995; Dakora, 1998; Muofhe & Dakora, 1999). The results of the current study similarly showed that the *Crotalarieae* genus *Lebeckia* is also nodulated by diverse rhizobia, which may be unique from previously described rhizobia (Figs 3.2, 3.3).

Taking into account the current taxonomic revision of *Lebeckia* (Le Roux & van Wyk, 2007; Boatwright & van Wyk, personal communication), it is of interest to compare the rhizobial groupings identified in this study with the host-specificity groups identified previously in Chapter 2 of this dissertation (Tables 2.2-2.4). The nodules of species in the shrubby trifoliate-leaf sections *Calobota* and *Stiza* yielded rhizobia only of the α-proteobacterial group (i.e. *Bradyrhizobium*, *Mesorhizobium,* and *Sinorhizobium* species), while the nodules of species in the suffrutescent needle-leaf section *Lebeckia*, yielded only β- proteobacterial rhizobia, except for *L. pauciflora* from which one α-proteobacterial isolate (WC21.1l) was obtained. Correspondingly, cross-inoculation across these major plant divisions generally failed (Table 2.4). Examination of more plant species will help to establish whether this is typical of genera in the two divisions of *Lebeckia*.

My study highlighted that *Lebeckia* is nodulated by members of the genera *Mesorhizobium, Sinorhizobium*, *Bradyrhizobium* and *Burkholderia*. The four genera were distinct from each other in growth and symbiotic characteristics (Chapter 2 of this dissertation), while SP-PCR revealed that clustering of the isolates was related both to geographical location and host plant species. The comparison of SP-PCR and 16S rRNA sequence data confirmed that SP-PCR allowed appreciable bacterial discrimination thus providing an effective screening technique for grouping taxonomically similar isolates. Phylogenetic analyses using 16S rRNA gene sequences enabled elucidation of the generic status of the various bacteria. However, this region is conserved, thus limiting its discriminatory power and restricting taxonomic resolution when comparing closely related strains (Vandamme *et al.*, 1996). Further taxonomic investigation of the isolates is clearly required, including the sequencing of additional marker genes, DNA-DNA hybridization, and biochemical analysis to clarify their taxonomic position. These polyphasic techniques should help to resolve

species identities of the various *Lebeckia* isolates examined in this study. This has relevance, considering present evidence that suffrutescent needle-leaf species of *Lebeckia* differ in their respective rhizobial affinities from species in the shrubby trifoliate-leaf division, which will become a separate genus (Boatwright & van Wyk, personal communication).

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3.7 TABLES

Table 3.1. The rhizobial type strains from the South African *Rhizobium* Culture Collection used for DNA fingerprinting comparison with DNA of *Lebeckia* rhizobia.

Species	Type strain[a]
R. tropici	LMG 9503
R. etli	LMG 17827
R. leguminosarum	LMG 8820
Br. elkanii	LMG 6134 or USDA 76
Br. japonicum	LMG 6138 or WB86
M. plurifarium	LMG 11892
S. meliloti	LMG 6133

[a]LMG, Laboratorium voor Microbiologie, Universiteit Gent, Belgium; USDA, U.S. Department of Agriculture, Beltsville, Maryland;

Table 3.2. Oligonucleotides and primers used in this study.

Primer	Sequence (5' to 3')	Target gene	Reference
RPO1	aattttcaagcgtcgtgcca	*Nif* gene and random	Richardson *et al.,* 1995
CRL7	gcccgccgcc	random	Mathis & MacMillin, 1996
16f 27	agagtttgatcctggctcag	16S rRNA	Lane, 1991
16r 1485	taccttgttacgacttcacccca	16S rRNA	Lane, 1991

Table 3.3. Accession numbers and strain identities of the top *blastn* GenBank hits for the 16S rRNA sequences of the *Lebeckia* rhizobia studied. As on 10 April 2008.

Isolate	*blastn* Top hit (Accession no.)	*blastn* Top hit (species identity)	% similarity	*Lebeckia* host	Habit	Locality
BW1LSab	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BW2LSa	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BW2LSb	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BW3LSc	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BW3LSd	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BH1LSc	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BH2LSa	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BH2LSc	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
BH3LSc	EF152475.1	*Sinorhizobium* sp. STM 4036	100	*L. spinescens*	Shrubby	Beaufort West
Betal106a	EU481825.1	*Bradyrhizobium elkanii*SEMIA938	99	*L. pungens*	Shrubby	Meiringspoort
Betal106d	AY923031.1	*Bradyrhizobium* sp.LMTR21	99	*L. pungens*	Shrubby	Meiringspoort
Betal106f	AB367695.1	*Bradyrhizobium* sp. KO14	100	*L. pungens*	Shrubby	Meiringspoort
NK22	EF638789.1	*Bradyrhizobium* sp. LAR-20	100	*L. sericea*	Shrubby	Kamiesberg
WC19.1a	EU5145528.1	*Mesorhizobium* sp. SCAU13	99	*L. cytisoides*	Shrubby	Citrusdal
WC19.1b	EU514526.1	*Mesorhizobium* sp. SCAU9	99	*L. cytisoides*	Shrubby	Citrusdal
WC23.1b	EU5145528.1	*Mesorhizobium* sp. SCAU13	100	*L. multiflora*	Shrubby	Lamberts bay
WC23.1c	EU514526.1	*Mesorhizobium* sp. SCAU9	100	*L. multiflora*	Shrubby	Lamberts bay
WC23.1d	EU514526.1	*Mesorhizobium* sp. SCAU9	100	*L. multiflora*	Shrubby	Lamberts bay
WC28.1f	EU514526.1	*Mesorhizobium* sp. SCAU13	99	*L. sericea*	Shrubby	Bitterfontein
WC28.1g	EF611375.1	*Mesorhizobium* sp. N46	100	*L. sericea*	Shrubby	Bitterfontein
WC28.1h	EU514526.1	*Mesorhizobium* sp. SCAU9	100	*L. sericea*	Shrubby	Bitterfontein
WC28.1i	EU514526.1	*Mesorhizobium* sp. SCAU13	99	*L. sericea*	Shrubby	Bitterfontein
WC28.1j	EU514526.1	*Mesorhizobium* sp. SCAU9	100	*L. sericea*	Shrubby	Bitterfontein
WC33b	EF611375.1	*Mesorhizobium* sp. N46	100	*L. sericea*	Shrubby	Bitterfontein
WC33c	EF611375.1	*Mesorhizobium* sp. N46	100	*L. sericea*	Shrubby	Bitterfontein
WC33e	EU514526.1	*Mesorhizobium* sp. SCAU9	100	*L. sericea*	Shrubby	Bitterfontein
WC33h	EF611375.1	*Mesorhizobium* sp. N46	99	*L. sericea*	Shrubby	Bitterfontein
WC5.4c	AY178072.1	*Burkholderia* sp. CI 3	100	*L. ambigua*	Suffrutescent	Modder R.
WC5.4d	AJ971352.1	*Burkholderia* sp. C1	100	*L. ambigua*	Suffrutescent	Modder R.
WC5.4e	AJ971352.1	*Burkholderia* sp. C1	100	*L. ambigua*	Suffrutescent	Modder R.
WC12.1a	EU514526.1	*Mesorhizobium* sp. SCAU9	100	*L. sepiaria*	Suffrutescent	Citrusdal
WC21.1b	AY691395.1	*Burkholderia* sp. hpud12.1	100	*L. pauciflora*	Suffrutescent	Cedarberg
WC21.1i	AY691395.1	*Burkholderia* sp. hpud12.1	100	*L. pauciflora*	Suffrutescent	Cedarberg
WC21.1j	AY691395.1	*Burkholderia* sp. hpud12.1	99	*L. pauciflora*	Suffrutescent	Cedarberg
WC21.1l	EU170551.1	*B. elkanii* CCBAU 15609	99	*L. pauciflora*	Suffrutescent	Cedarberg
WC26.1d	AY539823.1	*Burkholderia* sp. 59-VN4-1W	99	*L. meyeriana*	Suffrutescent	Clanwilliam
WC26.1e	AY539823.1	*Burkholderia* sp. 59-VN4-1W	99	*L. meyeriana*	Suffrutescent	Clanwilliam
WC26.1f	AY539823.1	*Burkholderia* sp. 59-VN4-1W	99	*L. meyeriana*	Suffrutescent	Clanwilliam
XHR1a	AF408947.1	*Bradyrhizobium* sp. Ellin 105	100	*L. simsiana*	Suffrutescent	Stellenbosch

3.8 FIGURES

See following pages.

RPO1+CRL7
RPO1_CRL7

% similarity

30 40 50 60 70 80 90 100

	RPO1	CRL7	Isolate number	Growth Rate	Host Species	Host Section	Geographic origin
			WC19.1b	M	*L. cytisoides*	*Calobota*	Citrusdal
			WC19.1c	M	*L. cytisoides*	*Calobota*	Citrusdal
			LMG 6133T	F	*Medicago* sp.	N/a	N/a
			LMG11892T	M	*Neptunia* sp.	N/a	N/a
			WC33e	M	*L. sericea*	*Calobota*	Bitterfontein
			NK22	M	*L. sericea*	*Calobota*	Kamiesberg
			WC28.1b	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1h	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1g	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1c	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1e	M	*L. sericea*	*Calobota*	Bitterfontein
C1			WC28.1f	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1a	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1i	M	*L. sericea*	*Calobota*	Bitterfontein
			WC28.1j	M	*L. sericea*	*Calobota*	Bitterfontein
			WC33d	M	*L. sericea*	*Calobota*	Bitterfontein
			WC33f	M	*L. sericea*	*Calobota*	Bitterfontein
			WC33a	M	*L. sericea*	*Calobota*	Bitterfontein
			WC33c	M	*L. sericea*	*Calobota*	Bitterfontein
			WC33g	M	*L. sericea*	*Calobota*	*Bitterfontein*
			WC33h	M	*L. sericea*	*Calobota*	Bitterfontein
			WC5.4c	F	*L. ambigua*	*Lebeckia*	Modder river
C2			LMG6138T	S	*Glycine max*	N/a	N/a
			WC26.1e1	F	*L. meyerianna*	*Lebeckia*	Clanwilliam
			WC26.1e	F	*L. meyerianna*	*Lebeckia*	Clanwilliam
			WC5.4a	F	*L. ambigua*	*Lebeckia*	Modder river
			WC5.4b	F	*L. ambigua*	*Lebeckia*	Modder river
			WC5.4d	F	*L. ambigua*	*Lebeckia*	Modder river
			WC12.1a	S	*L. sepiaria*	*Lebeckia*	Citrusdal
			WC23.1c	M	*L. multiflora*	*Calobota*	Lamberts bay
C3			WC26.1c	F	*L. meyerianna*	*Lebeckia*	Clanwilliam
			WC26.1d	F	*L. meyerianna*	*Lebeckia*	Clanwilliam
			WC26.1f	F	*L. meyerianna*	*Lebeckia*	Clanwilliam
			WC5.4f	F	*L. ambigua*	*Lebeckia*	Modder river
			WC5.4i	F	*L. ambigua*	*Lebeckia*	Modder river
			WC5.4g	F	*L. ambigua*	*Lebeckia*	Modder river
			WC5.4h	F	*L. ambigua*	*Lebeckia*	Modder river
			WC5.4e	F	*L. ambigua*	*Lebeckia*	Modder river
C4			WC23.1a	M	*L. multiflora*	*Calobota*	Lamberts bay
			WC23.1b	M	*L. multiflora*	*Calobota*	Lamberts bay
			LMG 9503T	F	*Leucaena* sp.	N/a	N/a
C5			WC23.1d	M	*L. multiflora*	*Calobota*	Lamberts bay
C6			XHR1a	Vs	*L. simsiana*	*Lebeckia*	Stellenbosch
			XHR1b	Vs	*L. simsiana*	*Lebeckia*	Stellenbosch
			LMG6134T	S	*Glycine max*	N/a	N/a
			LMG17827T	F	*Phaseolus vulgaris*	N/a	N/a
			LMG 8820T	F	*Trifolium* sp.	N/a	N/a
			B/W1iii	F	*L. spinescens*	*Calobota*	Beaufort West
			BW3LSd	F	*L. spinescens*	*Calobota*	Beaufort West
			BH2LSd	F	*L. spinescens*	*Calobota*	Beaufort West
			BH3LSa	F	*L. spinescens*	*Calobota*	Beaufort West
			BH3LSd	F	*L. spinescens*	*Calobota*	Beaufort West
			BH1LSb	F	*L. spinescens*	*Calobota*	Beaufort West
C7			BW2LSb	F	*L. spinescens*	*Calobota*	Beaufort West
			BH3LSc	F	*L. spinescens*	*Calobota*	Beaufort West
			BW3LSc	F	*L. spinescens*	*Calobota*	Beaufort West
			BW1LSab	F	*L. spinescens*	*Calobota*	Beaufort West
			BW2LSa	F	*L. spinescens*	*Calobota*	Beaufort West
			BH2LSc	F	*L. spinescens*	*Calobota*	Beaufort West
			BHLSb	F	*L. spinescens*	*Calobota*	Beaufort West
			BH2LSa	F	*L. spinescens*	*Calobota*	Beaufort West
			BH1LSa	F	*L. spinescens*	*Calobota*	Beaufort West
			BH1LSc	F	*L. spinescens*	*Calobota*	Beaufort West
C8			Betal106f	Vs	*L. pungens*	*Stiza*	Meiringspoort
			WC 21.1b	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1h	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1a	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1c	F	*L. pauciflora*	*Lebeckia*	Cedarberg
C9			WC 21.1e	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1f	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1d	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1j	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1g	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1i	F	*L. pauciflora*	*Lebeckia*	Cedarberg
			Betal106a	Vs	*L. pungens*	*Stiza*	Meiringspoort
			Betal106g	Vs	*L. pungens*	*Stiza*	Meiringspoort
			WC 21.1k	S	*L. pauciflora*	*Lebeckia*	Cedarberg
			WC 21.1l	S	*L. pauciflora*	*Lebeckia*	Cedarberg
C10			WC33b1	M	*L. sericea*	*Calobota*	Bitterfontein
			Betal106b	Vs	*L. pungens*	*Stiza*	Meiringspoort
			Betal106d	Vs	*L. pungens*	*Stiza*	Meiringspoort
			Betal106e	Vs	*L. pungens*	*Stiza*	Meiringspoort
			Betal106c	Vs	*L. pungens*	*Stiza*	Meiringspoort

Figure 3.1. UPGMA dendogram derived from combined RPO1 and CRL7 PCR-fingerprints of *Lebeckia* isolates and type strains of *Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium* species. The type strains are indicated with **T.** Symbols C1-C10 indicate major clusters and scale shows percentage similarity. The strains selected for sequencing are highlighted in red. The isolate number, growth rate, host species, host sections and geographic origin are indicated next to each DNA profile. LMG6133T represents *S. meliloti*, LMG11892 represents *M. plurifarium*, LMG6128 *B. japonicum*, LMG9503 *R. tropici*, LMG6134 *B. elkani*, LMG17827 *R. elti* and LMG8820 represents *R. leguminosarium*.
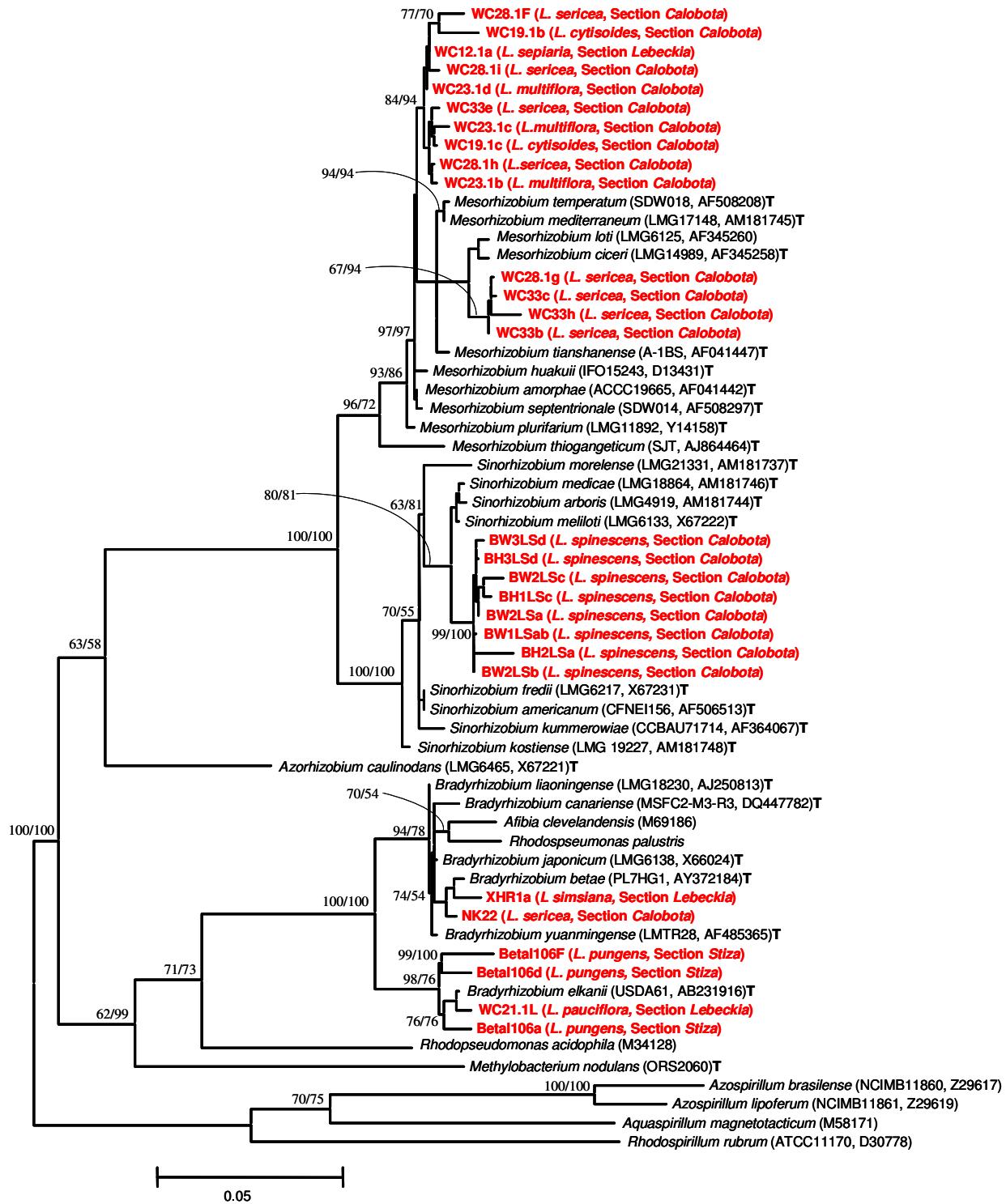
Figure 3.2. A maximum-likelihood (ML) species phylogeny for *Lebeckia* rhizobia, based on 16S rRNA nucleotide sequence data. South African *Lebeckia* rhizobia are indicated in red and bold. The rhizobia type strains are indicated with a **T**. The tree was rooted with *Azospirillum lipoferum* and *Azospirillum brasilense.*
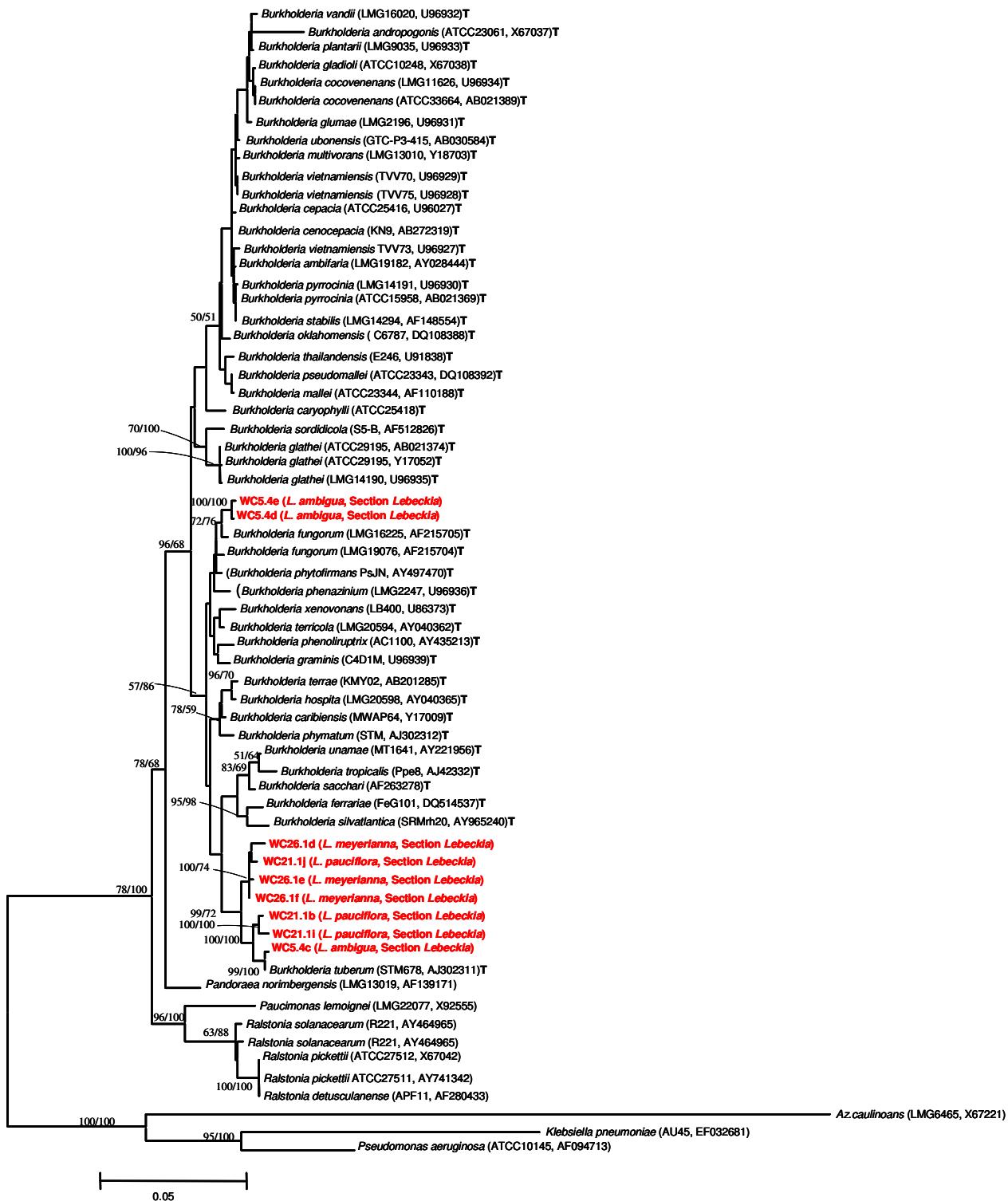
Figure 3.3. A maximum-likelihood (ML) species phylogeny for *Burkholderia* from *Lebeckia,* based on 16S rRNA nucleotide sequence data. South African *Lebeckia Burkholderia* are indicated in red and bold. The *Burkholderia* type strains are indicated with a **T**. The tree was rooted with *Ralstonia solanacearum and Paucimonas lemoignei.*

CHAPTER 4
CONCLUDING REMARKS

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CHAPTER 4

CONCLUDING REMARKS

Over the past decade or so there has been a world-wide surge in scientific and commercial interest in native legumes as potential crop plants with novel agricultural or industrial applications (Doyle, 1998; Doyle & Luckow, 2003; Graham & Vance, 2003; Sprent, 2007). The additional ecological benefits provided by many of these plants are the result of symbiotic nitrogen fixation by their associated root-nodule bacteria. Considerable effort has consequently been applied to the study of these important and often novel microsymbionts (Moulin *et al.,* 2001; Sy *et al.,* 2001; Rivas *et al.,* 2003; Perin *et al.*, 2006). In South Africa, with its rich heritage of indigenous legumes (Germishuizen & Meyer, 2003; Elliot *et al.,* 2007; Le Roux & van Wyk 2007; Boatwright *et al.*, 2007), the rooibos tea and honey bush tea legumes have already shown economic potential. Rooibos tea (*Aspalathus linearis*) and honey bush tea (*Cyclopia*) are now regarded as amongst the healthiest natural herbal teas, without additives, preservatives, colourants and caffeine free. *Lebeckia* spp. also have potential in veld reclamation and as forage legumes and ornamental plants, thus further studies on this legume and its association with symbiotic nitrogen-fixing rhizobia and their respective properties and taxonomic groupings are essential.

As part of an ongoing survey of South African rhizobia (Dagutat, 1995; Le Roux, 2003; Lindique, 2005), isolates from indigenous species of *Lebeckia* were examined for symbiotic ability using traditional root nodule bacteria technology, screened for genomic relatedness using DNA fingerprinting methods and their putative taxonomic positions determined using 16S rRNA gene sequencing (Laguerre *et al.*, 1994; Richardson *et al.*, 1995; Thies *et al.*, 2001).

The diverse nature of the rhizobia isolated from *Lebeckia* was clearly illustrated by the different rhizobial genera that were isolated, sometimes from the same plant species. DNA profiles showed that most of the isolates grouped according to host plant species rather than geographical location. The majority of the isolates were found to be classical genera of rhizobia belonging to the *α-Proteobacteria* (*Mesorhizobium, Sinorhizobium* and *Bradyrhizobium*) with a few isolates showing affinity to the *β*-proteobacterial genus *Burkholderia*. This species diversity appears to be related in both symbiotic and taxonomic properties to the diversity found amongst the 33-35

species of *Lebeckia*, a genus currently undergoing taxonomic revision (Le Roux & van Wyk, 2007; Boatwright *et al.,* 2007). *Lebeckia* may be divided into shrubby trifoliate leaf species and suffrutescent needle leaf species. Interestingly, the results of my cross-inoculation studies indicated that rhizobia isolated from one division rarely nodulated legumes of the other division and any nodules formed were ineffective, i.e. cannot fix nitrogen. In contrast, cross-inoculation between symbionts from within either division always resulted in nodulation. Such nodulation was effective when cross-inoculation was between species within the same section of a division, eg. *Calobota* plants, and ineffective when between different sections of that division, eg. *Stiza* and *Calobota*. Furthermore, species in the shrubby division yielded only *α-Proteobacteria* whereas suffrutescent species yielded predominantly *β-Proteobacteria*, *Burkholderia* being isolated only from species in the latter suffrutescent division. These results of consistent with current plant taxonomic studies elsewhere that will soon assign species in the shrubby division to a new genus separate from those in the suffrutescent division that will remain in the genus *Lebeckia* (Boatwright & van Wyk, personal communication).

Further investigation of isolates from the 10 *Lebeckia* species so far examined, well as new isolates from other species may reveal further insights into the taxonomy of the two legume divisions and their rhizobia. In addition, the phylogenetic groupings of rhizobia emerging from the present study suggest that new species of rhizobia specific for *Lebeckia* may exist. Further study of these using advanced taxonomic techniques such as DNA-DNA hybridization will be required to determine their exact identity.

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SUMMARY

The genus *Lebeckia* Thunb. (Family *Leguminosae*, subfamily *Papilionoideae*, tribe *Crotalarieae*) comprises some 33-35 plant species. In this study, the diversity of root-nodule bacteria (rhizobia) associated with *Lebeckia* species in South Africa was investigated. *Lebeckia* specimens were collected from nine localities in the western and southern Cape. Seventy nine isolates obtained from the root nodules of ten *Lebeckia* species were purified. The strains were confirmed as rhizobia by their ability to nodulate their host plants as well as the promiscuous legumes cowpea and siratro. All the isolates formed nodules and effectively fixed nitrogen on their respective *Lebeckia* hosts, whereas 56% of the strains were effective on cowpea and 77% on siratro. The isolates were further characterized using SP-PCR DNA fingerprinting followed by partial 16S rRNA gene sequencing of 39 selected isolates to confirm their taxonomic identity. DNA-fingerprinting profiles showed that most of the isolates grouped according to host plant species rather than geographical location. Based on 16S rRNA phylogenetic analysis, the isolates from *Lebeckia* comprised a wide diversity of nitrogen-fixing bacteria from both the *α*- and *β-Proteobacteria*. Amongst the *α-Proteobacteria* fast-growing isolates were all assigned to the genus *Sinorhizobium*, intermediate-growers were assigned to the genus *Mesorhizobium* and very slow-growers to the genus *Bradyrhizobium*. The *α*-proteobacterial rhizobia all nodulated shrubby trifoliate-leaf *Lebeckia* spp. In contrast, *β*-proteobacterial rhizobia related to the genus *Burkholderia* only nodulated suffrutescent unifoliate needle-leaf *Lebeckia* spp. Cross-inoculation studies suggest that nodulation is restricted to combinations of rhizobia and *Lebeckia* from either of the two *Lebeckia* plant types, but not across these groups. This finding has relevance to a current taxonomic revision of *Lebeckia* being undertaken elsewhere.

APPENDICES

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APPENDIX A: MEDIA, BUFFER AND REAGENTS

Nitrogen free nutrient solution (Hoagland's solution)

Hoagland's solution was prepared by dissolving the following ingredients in 20L sterile distilled water (SDW) and adjusted with KOH to pH 6.6-6.8.

KCl	100g
$Ca_3 (PO4)_2$	100g
$CaSO_4.2H_2O$	100g
$MgSO_4.7H_2O$	156g
Nafe solution	400ml
Trace element	40ml

Nafe solution consists of:

N_2EDTA	15.6g
$FeSO_4.7H_2O$	15g

Dissolve in 600ml and oxygenated by vigorous shaking until colour was dark brown. The volume was then made up to1500ml.

Trace element solution ingredients:

LiCl	0.2g
$CuSO_4.5H_2O$	1g
$ZnSO_4.7H_2O$	1g
TiO_2	1g
H_3BO_3	1g
$AL_2(SO4)_3$	1g
$SnCl._2.2H_2O$	0.5g
$MnCl_2.4H_2O$	7g
KI	0.5g
$NiSO_4.7H_2O$	1g
$Co (NO_3)_2.6H_2O$	1g
KBr	0.5g
$Na_2MoO_4.2H_2O$	5.4g

Dissolved in 1800ml distilled water and volume adjusted to 2000ml.

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Paraffin sand

15g paraffin wax

Dissolved in 1L benzene

Mixed with 10kg sifted sand and sterilized in a drying oven at 160°C for 2 hrs.

Yeast Mannitol Congo red (YM-CR) (Vincent 1970)

Mannitol	10g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2g
Yeast extracts	0.4g
NaCl	0.1g
4% (m/v) Congo red	10ml
Agar	15g

Yeast Mannitol Agar slants

Per 1000ml in distilled water:

Mannitol	10g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2g
Yeast extract	0.4g
NaCl	0.1g
$CaCO_3$	1g
Agar	15g

Water agar

Per 1000ml in distilled water

Agar	15g

Yeast Mannitol Broth

Per 1000ml of distilled water

Mannitol	10g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2g
Yeast extract	0.4g
NaCl	0.1g

Tryptone

Per 1000ml distilled water

Tryptone	5g
Yeast extract	3g

Wash buffer

NaCl (0.5M)	2.422g
Tris (100mM)	1.169g
EDTA (20mM)	1.168g

Dissolved in 200ml SDW, pH 8.0 adjusted with HCl.

Extraction buffer

Per 500ml SDW, pH 8.0 adjusted with HCl.

Tris (150mM)	9.084g
EDTA (40mM)	5.845g
NaCl (2M)	58.44g

Sodium Chloride-Tris-EDTA (STET) buffer

Per 200ml SDW, pH 8.0 adjusted with HCl.

NaCl (0.1M)	1.168g
Tris (50mM)	1.211g
EDTA (10mM)	0.584g
Triton X-100 (0.1%, v/v)	200µl

3% KOH solution

KOH 3g

SDW 100ml

44% (m/v) CaCl$_2$.2H2O

CaCl$_2$.2H2O   4.4g

Distilled water   10ml

Tris wash buffer

Per 200ml SDW, pH 8.0 adjusted with HCl

M Tris (100mM)   1.169g

NaCl (0.5 M)   2.422g

EDTA (20 mM)   1.168g

Extraction buffer

Per 500ml SDW, pH 8.0 adjusted with HCl

Tris (150mM)   9.084g

NaCl (2M)   58.44g

EDTA (40mM)   5.845g

10X TBE buffer

Tris (1M)   24.2g

EDTA (10mM)   0.584g

Borate (0.83M)   10.27g

SDW   200ml

1X TBE buffer

10X TBE buffer stock   200ml

SDW   1800ml

Agarose gel

For 1.5% and 2% agarose gels, mix 0.825g and 1.1g agarose, respectively with 55ml SDW

Chloroform: Iso-amylalcohol (24:1)

Chloroform	240ml
Iso-amylalcohol	10ml

5M NaCl

NaCl	58.44g
Distilled water	200ml

20% Glycerol

Per 1000ml solution

Concentrated glycerol	200ml
SDW	800ml

2 M NaOH

NaOH	2g
SDW	100ml

1.5 M KCl

KCl	111.84g
SDW	1000ml

70% ethanol

Per 1000ml solution

Absolute ethanol	700ml
Distilled water	300ml

0.25% Congo red

Congo red	1g
Distilled water	400ml

10% Sodium dodecyl sulphate (SDS)

Sodium lauryl sulphate	4g
SDW	40ml

APPENDIX B: β-RHIZOBIA 16S rRNA alignment

```
B. andropogonis (ATCC23061)T      aagtcgaacg gtaacaggga g--ct--tg- -ctccgctga cgagtggcga
B. fungorum (LMG19076)T           aagtcgaacg gcagcacggg a--gc--aa- -tcctggtgg cgagtggcga
B. caribiensi (MWAP64)T           aagtcggacg gcagcacggg g--gc--aa- -ccctggcgg cgagtggcga
B. caryophylii (ATCC25418)T       aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. cepacia (ATCC25416)T           aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. cocovenenas (ATCC33664)T       aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. cocovenenas (LMG11626)T        cattcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. fungorum (LMG16225)            aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. gladioli (ATCC10248)T          aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. glathei (LMG14190)T            aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. glathei (ATCC29195)T           aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. glathei (ATCC29195)T           aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. glumae (LMG2196)T              aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. graminis (C4D1M)T              aattcgaacg gcagcacggg a--gc--aa- -tcctggtgg cgattggcga
B. hospita (LMG20598)T            aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. mallei (ATCC23344)T            aagtcgaacg gcagcacgg- ---gcttcg- -gcctggtgg cgagtggtga
B. multivorans (LMG13010)T        aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. norimbergensis (Y09879)T       aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. phenazinium (LMG2247)T         -agtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. phenoliruptrix (AC1100)T       aagtcggacg gcagcgcggg c--tt--cg- -gcctggcgg cgagtggcga
B. phymatum (STM815)T             aagtcggacg gcagcgcggg c--tt--cg- -gcctggcgg cgagtggcga
B. phytofirmans (PsJN)T           aagtcgaacg gcagcacggg g--gc--aac -ccctggtgg cgagtggcga
B. pickettii (ATCC27511)T         aagtcgaacg gcagcatgat ctagcttgct agattgatgg cgagtggcga
B. pseudomallei (ATCC23343)T      aagtcgaacg gcagcacgg- ---gcttcg- -gcctggtgg cgagtggcga
B. pyrrocinia (LMG14191)T         ----cgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. pyrrocinia ATCC15958)T         aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. sacchari (AF263278)T           aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. silvatlantica (SRMrh-20)T      aagtcggacg gcagcgcgg- ---gcttcg- -gcctggcgg cgagtggcga
B. solanacearum (ATCC11696)T      aagtcgaacg gcagcggggg t-agcttgct -acctgccgg cgagtggcga
B. sordicola (S5-B)T              aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. stabilis (LMG14294)T           aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. terrae (KMY02)T                aagtcgaacg gcagcgcggg g--gc--aa- -ccctggcgg cgagtggcga
B. terriccola (LMG20594)T         aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. thailandensis (E264)T          aagtcgaacg gcagcgcgg- ---gcttcg- -gcctggcgg cgagtggcga
B. tropicalis (Ppe8)T             aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. tuberum (STM678)T              aagtcgaacg gcagcacgga c--tt--cg- -gtctggtgg cgagtggcga
B. unamae (MT1-641)T              aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. vandii (LMG16020)T             ---------- ---------- ---------- ---------- ----------
B. vietnamiensis (AMMD)T          aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. vietnamiensis (TVV75)T         aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. vietnamiensis (TVV70)T         aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. plantarii (LMG9035)T           aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. xenovorans (LB400)T            aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
B. cenocepacia (KN9)T             ---------- ---------- ---------- ---------- ----------
B. ferrariae (FeG101)T            aagtcggacg gcagcgcggg a--gc--aa- -tcctggcgg cgagtggcga
B. ambifaria (LMG19182)T          aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. oklahomensis (C6786)T          aagtcgaacg gcagcacggg t--gcttgc- -acctggtgg cgagtggcga
B. ubonensis (GTC-P3-415)T        aagtcgaacg gcagcacggg t--gcttgc- -a-ctggtgg cgagtggcga
WC5.4c L. ambigua SA              cagtcgaacg gcagcacgga c--tt--cg- -gtctggtgg cgagtggcga
WC5.4d L. ambigua SA              aagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
WC5.4e L. ambigua SA              cagtcgaacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
WC21.1i L. pauciflora SA          aagtcggacg gcagcacgga c--tt--cg- -gtctggtgg cgagtggcg-
WC21.1b L. pauciflora SA          aagtcggacg gcagcacgga c--tt--cg- -gtctggtgg cgagtggcg-
WC21.1j L. pauciflora SA          aagtcggacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
WC26.1d L. meyerianna SA          aagtcggacg gcagcacggg g--gc--aa- -ccctggtgg cgagtg--gg
WC26.1e L. meyerianna SA          aagtcggacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
WC26.1f L. meyerianna SA          cagtcggacg gcagcacggg g--gc--aa- -ccctggtgg cgagtggcga
P. lemoignei (LMG2207)            aagtcgaacg gcagcacggg t--rcttgc- -acctggtgg cgagtggcga
R. detusculanense (APF11)         aagtcgaacg gcagcatgat ctagcttgct agattgatgg cgagtggcga
R. solanacearum (R221)            aagtcgaacg gcagcggggg t-agcttgc- -tacctccgg cgagtggcga
R. pickettii (ATCC27512)          aagtcgaacg gcagcatgat ctagcttgct agattgatgg cgagtggcga
Az. caulinodans (LMG6465)         aagtcgagcg cc------- ---------- --cagcaatg ggagcggcag
P. aeruginosa (ATCC10145)         aagtcgagcg gatgaaggga ---gcttgc- --tcctggat tcagcggcag
K. pneumoniae (AU45)              aagtcgagcg gtagcacaga ga-gcttgct -ctcgggtga cgagcggcgg


                                           60         70         80         90        100
B. andropogonis (ATCC23061)T      acgggtgagt aatacatcgg aacgtgtcct gtagtggggg atag-cccgg
B. fungorum (LMG19076)T           acgggtgagt aatacatcgg aacgtgtcct gtagtggggg atag-cccgg
B. caribiensi (MWAP64)T           acgggtgagt aatacatcgg aacgtgtcct ggagtggggg atag--ccgg
B. caryophylii (ATCC25418)T       acgggtgagt aatacatcgg aacgtgtcct ggagtggggg atag-cccgg
B. cepacia (ATCC25416)T           acgggtgagt aatacatcgg aacatgtcct gtagtggggg atag-cccgg
B. cocovenenas (ATCC33664)T       acgggtgagt aatacatcgg aacatgtcct gtagtggggg atag-cccgg
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B. cocovenenas (LMG11626)T        acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. fungorum (LMG16225)            acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. gladioli (ATCC10248)T          acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. glathei (LMG14190)T            acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. glathei (ATCC29195)T           acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag--ccgg
B. glathei (ATCC29195)T           acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atagcccgg
B. glumae (LMG2196)T              acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. graminis (C4D1M)T              acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. hospita (LMG20598)T            acgggtgagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
B. mallei (ATCC23344)T            acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. multivorans (LMG13010)T        acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. norimbergensis (Y09879)T       acgggtgagt aatacatcgg aatgtacctt gtagtgggg atag-ctcgg
B. phenazinium (LMG2247)T         acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. phenoliruptrix (AC1100)T       acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. phymatum (STM815)T             acgggtgagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
B. phytofirmans (PsJN)T           acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. pickettii (ATCC27511)T         acgggtgagt aatacatcgg aacgtgccct gtagtgggg ataa-ctagt
B. pseudomallei (ATCC23343)T      acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. pyrrocinia (LMG14191)T         acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. pyrrocinia ATCC15958)T         acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. sacchari (AF263278)T           acgggtgagt aatacatcgg aacatgtcca gtagtgggg atag-cccgg
B. silvatlantica (SRMrh-20)T      acgggtgagt aatacatcgg aacatgtcca gtagtgggg atag-cccgg
B. solanacearum (ATCC11696)T      acgggtgagt aatacatcgg aacgtgccct gtagtgggg ataa-ctagt
B. sordicola (S5-B)T              acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. stabilis (LMG14294)T           acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. terrae (KMY02)T                acgggtgagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
B. terricola (LMG20594)T          acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. thailandensis (E264)T          acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. tropicalis (Ppe8)T             acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. tuberum (STM678)T              acgggtgagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
B. unamae (MT1-641)T              acgggtgagt aatacatcgg aacatgtcca gtagtgggg atag-cccgg
B. vandii (LMG16020)T             ------gatt aatacatcgg aacatgtcct gtactgggg atag-cccgg
B. vietnamiensis (AMMD)T          acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. vietnamiensis (TVV75)T         acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. vietnamiensis (TVV70)T         acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. plantarii (LMG9035)T           acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. xenovorans (LB400)T            acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
B. cenocepacia (KN9)T             -----tgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. ferrariae (FeG101)T            acgggtgagt aatacatcgg aacatgtcca gtagtgggg atag-cccgg
B. ambifaria (LMG19182)T          acgggtgagt aatacatcgg aacatgtcca gtagtgggg atag-cccgg
B. oklahomensis (C6786)T          acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
B. ubonensis (GTC-P3-415)T        acgggtgagt aatacatcgg aacatgtcct gtagtgggg atag-cccgg
WC5.4c L. ambigua SA              a-gttggagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
WC5.4d L. ambigua SA              acgggtgagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
WC5.4e L. ambigua SA              acgtgggagt aatacatcgg aacgtgtcct gtagtgggg atag-cccgg
WC21.1i L. pauciflora SA          acgtgtgagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
WC21.1b L. pauciflora SA          acgggagagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
WC21.1j L. pauciflora SA          cc--gttgag tatacatcgg aacgtgtcct ggagtgggg actg-gccgg
WC26.1d L. meyerianna SA          ccgtttaagt cctacatcgg aacgtgtcct ggagtgggg acg--tccgg
WC26.1e L. meyerianna SA          accggtta-- tatacatcgg aacgtgtcct ggagtgggg atag-ccttg
WC26.1f L. meyerianna SA          acgggttagt aatacatcgg aacgtgtcct ggagtgggg atag-cccgg
P. lemoignei (LMG2207)            acgggtgagt aatatatcgg aacgtgccca atagtgggg ataa-cgtag
R. detusculanense (APF11)         acgggtgagt aatacatcgg aacgtgccct gtagtgggg ataa-ctagt
R. solanacearum (R221)            acgggtgagt aatacatcgg aacgtgccct gtagtgggg ataa--ctat
R. pickettii (ATCC27512)          acgggtgagt aatacatcgg aacgtgccct gtagtgggg ataa-ctagt
Az. caulinodans (LMG6465)         acgggtgagt aacgcgtggg gatgtgccca atggtgcgga ataa-cccag
P. aeruginosa (ATCC10145)         acgggtgagt aatgcctagg aatctgcctg gtagtgggg ataa-cgtcc
K. pneumoniae (AU45)              acgggtgagt aatgtctggg aaactgcctg atggagggg ataa-ctact


                                         110        120        130        140        150
B. andropogonis (ATCC23061)T      cgaaagc--- attaataccg catacgatct aaggatgaaa gcgggggatc
B. fungorum (LMG19076)T           cgaaagccgg attaataccg catacgctct gcggaggaaa gcgggggatc
B. caribiensi (MWAP64)T           cgaaagccgg attaataccg catacgctct gtggaggaaa gcgggggatc
B. caryophylii (ATCC25418)T       cgaaagc--- attaataccg catacgatcg ggagaggaaa gcgggggacc
B. cepacia (ATCC25416)T           cgaaagccgg attaataccg catacgatcc acggatgaaa gcgggggacc
B. cocovenenas (ATCC33664)T       cgaaagccgg attaataccg catacgatcc acggatgaaa gcgggggacc
B. cocovenenas (LMG11626)T        cgaaagccgg attaataccg catacgatcc acggatgaaa gcgggggacc
B. fungorum (LMG16225)            cgaaagccgg attaataccg catacgctct acggaggaaa gggggggatc
B. gladioli (ATCC10248)T          cgaaagc--- attaataccg catacgatct acggatgaaa gcgggggacc
B. glathei (LMG14190)T            cgaaagccgg attaataccg catacgatct acggaagaaa gcgggggatc
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B. glumae (LMG2196)T              cgaaagccgg attaataccg catacgatct acggaagaaa gcgggggacc
B. graminis (C4D1M)T              cgaaagccgg attaataccg catacgctct gcggagaaaa gcgggggatc
B. hospita (LMG20598)T            cgaaagccgg attaataccg catacgatct ctggatgaaa gcgggggacc
B. mallei (ATCC23344)T            cgaaagccgg attaataccg catacgatct gaggatgaaa gcgggggacc
B. multivorans (LMG13010)T        cgaaagccgg attaataccg catacgatct acggatgaaa gcgggggacc
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B. norimbergensis (Y09879)T        cgaaagccgg attaataccg catacgctct gaggaggaaa gcggggggacc
B. phenazinium (LMG2247)T          cgaaagccgg attaataccg catacgctct acggaggaaa gggggggatc
B. phenoliruptrix (AC1100)T        cgaaagccgg attaataccg catacgctct gcggaggaaa gcggggggatc
B. phymatum (STM815)T              cgaaagccgg attaataccg catacgctct gaggaggaaa gcggggggatc
B. phytofirmans (PsJN)T            cgaaagccgg attaataccg catacgatct gtggatgaaa gcggggggatc
B. pickettii (ATCC27511)T          cgaaagatta gctaataccg catacgacct gaggtgaaa gtggggggacc
B. pseudomallei (ATCC23343)T       cgaaagccgg attaataccg catacgatct raggatgaaa gcggggggacc
B. pyrrocinia (LMG14191)T          cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggacc
B. pyrrocinia ATCC15958)T          cgaaagc--- -ttaataccg catacgatct acggatgaaa gcgggggacc
B. sacchari (AF263278)T            cgaaagccgg attaataccg catacgatct ttggatgaaa gcggggggacc
B. silvatlantica (SRMrh-20)T       cgaaagccgg attaataccg catacgctct gtggaggaaa gcggggggatc
B. solanacearum (ATCC11696)T       cgaaagactg gctaataccg catacgacct gaggtgaaa gtggggggacc
B. sordicola (S5-B)T               cgaaagccgg attaataccg catacgatct acggaagaaa gcggggggacc
B. stabilis (LMG14294)T            cgaaagccgg attaataccg catacgatct ayggatgaaa gcggggggacc
B. terrae (KMY02)T                 cgaaagccgg attaataccg catacgatct tgggatgaaa gcggggggacc
B. terriccola (LMG20594)T          cgaaagccgg attaataccg catacgctct gcggagaaa gcgggggctc
B. thailandensis (E264)T           cgaaagccgg attaataccg catacgatct gtggatgaaa gcggggggacc
B. tropicalis (Ppe8)T              cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggatc
B. tuberum (STM678)T               cgaaagccgg attaataccg catacgctcg ggagaggaaa gcggggggatc
B. unamae (MT1-641)T               cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggacc
B. vandii (LMG16020)T              cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggatc
B. vietnamiensis (AMMD)T           cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggacc
B. vietnamiensis (TVV75)T          cgaaagccgg attaataccg catacgatct atggatgaaa gcggggggacc
B. vietnamiensis (TVV70)T          cgaaagccgg attaataccg catacgatct atggatgaaa gcggggggacc
B. plantarii (LMG9035)T            cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggatc
B. xenovorans (LB400)T             cgaaagccgg attaataccg catacgctct gcggaggaaa gcggggggacc
B. cenocepacia (KN9)T              cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggacc
B. ferrariae (FeG101)T             cgaaagccgg attaataccg catacgatct atggatgaaa gcggggggatc
B. ambifaria (LMG19182)T           cgaaagccgg attaataccg catacgatct acggatgaaa gcggggggacc
B. oklahomensis (C6786)T           cgaaagccgg attaataccg catacgatct gaggatgaaa gcggggggacc
B. ubonensis (GTC-P3-415)T         cgaaagc--- attaataccg catacgatct acggatgaaa gcggggggacc
WC5.4c L. ambigua SA               cgaaagccgg attaataccg catacgctcg ggagaggaaa gcggggggatc
WC5.4d L. ambigua SA               cgaaagccgg attaataccg catacgctct gcggaggaaa gcggggggatc
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WC21.1i L. pauciflora SA           cgaaagccgg attaataccg catacgctct gaggaggaaa gcggggggatc
WC21.1b L. pauciflora SA           cgaaagccgg attaataccg catacgctct gaggaggaaa gcggggggatc
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WC26.1d L. meyerianna SA           cgaaagccgg attaataccg catacgctct gaagaggaaa gcggggggatc
WC26.1e L. meyerianna SA           cgaaagccgg attaataccg catacgctct gaagaggaaa gcggggggatc
WC26.1f L. meyerianna SA           cgaaagccgg attaataccg catacgctct gaagaggaaa gcggggggatc
P. lemoignei (LMG2207)             cgaaagttac gctaataccg catacgctct acggaggaaa gtggggggatc
R. detusculanense (APF11)          cgaaagatta gctaataccg catacgacct gaggtgaaa gtggggggacc
R. solanacearum (R221)             cgaaagactg gctaataccg catacgacct gaggtgaaa gtggggggacc
R. pickettii (ATCC27512)           cgaaagatta gctaataccg catacgacct gaggtgaaa gtggggggacc
Az. caulinodans (LMG6465)          ggaaacttgg attaataccg catgtgccct tcgggggaaa ----------
P. aeruginosa (ATCC10145)          ggaaacgggc gctaataccg catacgtcct gagggagaaa gtggggggatc
K. pneumoniae (AU45)               ggaaacggta gctaataccg cataatgtcg caagaccaaa gtggggggacc


                                   160        170        180        190        200
B. andropogonis (ATCC23061)T       --ttcggacc tcgcgctata ggg-gcggcc gatggcagat tagctagttg
B. fungorum (LMG19076)T            cttcgggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
B. caribiensi (MWAP64)T            --ttcgggcc tcgcgctcaa ggg-gcggcc gatggcagat tagctagttg
B. caryophylii (ATCC25418)T        --ttcgggcc tcgcgctcaa ggg-gcggcc gatggcggat tagctagttg
B. cepacia (ATCC25416)T            --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. cocovenenas (ATCC33664)T        --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. cocovenenas (LMG11626)T         --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. fungorum (LMG16225)             --ttaggacc tcccgctaca ggg-gcggcc gatggcagat tagctagttg
B. gladioli (ATCC10248)T           --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. glathei (LMG14190)T             --ttcggacc tcgcgctata ggg-gcggcc gatggcagat tagctagttg
B. glathei (ATCC29195)T            --ttcggacc tcgcgctata ggg-gcggcc gatggcagat tagctagttg
B. glathei (ATCC29195)T            --ttcggacc tcgcgc---- ggg-gcggcc gatggcagat tagctagttg
B. glumae (LMG2196)T               --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. graminis (C4D1M)T               cttcgggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
B. hospita (LMG20598)T             --gaaaggcc tcgcgctcaa ggg-gcggcc gatggcagat tagctagttg
B. mallei (ATCC23344)T             --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. multivorans (LMG13010)T         --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. norimbergensis (Y09879)T        --ttcgggcc tcgtgctaca aga-gcagcc gatgtcagat tagcttgttg
B. phenazinium (LMG2247)T          --ttaggacc tctcgctaca ggg-gcggcc gatggcagat tagctagttg
B. phenoliruptrix (AC1100)T        cttcgggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
B. phymatum (STM815)T              --ttcgggcc tcgcgctcaa ggg-gcggcc gatggcagat tagctagttg
B. phytofirmans (PsJN)T            --ttaggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
B. pickettii (ATCC27511)T          --gcaaggcc tcatgctata gga-gcggcc gatgtctgat tagctagttg
B. pseudomallei (ATCC23343)T       --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. pyrrocinia (LMG14191)T          --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. pyrrocinia ATCC15958)T          --ttcgggcc tcgcgcta-a ggg-ttggcc gatggctgat tagctagttg
B. sacchari (AF263278)T            --ttcgggcc tcgcgctatt ggg-gtggcc gatggcggat taggtagttg
B. silvatlantica (SRMrh-20)T       --gaaagacc tcgcgctatt ggg-gtggcc gatggcggat tagctagatg
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B. solanacearum (ATCC11696)T    --gcaaggcc tcatgctata gga-gcggcc gatgtctgat tagctagttg
B. sordicola (S5-B)T            --ttagggcc tcgcgctata ggg-gcggcc gatggcagat tagctagttg
B. stabilis (LMG14294)T         --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. terrae (KMY02)T              --gaaaggcc tcgcgctcaa ggg-gcggcc gatggcagat tagctagttg
B. terriccola (LMG20594)T       cttcgggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
B. thailandensis (E264)T        --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. tropicalis (Ppe8)T           --ttcgggcc tcgcgctata ggg-gcggcc gatggcggat tagctagttg
B. tuberum (STM678)T            tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
B. unamae (MT1-641)T            --ttcgggcc tcgcgctatt ggg-gtggcc gatggcggat tagctagttg
B. vandii (LMG16020)T           --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. vietnamiensis (AMMD)T        --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. vietnamiensis (TVV75)T       --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. vietnamiensis (TVV70)T       --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. plantarii (LMG9035)T         --tttggacc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. xenovorans (LB400)T          --ttcgggcc tcgcgctaca ggg-gcggcc gatggcagat tagctggttg
B. cenocepacia (KN9)T           --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. ferrariae (FeG101)T          --gcaagacc tcgcgctatt ggg-gtggcc gatggcggat tagctagttg
B. ambifaria (LMG19182)T        --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. oklahomensis (C6786)T        --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
B. ubonensis (GTC-P3-415)T      --ttcgggcc tcgcgctata ggg-ttggcc gatggctgat tagctagttg
WC5.4c L. ambigua SA            tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
WC5.4d L. ambigua SA            --ttaggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
WC5.4e L. ambigua SA            --ttaggacc tcgcgctaca ggg-gcggcc gatggcagat tagctagttg
WC21.1i L. pauciflora SA        tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
WC21.1b L. pauciflora SA        tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
WC21.1j L. pauciflora SA        tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
WC26.1d L. meyerianna SA        tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
WC26.1e L. meyerianna SA        tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
WC26.1f L. meyerianna SA        tttcgggacc tcgcgctcaa ggg-gcggcc gatggcagat taggtagttg
P. lemoignei (LMG2207)          --gcaagacc tcatgctatt gga-gcggcc gatatctgat tagctagttg
R. detusculanense (APF11)       --gcaagacc tcatgctata gga-gcggcc gatgtctgat tagctagttg
R. solanacearum (R221)          --gcaaggcc tcatgctata gga-gcggcc gatgtctgat tagctagttg
R. pickettii (ATCC27512)        --gcaaggcc tcatgctata gga-gcggcc gatgtctgat tagctagttg
Az. caulinodans (LMG6465)       ------gatt tatcgccatt gga-tcaacc cgcgtctgat tagctagttg
P. aeruginosa (ATCC10145)       --ttcgggcc tcacgctatc aga-tgagcc taggtcggat tagctagttg
K. pneumoniae (AU45)            --ttcgggcc tcatgccatc aga-tgtgcc cagatgggat tagctagtag


                                    210        220        230        240        250
B. andropogonis (ATCC23061)T    gtggggtaaa ggcctaccaa ggcgacgatc cgtagctggt ctgagaggac
B. fungorum (LMG19076)T         gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. caribiensi (MWAP64)T         gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. caryophylii (ATCC25418)T     gtggggtaaa ggcctaccaa ggcgacgatc cgtagctggt ctgagaggac
B. cepacia (ATCC25416)T         gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. cocovenenas (ATCC33664)T     gtggggtaaa ggcccaccaa ggcgacgatc agtagctggt ctgagaggac
B. cocovenenas (LMG11626)T      gtggggtaaa ggcccaccaa ggcgacgatc agtagctggt ctgagaggac
B. fungorum (LMG16225)          gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. gladioli (ATCC10248)T        gtggggtaaa ggcccaccaa ggcgacgatc agtagctggt ctgagaggac
B. glathei (LMG14190)T          gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. glathei (ATCC29195)T         gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. glathei (ATCC29195)T         gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. glumae (LMG2196)T            gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. graminis (C4D1M)T            gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. hospita (LMG20598)T          gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. mallei (ATCC23344)T          gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. multivorans (LMG13010)T      gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. norimbergensis (Y09879)T     gtgaggtaaa agctcaccaa ggcgacgatc tgtagctggt ctgagaggac
B. phenazinium (LMG2247)T       gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. phenoliruptrix (AC1100)T     gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. phymatum (STM815)T           gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. phytofirmans (PsJN)T         gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. pickettii (ATCC27511)T       gtgaggtaaa ggctcaccaa ggcgacgatc agtagctggt ctgagaggac
B. pseudomallei (ATCC23343)T    gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. pyrrocinia (LMG14191)T       gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. pyrrocinia ATCC15958)T       gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. sacchari (AF263278)T         gtggggtaaa ggcctaccaa gccgacgatc cgtagctggt ctgagaggac
B. silvatlantica (SRMrh-20)T    gtggggtaaa ggcctaccat ggcgacgatc cgtagctggt ctgagaggac
B. solanacearum (ATCC11696)T    gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. sordicola (S5-B)T            gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. stabilis (LMG14294)T         gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. terrae (KMY02)T              gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. terriccola (LMG20594)T       gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. thailandensis (E264)T        gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. tropicalis (Ppe8)T           gtgaggtaaa ggctcaccaa ggcgacgatc cgtagctggt ctgagaggac
B. tuberum (STM678)T            gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
B. unamae (MT1-641)T            gtggggtaaa ggctcaccaa ggcgacgatc cgtagctggt ctgagaggac
B. vandii (LMG16020)T           gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. vietnamiensis (AMMD)T        gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
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B. vietnamiensis (TVV75)T        gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. vietnamiensis (TVV70)T        gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. plantarii (LMG9035)T          gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. xenovorans (LB400)T           gtggggtaaa ggcctaccaa ggcgacgatc tgtagctggt ctgagaggac
B. cenocepacia (KN9)T            gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. ferrariae (FeG101)T           gtggggtaaa ggcctaccaa ggcgacgatc cgtagctggt ctgagaggac
B. ambifaria (LMG19182)T         gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. oklahomensis (C6786)T         gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
B. ubonensis (GTC-P3-415)T       gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac
WC5.4c L. ambigua SA             gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
WC5.4d L. ambigua SA             gtggggtaaa ggcctaccaa ggcgacgatc tgtaggtggt ctgagaggac
WC5.4e L. ambigua SA             gtggggtaaa ggcctaccaa ggcgacgatc tgtaggtggt ctgagaggac
WC21.1i L. pauciflora SA         gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
WC21.1b L. pauciflora SA         gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
WC21.1j L. pauciflora SA         gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
WC26.1d L. meyerianna SA         gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
WC26.1e L. meyerianna SA         gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
WC26.1f L. meyerianna SA         gtggggtaaa ggcctaccaa gccgacgatc tgtagctggt ctgagaggac
P. lemoignei (LMG2207)           gtggggtaat ggcccaccaa ggcgacgatc agtagctggt ctgagaggac
R. detusculanense (APF11)        gtgaggtaaa ggctcaccaa ggcgacgatc agtagctggt ctgagaggac
R. solanacearum (R221)           gtggggtaaa ggctcaccaa ggcgacgatc agtagctggt ctgagaggac
R. pickettii (ATCC27512)         gtgaggtaaa ggctcaccaa ggcgacgatc agtagctggt ctgagaggac
Az. caulinodans (LMG6465)        gtgaggtaaa ggctcaccaa ggcgacgatc agtagctggt ctgagaggat
P. aeruginosa (ATCC10145)        gtggggtaaa ggcctaccaa ggcgacgatc cgtaactggt ctgagaggat
K. pneumoniae (AU45)             gtggggtaac ggctcaccta ggcgacgatc cctagctggt ctgagaggat
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                                     260        270        280        290        300
B. andropogonis (ATCC23061)T     gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. fungorum (LMG19076)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. caribiensi (MWAP64)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. caryophylii (ATCC25418)T      gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. cepacia (ATCC25416)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. cocovenenas (ATCC33664)T      gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. cocovenenas (LMG11626)T       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. fungorum (LMG16225)           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. gladioli (ATCC10248)T         gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. glathei (LMG14190)T           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. glathei (ATCC29195)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. glathei (ATCC29195)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. glumae (LMG2196)T             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. graminis (C4D1M)T             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. hospita (LMG20598)T           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. mallei (ATCC23344)T           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. multivorans (LMG13010)T       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. norimbergensis (Y09879)T      gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. phenazinium (LMG2247)T        gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. phenoliruptrix (AC1100)T      gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. phymatum (STM815)T            gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. phytofirmans (PsJN)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. pickettii (ATCC27511)T        gatcagccac actgggactg agacacggcc cagactccta c-gggaggca
B. pseudomallei (ATCC23343)T     gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. pyrrocinia (LMG14191)T        gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. pyrrocinia ATCC15958)T        gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. sacchari (AF263278)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. silvatlantica (SRMrh-20)T     gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. solanacearum (ATCC11696)T     gatcagccac actgggactg agacacggcc cagactccta c-gggaggca
B. sordicola (S5-B)T             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. stabilis (LMG14294)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. terrae (KMY02)T               gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. terriccola (LMG20594)T        gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. thailandensis (E264)T         gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. tropicalis (Ppe8)T            gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. tuberum (STM678)T             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. unamae (MT1-641)T             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. vandii (LMG16020)T            gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. vietnamiensis (AMMD)T         gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. vietnamiensis (TVV75)T        gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. vietnamiensis (TVV70)T        gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. plantarii (LMG9035)T          gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. xenovorans (LB400)T           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. cenocepacia (KN9)T            gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. ferrariae (FeG101)T           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. ambifaria (LMG19182)T         gaccagccac actgggactg agacacggcc cagactccta cgggaagca
B. oklahomensis (C6786)T         gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
B. ubonensis (GTC-P3-415)T       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC5.4c L. ambigua SA             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC5.4d L. ambigua SA             gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
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WC5.4e L. ambigua SA           gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC21.1i L. pauciflora SA       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC21.1b L. pauciflora SA       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC21.1j L. pauciflora SA       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC26.1d L. meyerianna SA       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC26.1e L. meyerianna SA       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
WC26.1f L. meyerianna SA       gaccagccac actgggactg agacacggcc cagactccta c-gggaggca
P. lemoignei (LMG2207)         gaccarccac actgggactg agacacggcc cagactccta c-gggaggca
R. detusculanense (APF11)      gatcagccac actgggactg agacacggcc cagactccta c-gggaggca
R. solanacearum (R221)         gatcagccac actgggactg agacacggcc cagactccta c-gggaggca
R. pickettii (ATCC27512)       gatcagccac actgggactg agacacggcc cagactccta c-gggaggca
Az. caulinodans (LMG6465)      gatcagccac actgggactg agacacggcc cagactccta c-gggaggca
P. aeruginosa (ATCC10145)      gatcagtcac actggaactg agacacggtc cagactccta c-gggaggca
K. pneumoniae (AU45)           gaccagccac actggaactg agacacggtc cagactccta c-gggaggca

                                       310        320        330        340        350
B. andropogonis (ATCC23061)T   gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. fungorum (LMG19076)T        gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. caribiensi (MWAP64)T        gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. caryophylii (ATCC25418)T    gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. cepacia (ATCC25416)T        gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. cocovenenas (ATCC33664)T    gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. cocovenenas (LMG11626)T     gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. fungorum (LMG16225)         gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. gladioli (ATCC10248)T       gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. glathei (LMG14190)T         gcagtgggga attttggaca atgggggaaa ccctgatcca gcaatgccgc
B. glathei (ATCC29195)T        gcagtgggga attttggaca atgggggaaa ccctgatcca gcaatgccgc
B. glathei (ATCC29195)T        gcagtgggga attttggaca atgggggaaa ccctgatcca gcaatgccgc
B. glumae (LMG2196)T           gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. graminis (C4D1M)T           gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. hospita (LMG20598)T         gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. mallei (ATCC23344)T         gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. multivorans (LMG13010)T     gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. norimbergensis (Y09879)T    gcagtgggga attttggaca atgggcgaaa g-ctgatcca gcaatgccgc
B. phenazinium (LMG2247)T      gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. phenoliruptrix (AC1100)T    gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. phymatum (STM815)T          gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. phytofirmans (PsJN)T        gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. pickettii (ATCC27511)T      gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. pseudomallei (ATCC23343)T   gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. pyrrocinia (LMG14191)T      gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. pyrrocinia ATCC15958)T      gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. sacchari (AF263278)T        gcagtgggga attttggaca atgggggaaa ccctgatcca gcaatgccgc
B. silvatlantica (SRMrh-20)T   gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. solanacearum (ATCC11696)T   gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. sordicola (S5-B)T           gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. stabilis (LMG14294)T        gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. terrae (KMY02)T             gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
B. terriccola (LMG20594)T      gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. thailandensis (E264)T       gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. tropicalis (Ppe8)T          gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. tuberum (STM678)T           gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. unamae (MT1-641)T           gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. vandii (LMG16020)T          gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. vietnamiensis (AMMD)T       gcagtgggga attatggaca atgggcgaaa gcctgatcca gcaatgccgc
B. vietnamiensis (TVV75)T      gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. vietnamiensis (TVV70)T      gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. plantarii (LMG9035)T        gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. xenovorans (LB400)T         gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. cenocepacia (KN9)T          gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. ferrariae (FeG101)T         gcagtgggga attttggaca atgggggcaa ccctgatcca -caatgccgc
B. ambifaria (LMG19182)T       gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
B. oklahomensis (C6786)T       gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
B. ubonensis (GTC-P3-415)T     gcagtgggga attttggaca atgggcg-aa -cctgatcca gcaatgccgc
WC5.4c L. ambigua SA           gcagtgggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc
WC5.4d L. ambigua SA           gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC5.4e L. ambigua SA           gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC21.1i L. pauciflora SA       gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC21.1b L. pauciflora SA       gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC21.1j L. pauciflora SA       gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC26.1d L. meyerianna SA       gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC26.1e L. meyerianna SA       gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
WC26.1f L. meyerianna SA       gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
P. lemoignei (LMG2207)         gcagtgggga attttggaca atggggggcaa ccctgatcca gcaatgccgc
R. detusculanense (APF11)      gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
R. solanacearum (R221)         gcagtgggga attttggaca atgggggcaa ccctgatcca gcaatgccgc
R. pickettii (ATCC27512)       gcagtgggga attttggaca atgggcgaaa gcctgatcca gcaatgccgc
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Az. caulinodans (LMG6465)         gcagtgggga atattggaca atgggcgcaa gcctgatcca gccatgccgc
P. aeruginosa (ATCC10145)         gcagtgggga atattggaca atgggcgaaa gcctgatcca gccatgccgc
K. pneumoniae (AU45)              gcagtgggga atattgcaca atgggcgcaa gcctgatgca gccatgccgc


                                           360        370        380        390        400
B. andropogonis (ATCC23061)T      gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtt cgggaagaaa
B. fungorum (LMG19076)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. caribiensi (MWAP64)T           gtgtgtgaag aaggc-ttcg ggttgtaaag cacttttgtc cggaaagaaa
B. caryophylii (ATCC25418)T       gtgtgcgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. cepacia (ATCC25416)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. cocovenenas (ATCC33664)T       gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. cocovenenas (LMG11626)T        gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. fungorum (LMG16225)            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. gladioli (ATCC10248)T          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. glathei (LMG14190)T            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. glathei (ATCC29195)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. glathei (ATCC29195)T           gtgtgtgaac aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. glumae (LMG2196)T              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. graminis (C4D1M)T              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. hospita (LMG20598)T            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. mallei (ATCC23344)T            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. multivorans (LMG13010)T        gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. norimbergensis (Y09879)T       gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. phenazinium (LMG2247)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. phenoliruptrix (AC1100)T       gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. phymatum (STM815)T             gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. phytofirmans (PsJN)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. pickettii (ATCC27511)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. pseudomallei (ATCC23343)T      gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. pyrrocinia (LMG14191)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. pyrrocinia ATCC15958)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. sacchari (AF263278)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. silvatlantica (SRMrh-20)T      gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. solanacearum (ATCC11696)T      gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. sordicola (S5-B)T              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. stabilis (LMG14294)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. terrae (KMY02)T                gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. terriccola (LMG20594)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. thailandensis (E264)T          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. tropicalis (Ppe8)T             gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. tuberum (STM678)T              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. unamae (MT1-641)T              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. vandii (LMG16020)T             gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. vietnamiensis (AMMD)T          gtgtgtgaag aaggccttcg ggttgttaag cacttttgtc cggaaagaaa
B. vietnamiensis (TVV75)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. vietnamiensis (TVV70)T         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. plantarii (LMG9035)T           gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. xenovorans (LB400)T            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. cenocepacia (KN9)T             gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. ferrariae (FeG101)T            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cgggaagaaa
B. ambifaria (LMG19182)T          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. oklahomensis (C6786)T          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
B. ubonensis (GTC-P3-415)T        gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
WC5.4c L. ambigua SA              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
WC5.4d L. ambigua SA              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
WC5.4e L. ambigua SA              gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
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WC26.1d L. meyerianna SA          gtgtgtgaag aaggccttcg ggttgtaaag caattttgtc cggaaagaaa
WC26.1e L. meyerianna SA          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
WC26.1f L. meyerianna SA          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
P. lemoignei (LMG2207)            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc aggaacgaaa
R. detusculanense (APF11)         gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc aggaaagaaa
R. solanacearum (R221)            gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
R. pickettii (ATCC27512)          gtgtgtgaag aaggccttcg ggttgtaaag cacttttgtc cggaaagaaa
Az. caulinodans (LMG6465)         gtgagtgatg aaggccttag ggttgtaaag ctctttcgcc ggtgaagata
P. aeruginosa (ATCC10145)         gtgtgtgaag aaggtcttcg gattgtaaag cacttaagt tgggaggaag
K. pneumoniae (AU45)              gtgtgtgaag aaggccttcg ggttgtaaag cacttcagc ggggaggaag


                                           410        420        430        440        450
B. andropogonis (ATCC23061)T      aggcgagggc taatatcctt tgctgatgac ggtaccggaa gaataagcac
B. fungorum (LMG19076)T           acctcgtggt taataccgt ggggatgac ggtaccggaa gaataagcac
B. caribiensi (MWAP64)T           accgcttctc taatacaggg gcgggatgac ggtaccggaa gaataagcac
B. caryophylii (ATCC25418)T       tcctgcctga taataccggg cggggatgac ggtaccggaa gaataagcac
B. cepacia (ATCC25416)T           tccttggctc taatacagtc ggggatgac ggtaccggaa gaataagcac
B. cocovenenas (ATCC33664)T       tcctgagggc taatatcctt cggggatgac ggtaccggaa gaataagcac
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B. cocovenenas (LMG11626)T         tcctgagggc taatatcctt cggggatgac ggtaccggaa gaataagcac
B. fungorum (LMG16225)             acgccgtggt taataacccgt ggcggatgac ggtaccggaa gaataagcac
B. gladioli (ATCC10248)T           tcctgagggc taatatcctt cggggatgac ggtaccggaa gaataagcac
B. glathei (LMG14190)T             acttcggggc taataacctct ggaggatgac ggtaccggaa gaataagcac
B. glathei (ATCC29195)T            acttcggggc taataacctct ggaggatgac ggtaccggaa gaataagcac
B. glathei (ATCC29195)T            acttcggggc taataacctct ggaggatgac ggtaccggaa gaataagcac
B. glumae (LMG2196)T               tcctgagggc taatatcctt cggggatgac ggtaccggaa gaataagcac
B. graminis (C4D1M)T               actgcctggc taatatccgg gcgggatgac ggtaccggaa gaataagcac
B. hospita (LMG20598)T             accgcytskc taatacaggg gkgggatgac ggtaccggaa gaataagcac
B. mallei (ATCC23344)T             tcattctggc taatacaccg agtggatgac ggtaccggaa gaataagcac
B. multivorans (LMG13010)T         tccttggctc taatacagtc ggggatgac ggtaccggaa gaataagcac
B. norimbergensis (Y09879)T        tcctttgggt taatacctcg ggggatgac ggtaccggaa gaataagcac
B. phenazinium (LMG2247)T          tcctctgggt taatacccccg ggggatgac ggtaccggaa gaataagcac
B. phenoliruptrix (AC1100)T        acgtcgtggc taatacccgt ggcggatgac ggtaccggaa gaataagcac
B. phymatum (STM815)T              acttcgccgc caatacggt ggaggatgac ggtaccggaa gaataagcac
B. phytofirmans (PsJN)T            acctcttggt taatacctga ggggatgac ggtaccggaa gaataagcac
B. pickettii (ATCC27511)T          tggctctggt taatacctgg ggtcgatgac ggtaccggaa gaataaggac
B. pseudomallei (ATCC23343)T       tcattctggc taatacccgg agtggatgac ggtaccggaa gaataagcac
B. pyrrocinia (LMG14191)T          tccttggttc taatataacc ggggatgac ggtaccggaa gaataagcac
B. pyrrocinia ATCC15958)T          tccttggttc taatatagcc ggggatgac ggtaccggaa gaataagcac
B. sacchari (AF263278)T            tcctttgggg taatacccccg ggggatgac ggtaccggaa gaataagcac
B. silvatlantica (SRMrh-20)T       tcctttttcga taatacccgga aggggatgac ggtaccggaa gaataagcac
B. solanacearum (ATCC11696)T       tcgcttcggt taatacctgg agtggatgac ggtaccggaa gaataaggac
B. sordicola (S5-B)T               accttcgctc taatacagtg gagggatgac ggtaccggaa gaataagcac
B. stabilis (LMG14294)T            tccttggttc taatatagcc ggggatgac ggtaccggaa gaataagcac
B. terrae (KMY02)T                 acctccgtcc taatacggtg ggggatgac ggtaccggaa gaataagcac
B. terriccola (LMG20594)T          acctcagccc taatacggcg ggggatgac ggtaccggaa gaataagcac
B. thailandensis (E264)T           tcatcctggc taataaccgg ggtggatgac ggtaccggaa gaataagcac
B. tropicalis (Ppe8)T              tccctggtcc taatatggcc ggggatgac ggtaccggaa gaataagcac
B. tuberum (STM678)T               acctcgtggc taatatccgt gagggatgac ggtaccggaa gaataagcac
B. unamae (MT1-641)T               tccttttgggg taatacccta ggggatgac ggtaccggaa gaataagcac
B. vandii (LMG16020)T              tcctgagggc taatatcctt cggggatgac ggtaccggaa gaataagcac
B. vietnamiensis (AMMD)T           tccttggttc taatatagcc ggggatgac ggtaccggaa gaataagca-
B. vietnamiensis (TVV75)T          tccttggctc taatacagtc ggggatgac ggtaccggaa gaataagcac
B. vietnamiensis (TVV70)T          tccttggctc taatacagtc ggggatgac ggtaccggaa gaataagcac
B. plantarii (LMG9035)T            tcctgagggc taatatcctt cggggatgac ggtaccggaa gaataagcac
B. xenovorans (LB400)T             acctcgtgccc taatacggtg ggggatgac ggtaccggaa gaataagcac
B. cenocepacia (KN9)T              tccttggctc taatacagtc ggggatgac ggtaccggaa gaataagcac
B. ferrariae (FeG101)T             tcccctgccc taatacggcg ggggatgac ggtaccggaa gaataagcac
B. ambifaria (LMG19182)T           tccttggttc taatatagcc ggggatgac ggtaccggaa gaataagcac
B. oklahomensis (C6786)T           tcctaccgac taatactggg tggggatgac ggtaccggaa gaataagcac
B. ubonensis (GTC-P3-415)T         tccttggccc taatatggcc ggggatgac ggtaccggaa gaataagcac
WC5.4c L. ambigua SA               acctcgtggc taatatccgt gagggatgac ggtaccggaa gaataagcac
WC5.4d L. ambigua SA               actcccgggt taatacccctg ggggatgac ggtaccggaa gaataagcac
WC5.4e L. ambigua SA               actcccgggt taatacccctg ggggatgac ggtaccggaa gaataagcac
WC21.1i L. pauciflora SA           gctccggggc taataccccct ggggatgac ggtaccggaa gaataagcac
WC21.1b L. pauciflora SA           gctccgggggc taataccccct ggggatgac ggtaccggaa gaataagcac
WC21.1j L. pauciflora SA           gcattcctgt taataccggg ggkggatgac ggtaccggaa gaataagcac
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WC26.1e L. meyerianna SA           gcattcctgt taataccggg ggtggatgac ggtaccggaa gaataagcac
WC26.1f L. meyerianna SA           gcattcctgt taataccggg ggtggatgac ggtaccggaa gaataagcac
P. lemoignei (LMG2207)             cggagctggc taatacctgg ctctaatgac ggtacctgaa gaataagcac
R. detusculanense (APF11)          tggctctggt taatacctgg ggtcgatgac ggtaccggaa gaataaggac
R. solanacearum (R221)             tcgcttcggt taatacctgg agtggatgac ggtaccggaa gaataaggac
R. pickettii (ATCC27512)           tggctctggt taatacctgg ggtcgatgac ggtaccggaa gaataaggac
Az. caulinodans (LMG6465)          ---------- ---------- -----atgac ggtaaccgga gaagaagccc
P. aeruginosa (ATCC10145)          ggcagtaagt taataccttg ctgtttttgac gttaccaaca gaataagcac
K. pneumoniae (AU45)               gcggtgaggt taatagcctc atcgattgac gttacccgca gaagaagcac


                                          460        470        480        490        500
B. andropogonis (ATCC23061)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. fungorum (LMG19076)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. caribiensi (MWAP64)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. caryophylii (ATCC25418)T        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. cepacia (ATCC25416)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. cocovenenas (ATCC33664)T        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. cocovenenas (LMG11626)T         cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. fungorum (LMG16225)             cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. gladioli (ATCC10248)T           cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. glathei (LMG14190)T             cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. glathei (ATCC29195)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
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B. glumae (LMG2196)T               cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. graminis (C4D1M)T               cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. hospita (LMG20598)T             cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. mallei (ATCC23344)T             cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. multivorans (LMG13010)T         cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
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B. norimbergensis (Y09879)T     cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. phenazinium (LMG2247)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. phenoliruptrix (AC1100)T     cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. phymatum (STM815)T           cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. phytofirmans (PsJN)T         cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. pickettii (ATCC27511)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtccaagcg
B. pseudomallei (ATCC23343)T    cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. pyrrocinia (LMG14191)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. pyrrocinia ATCC15958)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. sacchari (AF263278)T         cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. silvatlantica (SRMrh-20)T    cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. solanacearum (ATCC11696)T    cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtccaagcg
B. sordicola (S5-B)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. stabilis (LMG14294)T         cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. terrae (KMY02)T              cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. terriccola (LMG20594)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. thailandensis (E264)T        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. tropicalis (Ppe8)T           cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. tuberum (STM678)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. unamae (MT1-641)T            cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. vandii (LMG16020)T           cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. vietnamiensis (AMMD)T        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. vietnamiensis (TVV75)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. vietnamiensis (TVV70)T       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. plantarii (LMG9035)T         cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. xenovorans (LB400)T          cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcaagcg
B. cenocepacia (KN9)T           cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. ferrariae (FeG101)T          cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. ambifaria (LMG19182)T        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. oklahomensis (C6786)T        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
B. ubonensis (GTC-P3-415)T      cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
WC5.4c L. ambigua SA            cggctaacta cgtgccagua -gccgcgg-t aatacgta-g ggtgcgagcg
WC5.4d L. ambigua SA            cggctaacta cgtgccagua -gccgcgg-t aatacgta-g ggtgcgagcg
WC5.4e L. ambigua SA            cggctaacta cgtgccagua -gccgcgg-t aatacgta-g ggtgcgagcg
WC21.1i L. pauciflora SA        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
WC21.1b L. pauciflora SA        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
WC21.1j L. pauciflora SA        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
WC26.1d L. meyerianna SA        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
WC26.1e L. meyerianna SA        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
WC26.1f L. meyerianna SA        cggctaacta cgtgccagca ggccgcggtt aatacgtagg ggtgcgagcg
P. lemoignei (LMG2207)          cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtgcgagcg
R. detusculanense (APF11)       cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtccaagcg
R. solanacearum (R221)          cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtccaagcg
R. pickettii (ATCC27512)        cggctaacta cgtgccagca -gccgcgg-t aatacgta-g ggtccaagcg
Az. caulinodans (LMG6465)       cggctaactt cgtgccagca -gccgcgg-t aatacgaa-g ggggcaagcg
P. aeruginosa (ATCC10145)       cggctaactt cgtgccagca -gccgcgg-t aatacgaa-g ggtgcaagcg
K. pneumoniae (AU45)            cggctaactc cgtgccagca -gccgcgg-t aatacgga-g ggtgcaagcg

                                510        520        530        540        550
B. andropogonis (ATCC23061)T    ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. fungorum (LMG19076)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
B. caribiensi (MWAP64)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagacc
B. caryophylii (ATCC25418)T     ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagacc
B. cepacia (ATCC25416)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. cocovenenas (ATCC33664)T     ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. cocovenenas (LMG11626)T      ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. fungorum (LMG16225)          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtc cgctaagaca
B. gladioli (ATCC10248)T        ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. glathei (LMG14190)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtc tgttaagaca
B. glathei (ATCC29195)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtc tgttaagaca
B. glathei (ATCC29195)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtc tgttaagaca
B. glumae (LMG2196)T            ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. graminis (C4D1M)T            ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagacc
B. hospita (LMG20598)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagacc
B. mallei (ATCC23344)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. multivorans (LMG13010)T      ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtc tgttaagaca
B. norimbergensis (Y09879)T     ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt ttgtaagacg
B. phenazinium (LMG2247)T       ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
B. phenoliruptrix (AC1100)T     ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
B. phymatum (STM815)T           ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagacc
B. phytofirmans (PsJN)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
B. pickettii (ATCC27511)T       ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt gtgcaagacc
B. pseudomallei (ATCC23343)T    ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. pyrrocinia (LMG14191)T       ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. pyrrocinia ATCC15958)T       ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. sacchari (AF263278)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg atgcaagacc
B. silvatlantica (SRMrh-20)T    ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg atgtaagacc
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B. solanacearum (ATCC11696)T      ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt gtgcaagacc
B. sordicola (S5-B)T              ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgttaagaca
B. stabilis (LMG14294)T           ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaaracc
B. terrae (KMY02)T                ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagacc
B. terriccola (LMG20594)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtc cgctaagaca
B. thailandensis (E264)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. tropicalis (Ppe8)T             ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg atgtaagacc
B. tuberum (STM678)T              ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
B. unamae (MT1-641)T              ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg atgtaagacc
B. vandii (LMG16020)T             ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. vietnamiensis (AMMD)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaag--c
B. vietnamiensis (TVV75)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. vietnamiensis (TVV70)T         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. plantarii (LMG9035)T           ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. xenovorans (LB400)T            ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
B. cenocepacia (KN9)T             ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. ferrariae (FeG101)T            ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg atgtaagacc
B. ambifaria (LMG19182)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
B. oklahomensis (C6786)T          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgttaagacc
B. ubonensis (GTC-P3-415)T        ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt tgctaagacc
WC5.4c L. ambigua SA              ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
WC5.4d L. ambigua SA              ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
WC5.4e L. ambigua SA              ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt cgctaagaca
WC21.1i L. pauciflora SA          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
WC21.1b L. pauciflora SA          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
WC21.1j L. pauciflora SA          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
WC26.1d L. meyerianna SA          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
WC26.1e L. meyerianna SA          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
WC26.1f L. meyerianna SA          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtg ctgtaagacc
P. lemoignei (LMG2207)            ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt gtgcaagaca
R. detusculanense (APF11)         ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt gtgcaagacc
R. solanacearum (R221)            ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt gtgcaagacc
R. pickettii (ATCC27512)          ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt gtgcaagacc
Az. caulinodans (LMG6465)         ttgctcggaa tcactgggcg taaagcgcac gtaggcggat cgttaagtca
P. aeruginosa (ATCC10145)         ttaatcggaa ttactgggcg taaagcgcgc gtaggtggtt cagcaagttg
K. pneumoniae (AU45)              ttaatcggaa ttactgggcg taaagcgcac gcaggcggtc tgtcaagtcg

                                         560        570        580        590        600
B. andropogonis (ATCC23061)T      gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcagg
B. fungorum (LMG19076)T           gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. caribiensi (MWAP64)T           gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactggcggg
B. caryophylii (ATCC25418)T       gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactggcggg
B. cepacia (ATCC25416)T           gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcagg
B. cocovenenas (ATCC33664)T       gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
B. cocovenenas (LMG11626)T        gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
B. fungorum (LMG16225)            gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. gladioli (ATCC10248)T          gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
B. glathei (LMG14190)T            gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcagg
B. glathei (ATCC29195)T           gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcagg
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B. glumae (LMG2196)T              gatgtgaaat ccccgggctc aacctgggaa ctgcatttgt gactggcagg
B. graminis (C4D1M)T              gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. hospita (LMG20598)T            gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactggcggg
B. mallei (ATCC23344)T            gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. multivorans (LMG13010)T        gatgtgaaat ccccgggctc aacctgggaa ctgcatttgt gactggcagg
B. norimbergensis (Y09879)T       gatgtgaaat ccccgggctt aacctgggaa ctgcattcgt gactgcaagg
B. phenazinium (LMG2247)T         gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. phenoliruptrix (AC1100)T       gatgtgaaat ccccgggctc aacctgggaa ctgcatttgt gactggcggg
B. phymatum (STM815)T             gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. phytofirmans (PsJN)T           gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. pickettii (ATCC27511)T         gatgtgaaat ccccgagctt aacttgggaa ttgcatttgt gactgcacgg
B. pseudomallei (ATCC23343)T      gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. pyrrocinia (LMG14191)T         gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. pyrrocinia ATCC15958)T         gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. sacchari (AF263278)T           gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcatcg
B. silvatlantica (SRMrh-20)T      gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcatcg
B. solanacearum (ATCC11696)T      gatgtgaaat ccccgggctt aacctgggaa ttgcattggt gactgcacgg
B. sordicola (S5-B)T              gatgtgaaat ccccgggctc aacctgggaa ctgcatttgt gactggcgag
B. stabilis (LMG14294)T           gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. terrae (KMY02)T                gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. terriccola (LMG20594)T         gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. thailandensis (E264)T          gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. tropicalis (Ppe8)T             gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactgcatcg
B. tuberum (STM678)T              gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
B. unamae (MT1-641)T              gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactgcatcg
B. vandii (LMG16020)T             gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
B. vietnamiensis (AMMD)T          gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
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B. vietnamiensis (TVV75)T        gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. vietnamiensis (TVV70)T        gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. plantarii (LMG9035)T          gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
B. xenovorans (LB400)T           gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
B. cenocepacia (KN9)T            gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. ferrariae (FeG101)T           gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactgcattg
B. ambifaria (LMG19182)T         gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
B. oklahomensis (C6786)T         gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcaag
B. ubonensis (GTC-P3-415)T       gatgtgaaat ccccgggctc aacctgggaa ctgcattggt gactggcagg
WC5.4c L. ambigua SA             gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
WC5.4d L. ambigua SA             gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
WC5.4e L. ambigua SA             gatgtgaaat ccccgggctt aacctgggaa ctgcatttgt gactggcggg
WC21.1i L. pauciflora SA         gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
WC21.1b L. pauciflora SA         gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
WC21.1j L. pauciflora SA         gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
WC26.1d L. meyerianna SA         gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
WC26.1e L. meyerianna SA         gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
WC26.1f L. meyerianna SA         gatgtgaaat ccccgggctt aacctgggaa ctgcattggt gactgcagcg
P. lemoignei (LMG2207)           gaggtgaaat ccccgagctt aacttgggaa ctgcctttgt gactgcatgg
R. detusculanense (APF11)        gatgtgaaat ccccgagctt aacttgggaa ttgcattggt gactgcacgg
R. solanacearum (R221)           gatgtgaaat ccccgggctt aacctgggaa ttgcattggt gactgcacgg
R. pickettii (ATCC27512)         gatgtgaaat ccccgagctt aacttgggaa ttgcattggt gactgcacgg
Az. caulinodans (LMG6465)        ggggtgaaag cctggagctc aactccagaa ctgcccttga tactggcgat
P. aeruginosa (ATCC10145)        gatgtgaaat ccccgggctc aacctgggaa ctgcatccaa aactactgag
K. pneumoniae (AU45)             gatgtgaaat ccccgggctc aacctgggaa ctgcattcga aactggcagg

                                 610        620        630        640        650
B. andropogonis (ATCC23061)T     ctagagtgtg gcaga-ggg- gggtagaatt -ccgcgtgta -gcagtgaaa
B. fungorum (LMG19076)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. caribiensi (MWAP64)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. caryophylii (ATCC25418)T      ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. cepacia (ATCC25416)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. cocovenenas (ATCC33664)T      ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. cocovenenas (LMG11626)T       ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. fungorum (LMG16225)           ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. gladioli (ATCC10248)T         ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. glathei (LMG14190)T           ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. glathei (ATCC29195)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. glathei (ATCC29195)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. glumae (LMG2196)T             ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. graminis (C4D1M)T             ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. hospita (LMG20598)T           ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. mallei (ATCC23344)T           ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. multivorans (LMG13010)T       ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. norimbergensis (Y09879)T      ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. phenazinium (LMG2247)T        ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. phenoliruptrix (AC1100)T      ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. phymatum (STM815)T            ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. phytofirmans (PsJN)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. pickettii (ATCC27511)T        ctagagtgtg tcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. pseudomallei (ATCC23343)T     ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. pyrrocinia (LMG14191)T        ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. pyrrocinia ATCC15958)T        ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. sacchari (AF263278)T          ctggagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. silvatlantica (SRMrh-20)T     ctggagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. solanacearum (ATCC11696)T     ctagagtgtg tcaga-ggg- aggtagaatt -ccacgtgta -gcagtgaaa
B. sordicola (S5-B)T             ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. stabilis (LMG14294)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. terrae (KMY02)T               ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. terriccola (LMG20594)T        ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. thailandensis (E264)T         ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. tropicalis (Ppe8)T            cttgagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. tuberum (STM678)T             ctggagtatg gcaga-ggg- gggtggaatt -ccacgtgta -gcagtgaaa
B. unamae (MT1-641)T             ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. vandii (LMG16020)T            ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. vietnamiensis (AMMD)T         ctagagtatg gcag---gg- gggtag-att -ccacgtgta -gcagtgaaa
B. vietnamiensis (TVV75)T        ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
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B. plantarii (LMG9035)T          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. xenovorans (LB400)T           ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. cenocepacia (KN9)T            ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. ferrariae (FeG101)T           ctcgagtatg gcaga-ggg- gggtggaatt -ccacgtgta -gcagtgaaa
B. ambifaria (LMG19182)T         ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta ggcagtgaaa
B. oklahomensis (C6786)T         ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
B. ubonensis (GTC-P3-415)T       ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
WC5.4c L. ambigua SA             ctggagtatg gcaga-ggg- gggtggaatt -ccacgtgta -gcagtgaaa
WC5.4d L. ambigua SA             ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
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WC5.4e  L. ambigua SA          ctagagtatg gcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
WC21.1i L. pauciflora SA       ctggagtatg gcaga-ggg- gggtggaatt -ccacgtgta -gcagtgaaa
WC21.1b L. pauciflora SA       ctggagtatg gcagagggg- gggtggaatt -ccacgtgta -gcagtgaaa
WC21.1j L. pauciflora SA       ctggagtatg gcaga-gggc gggtggaatt -ccacgtgta -gcagtgaaa
WC26.1d L. meyerianna SA       ctggagtatg gcaga-ggg- gggtggaatt -ccacgtgta -gcagtgaaa
WC26.1e L. meyerianna SA       ctggagtatg gcaga-ggg- gggtggaatt cccacgtgta -gcagtgaaa
WC26.1f L. meyerianna SA       ctggagtatg gcaga-ggg- gggtggaatt -ccacgtgta -gcagtgaaa
P. lemoignei (LMG2207)         ctagagtgtg tcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
R. detusculanense (APF11)      ctagagtgtg tcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
R. solanacearum (R221)         ctagagtgtg tcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
R. pickettii (ATCC27512)       ctagagtgtg tcaga-ggg- gggtagaatt -ccacgtgta -gcagtgaaa
Az. caulinodans (LMG6465)      cttgagttcg agaga-ggt- tggtggaact -ccgagtgta -gaggtgaaa
P. aeruginosa (ATCC10145)      ctagagtacg gtaga-ggg- tggtggaatt -tcctgtgta -gcggtgaaa
K. pneumoniae (AU45)           ctagagtctt gtaga-ggg- gggtagaatt -ccaggtgta -gcggtgaaa


                                    660        670        680        690        700
B. andropogonis (ATCC23061)T   tgcgtagata tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. fungorum (LMG19076)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. caribiensi (MWAP64)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. caryophylii (ATCC25418)T    tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. cepacia (ATCC25416)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. cocovenenas (ATCC33664)T    tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. cocovenenas (LMG11626)T     tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. fungorum (LMG16225)         tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. gladioli (ATCC10248)T       tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. glathei (LMG14190)T         tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. glathei (ATCC29195)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
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B. hospita (LMG20598)T         tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. mallei (ATCC23344)T         tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. multivorans (LMG13010)T     tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. norimbergensis (Y09879)T    tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgcggca
B. phenazinium (LMG2247)T      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. phenoliruptrix (AC1100)T    tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. phymatum (STM815)T          tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. phytofirmans (PsJN)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. pickettii (ATCC27511)T      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggata
B. pseudomallei (ATCC23343)T   tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. pyrrocinia (LMG14191)T      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. pyrrocinia ATCC15958)T      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. sacchari (AF263278)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. silvatlantica (SRMrh-20)T   tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. solanacearum (ATCC11696)T   tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc tcctgggata
B. sordicola (S5-B)T           tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. stabilis (LMG14294)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. terrae (KMY02)T             tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. terriccola (LMG20594)T      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. thailandensis (E264)T       tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. tropicalis (Ppe8)T          tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggtca
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B. unamae (MT1-641)T           tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggtca
B. vandii (LMG16020)T          tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
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B. vietnamiensis (TVV75)T      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
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B. plantarii (LMG9035)T        tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. xenovorans (LB400)T         tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. cenocepacia (KN9)T          tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. ferrariae (FeG101)T         tgcgtagaga tgtggaggaa caccgatggc gaaggcagcc ccctgggcca
B. ambifaria (LMG19182)T       tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. oklahomensis (C6786)T       tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
B. ubonensis (GTC-P3-415)T     tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
WC5.4c  L. ambigua SA          tgcgtagaga tgtggaggaa caccgatggc gaaggcagcc ccctgggcca
WC5.4d  L. ambigua SA          tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggcca
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WC21.1i L. pauciflora SA       tgcgtagaga tgtggaggaa caccgatggc gaaggcagcc ccctgggcca
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P. lemoignei (LMG2207)         tgcgtagata tgtggaggaa taccgatggc gaaggcagcc ccctgggata
R. detusculanense (APF11)      tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggata
R. solanacearum (R221)         tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggata
R. pickettii (ATCC27512)       tgcgtagaga tgtggaggaa taccgatggc gaaggcagcc ccctgggata
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Az. caulinodans (LMG6465)        ttcgtagata ttcggaagaa caccagtggc gaaggcggcc aactggctcg
P. aeruginosa (ATCC10145)        tgcgtagata taggaaggaa caccagtggc gaaggcgacc acctggactg
K. pneumoniae (AU45)             tgcgtagaga tctggaggaa taccggtggc gaaggcggcc ccctggacaa


                                        710        720        730        740        750
B. andropogonis (ATCC23061)T     atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. fungorum (LMG19076)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. caribiensi (MWAP64)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. caryophylii (ATCC25418)T      atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. cepacia (ATCC25416)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. cocovenenas (ATCC33664)T      atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. cocovenenas (LMG11626)T       atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. fungorum (LMG16225)           atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. gladioli (ATCC10248)T         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. glathei (LMG14190)T           atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. glathei (ATCC29195)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. glathei (ATCC29195)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. glumae (LMG2196)T             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. graminis (C4D1M)T             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. hospita (LMG20598)T           atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. mallei (ATCC23344)T           atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. multivorans (LMG13010)T       atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. norimbergensis (Y09879)T      atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. phenazinium (LMG2247)T        atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. phenoliruptrix (AC1100)T      atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. phymatum (STM815)T            atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. phytofirmans (PsJN)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. pickettii (ATCC27511)T        acactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. pseudomallei (ATCC23343)T     atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. pyrrocinia (LMG14191)T        atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. pyrrocinia ATCC15958)T        atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. sacchari (AF263278)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. silvatlantica (SRMrh-20)T     atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. solanacearum (ATCC11696)T     acactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. sordicola (S5-B)T             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. stabilis (LMG14294)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. terrae (KMY02)T               atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. terriccola (LMG20594)T        atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. thailandensis (E264)T         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. tropicalis (Ppe8)T            atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. tuberum (STM678)T             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. unamae (MT1-641)T             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. vandii (LMG16020)T            atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. vietnamiensis (AMMD)T         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
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B. plantarii (LMG9035)T          atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. xenovorans (LB400)T           atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. cenocepacia (KN9)T            atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. ferrariae (FeG101)T           atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. ambifaria (LMG19182)T         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. oklahomensis (C6786)T         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
B. ubonensis (GTC-P3-415)T       atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
WC5.4c L. ambigua SA             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
WC5.4d L. ambigua SA             atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
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WC26.1d L. meyerianna SA         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
WC26.1e L. meyerianna SA         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
WC26.1f L. meyerianna SA         atactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
P. lemoignei (LMG2207)           acactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
R. detusculanense (APF11)        acactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
R. solanacearum (R221)           acactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
R. pickettii (ATCC27512)         acactgacgc tcatgcacga aagcgtgggg agcaaacagg attagatacc
Az. caulinodans (LMG6465)        atactgacgc tgaggtgcga aagcgtgggg agcaaacagg attagatacc
P. aeruginosa (ATCC10145)        atactgacac tgaggtgcga aagcgtgggg agcaaacagg attagatacc
K. pneumoniae (AU45)             agactgacgc tcaggtgcga aagcgtgggg agcaaacagg attagatacc


                                        760        770        780        790        800
B. andropogonis (ATCC23061)T     ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. fungorum (LMG19076)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. caribiensi (MWAP64)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. caryophylii (ATCC25418)T      ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. cepacia (ATCC25416)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. cocovenenas (ATCC33664)T      ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
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B. cocovenenas (LMG11626)T        ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. fungorum (LMG16225)            ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. gladioli (ATCC10248)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. glathei (LMG14190)T            ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
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B. glumae (LMG2196)T              ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. graminis (C4D1M)T              ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-ccttcat
B. hospita (LMG20598)T            ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. mallei (ATCC23344)T            ctggtagtcc acgcccaaa cgatgtcaa- ctagttgttg gg-gattcat
B. multivorans (LMG13010)T        ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. norimbergensis (Y09879)T       ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. phenazinium (LMG2247)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. phenoliruptrix (AC1100)T       ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-ccttcat
B. phymatum (STM815)T             ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. phytofirmans (PsJN)T           ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. pickettii (ATCC27511)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. pseudomallei (ATCC23343)T      ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. pyrrocinia (LMG14191)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. pyrrocinia ATCC15958)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. sacchari (AF263278)T           ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-ccttcat
B. silvatlantica (SRMrh-20)T      ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-ccttcac
B. solanacearum (ATCC11696)T      ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. sordicola (S5-B)T              ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. stabilis (LMG14294)T           ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. terrae (KMY02)T                ctggtagtcc acgccctaaa cgatgtcaa- ctagttgtcg gg-tcttcat
B. terriccola (LMG20594)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-ccttcat
B. thailandensis (E264)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. tropicalis (Ppe8)T             ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-tcttcat
B. tuberum (STM678)T              ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-ccttcat
B. unamae (MT1-641)T              ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-tcttcat
B. vandii (LMG16020)T             ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. vietnamiensis (AMMD)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. vietnamiensis (TVV75)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. vietnamiensis (TVV70)T         ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. plantarii (LMG9035)T           ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. xenovorans (LB400)T            ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-tcttcat
B. cenocepacia (KN9)T             ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. ferrariae (FeG101)T            ctggtagtcc acgccctaaa cgatgtcaa- ctggttgtcg gg-ccttcat
B. ambifaria (LMG19182)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. oklahomensis (C6786)T          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
B. ubonensis (GTC-P3-415)T        ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
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R. solanacearum (R221)            ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
R. pickettii (ATCC27512)          ctggtagtcc acgccctaaa cgatgtcaa- ctagttgttg gg-gattcat
Az. caulinodans (LMG6465)         ctggtagtcc acgccgtaaa cgatggatg- ctagccgttg gg-gagcttg
P. aeruginosa (ATCC10145)         ctggtagtcc acgccgtaaa cgatgtcga- ctagccgttg ggatccttga
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                                    810        820        830        840        850
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B. fungorum (LMG19076)T           tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. caribiensi (MWAP64)T           tgacttggta acgaagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. caryophylii (ATCC25418)T       ttccttagta acgaagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. cepacia (ATCC25416)T           ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. cocovenenas (ATCC33664)T       ttccttagta acgta--taa cgcgtgaagt tgaccgcct- ggggagtacg
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B. fungorum (LMG16225)            tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
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B. mallei (ATCC23344)T            ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
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B. phenoliruptrix (AC1100)T      tggcttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. phymatum (STM815)T            tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. phytofirmans (PsJN)T          tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. pickettii (ATCC27511)T        ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. pseudomallei (ATCC23343)T     ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. pyrrocinia (LMG14191)T        ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. pyrrocinia ATCC15958)T        ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. sacchari (AF263278)T          tggcttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. silvatlantica (SRMrh-20)T     tggcttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. solanacearum (ATCC11696)T     ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. sordicola (S5-B)T             ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. stabilis (LMG14294)T          ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. terrae (KMY02)T               tgacttggta acgaagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. terriccola (LMG20594)T        tggcttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. thailandensis (E264)T         ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. tropicalis (Ppe8)T            tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. tuberum (STM678)T             tggcttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. unamae (MT1-641)T             tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. vandii (LMG16020)T            ttccttagta acgtagctga cgcgtgaagt tgaccgcct- ggggagtacg
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B. vietnamiensis (TVV75)T        ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
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B. xenovorans (LB400)T           tgacttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. cenocepacia (KN9)T            ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
B. ferrariae (FeG101)T           tggcttggta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
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B. ubonensis (GTC-P3-415)T       ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
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P. lemoignei (LMG2207)           tgacttggta acgcagctaa cgcgtgaagt tgaccgcct- ggggagtacg
R. detusculanense (APF11)        ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
R. solanacearum (R221)           ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
R. pickettii (ATCC27512)         ttccttagta acgtagctaa cgcgtgaagt tgaccgcct- ggggagtacg
Az. caulinodans (LMG6465)        ctcttcagtg gcgcagctaa cgccttaagc atcccgcct- ggggagtacg
P. aeruginosa (ATCC10145)        gatcttagtg gcgcagctaa cgcgataagc cgaccgcct- ggggagtacg
K. pneumoniae (AU45)             ggcgtggctt ccggagctaa cgcgttaaat cgaccgcct- ggggagtacg

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B. andropogonis (ATCC23061)T     gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. fungorum (LMG19076)T          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. caribiensi (MWAP64)T          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. caryophylii (ATCC25418)T      gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. cepacia (ATCC25416)T          gtcgcaagat taaaactcaa aggaattgac ggga--ccgc acaagcggtg
B. cocovenenas (ATCC33664)T      gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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B. fungorum (LMG16225)           gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. gladioli (ATCC10248)T         gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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B. multivorans (LMG13010)T       gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. norimbergensis (Y09879)T      gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. phenazinium (LMG2247)T        gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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B. sacchari (AF263278)T          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. silvatlantica (SRMrh-20)T     gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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B. solanacearum (ATCC11696)T      gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. sordicola (S5-B)T              gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. stabilis (LMG14294)T           gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. terrae (KMY02)T                gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. terriccola (LMG20594)T         gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. thailandensis (E264)T          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. tropicalis (Ppe8)T             gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. tuberum (STM678)T              gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. unamae (MT1-641)T              gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. vandii (LMG16020)T             gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. vietnamiensis (AMMD)T          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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B. oklahomensis (C6786)T          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
B. ubonensis (GTC-P3-415)T        gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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P. lemoignei (LMG2207)            gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagcggtg
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R. pickettii (ATCC27512)          gtcgcaagat taaaactcaa aggaattgac ggggacccgc acaagc-gtg
Az. caulinodans (LMG6465)         gtcgcaagat taaaactcaa aggaattgac ggggg-ccgc acaagcggtg
P. aeruginosa (ATCC10145)         gccgcaaggt taaaactcaa atgaattgac ggggccccgc acaagcggtg
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B. fungorum (LMG19076)T           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. caribiensi (MWAP64)T           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. caryophylii (ATCC25418)T       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
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B. mallei (ATCC23344)T            gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. multivorans (LMG13010)T        gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. norimbergensis (Y09879)T       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. phenazinium (LMG2247)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. phenoliruptrix (AC1100)T       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. phymatum (STM815)T             gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. phytofirmans (PsJN)T           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. pickettii (ATCC27511)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. pseudomallei (ATCC23343)T      gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. pyrrocinia (LMG14191)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. pyrrocinia ATCC15958)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. sacchari (AF263278)T           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. silvatlantica (SRMrh-20)T      gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. solanacearum (ATCC11696)T      gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. sordicola (S5-B)T              gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. stabilis (LMG14294)T           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. terrae (KMY02)T                gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. terriccola (LMG20594)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. thailandensis (E264)T          gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. tropicalis (Ppe8)T             gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. tuberum (STM678)T              gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. unamae (MT1-641)T              gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. vandii (LMG16020)T             gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. vietnamiensis (AMMD)T          gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
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B. vietnamiensis (TVV75)T      gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. vietnamiensis (TVV70)T      gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. plantarii (LMG9035)T        gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. xenovorans (LB400)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. cenocepacia (KN9)T          gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. ferrariae (FeG101)T         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. ambifaria (LMG19182)T       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. oklahomensis (C6786)T       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
B. ubonensis (GTC-P3-415)T     gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
WC5.4c L. ambigua SA           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
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WC5.4e L. ambigua SA           gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
WC21.1i L. pauciflora SA       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
WC21.1b L. pauciflora SA       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
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WC26.1d L. meyerianna SA       gatgatgtgg attaattcga tgcaacgcga aaaaccttta ccta-ccctt
WC26.1e L. meyerianna SA       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
WC26.1f L. meyerianna SA       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
P. lemoignei (LMG2207)         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
R. detusculanense (APF11)      gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
R. solanacearum (R221)         gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
R. pickettii (ATCC27512)       gatgatgtgg attaattcga tgcaacgcga aaaacc-tta ccta-ccctt
Az. caulinodans (LMG6465)      gagcatgtgg tttaattcga agcaacgcgc agaacc-tta ccag-ccttt
P. aeruginosa (ATCC10145)      gagcatgtgg tttaattcga agcaacgcga agaacc-tta cctg-gcctt
K. pneumoniae (AU45)           gagcatgtgg tttaattcga tgcaacgcga agaacc-tta cctg-gtctt

                                       960        970        980        990       1000
B. andropogonis (ATCC23061)T   gacatggtcg gaacccttga gagatcag-g gggtgctcga aagagaaccg
B. fungorum (LMG19076)T        gacatgtatg gaaccctgct gagaggtg-g gggtgcccga aagggagcca
B. caribiensi (MWAP64)T        gacatgtacg gaaccttgct gagaggtg-a gggtgcccga aagggagccg
B. caryophylii (ATCC25418)T    gacatggacg gaatcccgct gagaggtg-g gagtgctcga aagagaaccg
B. cepacia (ATCC25416)T        gacatggtcg gaatcctgct gagaggcg-g gagtgctcga aagagaaccg
B. cocovenenas (ATCC33664)T    gacatggtcg gaatcctgga gagatctg-g gagtgctcga aagagaaccg
B. cocovenenas (LMG11626)T     gacatggtcg gaatcctgga gagatctg-g gagtgctcga aagagaaccg
B. fungorum (LMG16225)         gacatgtatg gaatcctgct gagaggtg-g gagtgcccga aagggagcca
B. gladioli (ATCC10248)T       gacatggtcg gaatcctaga gagatctg-g gagtgctcga aagagaaccg
B. glathei (LMG14190)T         gacatggtcg gaaccctggt gagagctg-g gggtgctcga aagagaaccg
B. glathei (ATCC29195)T        gacatggtcg gaaccctggt gagagctg-g gggtgctcga aagagaaccg
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B. glumae (LMG2196)T           gacatggtcg gaatcctgag ga-actcg-g gagtgctcga aagagaaccg
B. graminis (C4D1M)T           gacatgtacg gaagtccgct gagaggtg-g atgtgcccga aagggagccg
B. hospita (LMG20598)T         gacatgtacg gaaccttgcc gagaggtg-a gggtgcccga aagggagccg
B. mallei (ATCC23344)T         gacatggtcg gaagcccgat gagagttg-g gcgtgctcga aagagaaccg
B. multivorans (LMG13010)T     gacatggtcg gaatcctgaa gagattcg-g gagtgctcga aagagaaccg
B. norimbergensis (Y09879)T    gacatgtacg gaatcttgct gaaaggtg-a gagtgctcga aagagagccg
B. phenazinium (LMG2247)T      gacatggtcg gaaccctgct gaaaggtg-g gggtgctcga aagagaaccg
B. phenoliruptrix (AC1100)T    gacatggatg gaatcccgct gagaggtg-g gagtgcccga aagggagcca
B. phymatum (STM815)T          gacatgtacg gaaccttgcc gagaggtg-a gggtgcccga aagggagccg
B. phytofirmans (PsJN)T        gacatgtatg gaatcctgct gagaggtg-g gagtgcccga aagggagcca
B. pickettii (ATCC27511)T      gacatgccac taacgaagca gagatgcatt aggtgctcga aagagaaagt
B. pseudomallei (ATCC23343)T   gacatggtcg gaagcccgat gagagttg-g gcgtgctcga aagagaaccg
B. pyrrocinia (LMG14191)T      gacatggtcg gaatcctgct gagaggtg-g gagtgctcga aagagaaccg
B. pyrrocinia ATCC15958)T      gacatggtcg gaatcctgct gagaggtg-g gagtgctcga aagagaaccg
B. sacchari (AF263278)T        gacatgtacg gaatcctgct gagaggcg-g gagtgcccga aagggagccg
B. silvatlantica (SRMrh-20)T   gacatggacg gaagaccggt gagagctg-g ttgtgcccga aagggagccg
B. solanacearum (ATCC11696)T   gacatgccac taacgaagca gagatgcatt aggtgctcga aagagaaagt
B. sordicola (S5-B)T           gacatggtcg gaacctgct gaaaggct-g gggtgctcga aagagaaccg
B. stabilis (LMG14294)T        gacatggtcg gaatcctgct gagaggtg-g gagtgctcga aagagaaccg
B. terrae (KMY02)T             gacatgtacg gaaccttgct gagaggtg-a gggtgcccga aagggagccg
B. terricola (LMG20594)T       gacatgtacg gaatcctgct gagaggcg-g gagtgcccga aagggagccg
B. thailandensis (E264)T       gacatggtcg gaatcctgct gagaggcg-g gagtgctcga aagagaaccg
B. tropicalis (Ppe8)T          gacatgtacg gaattccgct gagaggtg-a aagtgcccga aagggagccg
B. tuberum (STM678)T           gacatgtatg gaatcctgcc gagaggcg-g gagtgcccga aagggagccg
B. unamae (MT1-641)T           gacatggtcg gaatcctgct gagaggtg-g gagtgcccga aagggagcca
B. vandii (LMG16020)T          gacatggtcg gaatcctgag gagactcg-g gagtgctcga aagagaaccg
B. vietnamiensis (AMMD)T       gacatggtcg gaatcctgct gagaggtg-g gagtgctcga aagagaaccg
B. vietnamiensis (TVV75)T      gacatggtcg gaatcctgaa gagattcg-g gagtgctcga aagagaaccg
B. vietnamiensis (TVV70)T      gacatggtcg gaatcctgct gagattcg-g gagtgctcga aagagaaccg
B. plantarii (LMG9035)T        gacatggtcg gaatcccgaa gagatttg-g gagtgctcga aagagaaccg
B. xenovorans (LB400)T         gacatgtatg gaagtctgct gagaggtg-g atgtgcccga aagggagcca
B. cenocepacia (KN9)T          gacatggtcg gaatcctgcc gagaggtg-g gagtgctcga aagagaaccg
B. ferrariae (FeG101)T         gacatgtacg gaagtctgcc gagaggtg-g atgtgcccga aagggagccg
B. ambifaria (LMG19182)T       gacatggtcg gaatcctgct gagagggg-g gagtgctcga aagagaaccg
B. oklahomensis (C6786)T       gacatggtcg gaatcctgct gagaggtg-g gagtgctcga aagagaaccg
B. ubonensis (GTC-P3-415)T     gacatggtcg gaatcctgaa gagattcg-g gagtgctcga aagagaaccg
WC5.4c L. ambigua SA           gacatgtatg gaatcctgcc gagaggcg-g gagtgcccga aagggagcca
WC5.4d L. ambigua SA           gacatgtatg gaacctggct gagaggtc-a gggtgcccga aagggagcca
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WC5.4e  L. ambigua SA             gacatgtatg gaacctggct gagaggtc-a gggtgcccga aagggagcca
WC21.1i L. pauciflora SA          gacatgtatg gaatcctggc gagagccg-g gggtgcccga aagggagcca
WC21.1b L. pauciflora SA          gacatgtatg gaatcctggc gagagccg-g gagtgcccga aagggagcca
WC21.1j L. pauciflora SA          gacatgtatg gaatcctggt gagagccg-g gagtgcccga aagggagcca
WC26.1d L. meyerianna SA          gacatgtatg gaatcctggt gagagccg-g gagtgcccga aagggagcca
WC26.1e L. meyerianna SA          gacatgtatg gaatcctggt gagagccg-g gagtgcccga aagggagcca
WC26.1f L. meyerianna SA          gacatgtatg gaatcctggt gagagccg-g gagtgcccga aagggagcca
P. lemoignei (LMG2207)            gacatgtacg gaatccttca gagatgga-g gagtgctcga aagagagccg
R. detusculanense (APF11)         gacatgccac taacgaagca gagatgcatt aggtgctcga aagagaaagt
R. solanacearum (R221)            gacatgccac taacgaagca gagatgcatt aggtg-tcga aagagaaagt
R. pickettii (ATCC27512)          gacatgccac taacgaagca gagatgcatt aggtgctcga aagagaaagt
Az. caulinodans (LMG6465)         gacatggcag gacgacttcc ggagacgg-a tttcttccag caatggacct
P. aeruginosa (ATCC10145)         gacatgctga gaactttcca gagatgga-t tggtgcct-- tcgggaactc
K. pneumoniae (AU45)              gacatccaca gaactttcca gagatgga-t tggtgcct-- tcgggaactg

                                          1010       1020       1030       1040       1050
B. andropogonis (ATCC23061)T      atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. fungorum (LMG19076)T           taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. caribiensi (MWAP64)T           taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. caryophylii (ATCC25418)T       tcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. cepacia (ATCC25416)T           gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. cocovenenas (ATCC33664)T       atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. cocovenenas (LMG11626)T        atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. fungorum (LMG16225)            taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. gladioli (ATCC10248)T          atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. glathei (LMG14190)T            acacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. glathei (ATCC29195)T           acacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
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B. glumae (LMG2196)T              atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. graminis (C4D1M)T              taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
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B. multivorans (LMG13010)T        gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. norimbergensis (Y09879)T       taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. phenazinium (LMG2247)T         atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. phenoliruptrix (AC1100)T       tcacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. phymatum (STM815)T             taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. phytofirmans (PsJN)T           taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. pickettii (ATCC27511)T         ggacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. pseudomallei (ATCC23343)T      gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. pyrrocinia (LMG14191)T         atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. pyrrocinia ATCC15958)T         atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. sacchari (AF263278)T           taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. silvatlantica (SRMrh-20)T      tcacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. solanacearum (ATCC11696)T      ggacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. sordicola (S5-B)T              atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. stabilis (LMG14294)T           atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. terrae (KMY02)T                taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. terriccola (LMG20594)T         taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. thailandensis (E264)T          gcgcacaggt actgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. tropicalis (Ppe8)T             taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. tuberum (STM678)T              taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. unamae (MT1-641)T              taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. vandii (LMG16020)T             atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. vietnamiensis (AMMD)T          gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. vietnamiensis (TVV75)T         gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. vietnamiensis (TVV70)T         gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. plantarii (LMG9035)T           atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. xenovorans (LB400)T            taacacaggt gctgcatggc tgtcgac-ag ctcgtgtcgt gagatgttgg
B. cenocepacia (KN9)T             gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. ferrariae (FeG101)T            taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. ambifaria (LMG19182)T          gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. oklahomensis (C6786)T          atacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
B. ubonensis (GTC-P3-415)T        gcgcacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
WC5.4c  L. ambigua SA             taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
WC5.4d  L. ambigua SA             taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
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WC21.1i L. pauciflora SA          taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
WC21.1b L. pauciflora SA          taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
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WC26.1f L. meyerianna SA          taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
P. lemoignei (LMG2207)            taacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
R. detusculanense (APF11)         ggacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
R. solanacearum (R221)            ggacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
R. pickettii (ATCC27512)          ggacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
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Az. caulinodans (LMG6465)          gcacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
P. aeruginosa (ATCC10145)          agacacaggt gctgcatggc tgtcgtc-ag ctcgtgtcgt gagatgttgg
K. pneumoniae (AU45)               tgagacaggt gctgcatggc tgtcgtc-ag ctcgtgttgt gaaatgttgg


                                       1060       1070       1080       1090       1100
B. andropogonis (ATCC23061)T       gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. fungorum (LMG19076)T            gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. caribiensi (MWAP64)T            gttaagtccc gcaacgagcg caacccttgt ccctagttcg ta------cg
B. caryophylii (ATCC25418)T        gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. cepacia (ATCC25416)T            gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. cocovenenas (ATCC33664)T        gttaagtccc gcaacgagcg caacccttgt ccttag---- -a------cg
B. cocovenenas (LMG11626)T         gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. fungorum (LMG16225)             gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. gladioli (ATCC10248)T           gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. glathei (LMG14190)T             gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. glathei (ATCC29195)T            gttaagtccc gcaacgagcg caacccttgt ccttagttcg ta------cg
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B. glumae (LMG2196)T               gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. graminis (C4D1M)T               gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. hospita (LMG20598)T             gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. mallei (ATCC23344)T             gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. multivorans (LMG13010)T         gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. norimbergensis (Y09879)T        gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. phenazinium (LMG2247)T          gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. phenoliruptrix (AC1100)T        gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. phymatum (STM815)T              gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. phytofirmans (PsJN)T            gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
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B. pseudomallei (ATCC23343)T       gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
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B. sacchari (AF263278)T            gttaagtccc gcaacgagcg caacccttgt ccccagttgc ta------cg
B. silvatlantica (SRMrh-20)T       gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. solanacearum (ATCC11696)T       gttaagtccc gcaacgagcg caacccttgt ctctagttgc ta------cg
B. sordicola (S5-B)T               gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
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B. terrae (KMY02)T                 gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. terriccola (LMG20594)T          gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. thailandensis (E264)T           gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. tropicalis (Ppe8)T              gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. tuberum (STM678)T               gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. unamae (MT1-641)T               gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
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B. xenovorans (LB400)T             gttaagtccc gcaacgagcg caccccttgt ccctagttgc ta------cg
B. cenocepacia (KN9)T              gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
B. ferrariae (FeG101)T             gttaagtccc gcaacgagcg caacccttgt ccctagttgc ta------cg
B. ambifaria (LMG19182)T           gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
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B. ubonensis (GTC-P3-415)T         gttaagtccc gcaacgagcg caacccttgt ccttagttgc ta------cg
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P. lemoignei (LMG2207)             gttaagtccc gcaacgagcg caacccttgt cattagttgc ta------cg
R. detusculanense (APF11)          gttaagtccc gcaacgagcg caacccttgt ctctagttgc ta------cg
R. solanacearum (R221)             gttaagtccc gcaacgagcg caacccttgt ctctagttgc ta------cg
R. pickettii (ATCC27512)           gttaagtccc gcaacgagcg caacccttgt ctctagttgc ta------cg
Az. caulinodans (LMG6465)          gttaagtccc gcaacgagcg caaccctcgc ctttagttgc catcatt-ca
P. aeruginosa (ATCC10145)          gttaagtccc gtaacgagcg caacccttgt ccttagttac cagcacctcg
K. pneumoniae (AU45)               gttaagtccc gcaacgagcg caacccttat cctttgttgc cagcggttag


                                       1110       1120       1130       1140       1150
B. andropogonis (ATCC23061)T       caagggcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. fungorum (LMG19076)T            caagagcact ccaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. caribiensi (MWAP64)T            caagagcact ctaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. caryophylii (ATCC25418)T        caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. cepacia (ATCC25416)T            caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. cocovenenas (ATCC33664)T        caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
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B. cocovenenas (LMG11626)T       caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. fungorum (LMG16225)           caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. gladioli (ATCC10248)T         caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. glathei (LMG14190)T           caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. glathei (ATCC29195)T          caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. glathei (ATCC29195)T          caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. glumae (LMG2196)T             caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. graminis (C4D1M)T             caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. hospita (LMG20598)T           caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. mallei (ATCC23344)T           caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. multivorans (LMG13010)T       caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. norimbergensis (Y09879)T      caagagcact ctaaggagac tgccggtgac aaacc--gag gaaggtgggg
B. phenazinium (LMG2247)T        caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
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B. phymatum (STM815)T            caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. phytofirmans (PsJN)T          caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. pickettii (ATCC27511)T        aaagggcact ctagagagac tgccggtgac aaaccg-gag gaaggtgggg
B. pseudomallei (ATCC23343)T     caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. pyrrocinia (LMG14191)T        caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
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B. sacchari (AF263278)T          caagagcact ccgggagac tgccggtgac aaaccg-gag gaaggtgggg
B. silvatlantica (SRMrh-20)T     caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. solanacearum (ATCC11696)T     aaagggcact ctagagagac tgccggtgac aaaccg-gag gaaggtgggg
B. sordicola (S5-B)T             caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. stabilis (LMG14294)T          caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. terrae (KMY02)T               caagagcact ctagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. terriccola (LMG20594)T        caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
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B. tuberum (STM678)T             caagagcact ccagggagg tgccggtgac aaaccg-gag gaaggtgggg
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B. vietnamiensis (AMMD)T         caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
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B. plantarii (LMG9035)T          caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. xenovorans (LB400)T           caagagcact ccagggagac tgccggtgac aacccg-gag gaaggtgggg
B. cenocepacia (KN9)T            caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. ferrariae (FeG101)T           caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
B. ambifaria (LMG19182)T         caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. oklahomensis (C6786)T         caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
B. ubonensis (GTC-P3-415)T       caagagcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
WC5.4c L. ambigua SA             caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
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WC21.1i L. pauciflora SA         caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
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WC21.1j L. pauciflora SA         caagagcact ccagggagac tgccggtgac aaaccg-gag gaaggtgggg
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P. lemoignei (LMG2207)           aaagggcact ctaatgagac tgccggtgac aaaccg-gag gaaggtgggg
R. detusculanense (APF11)        aaagggcact ctagagagac tgccggtgac aaaccg-gag gaaggtgggg
R. solanacearum (R221)           aaagggcact ctagagagac tgccggtgac aaaccg-gag gaaggtgggg
R. pickettii (ATCC27512)         aaagggcact ctagagagac tgccggtgac aaaccg-gag gaaggtgggg
Az. caulinodans (LMG6465)        gttgggcact ctaaagggac tgccggtgat aagccgcgag gaaggtgggg
P. aeruginosa (ATCC10145)        ggtgggcact ctaaggagac tgccggtgac aaaccg-gag gaaggtgggg
K. pneumoniae (AU45)             gccgggaact caaaggagac tgccagtgat aaactg-gag gaaggtgggg

                                     1160       1170       1180       1190       1200
B. andropogonis (ATCC23061)T     atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. fungorum (LMG19076)T          atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. caribiensi (MWAP64)T          atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. caryophylii (ATCC25418)T      atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. cepacia (ATCC25416)T          atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. cocovenenas (ATCC33664)T      atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. cocovenenas (LMG11626)T       atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. fungorum (LMG16225)           atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. gladioli (ATCC10248)T         atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
B. glathei (LMG14190)T           atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
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B. mallei (ATCC23344)T           atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
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P. lemoignei (LMG2207)               atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
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R. pickettii (ATCC27512)             atgacgtcaa gtcctcatgg cccttatggg tagggcttca cacgtcatac
Az. caulinodans (LMG6465)            atgacgtcaa gtcctcatgg cccttacggg ctgggctaca cacgtgctac
P. aeruginosa (ATCC10145)            atgacgtcaa gtcatcatgg cccttacggc cagggctaca cacgtgctac
K. pneumoniae (AU45)                 atgacgtcaa gtcatcatgg cccttacgac cagggctaca cacgtgctac


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B. caribiensi (MWAP64)T              aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
B. caryophylii (ATCC25418)T          aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. cepacia (ATCC25416)T              aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. cocovenenas (ATCC33664)T          aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
B. cocovenenas (LMG11626)T           aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
B. fungorum (LMG16225)               aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. gladioli (ATCC10248)T             aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
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B. multivorans (LMG13010)T           aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. norimbergensis (Y09879)T          aatggtcggt acagagggct gccaaatcgc gagatggagc taaccccaga
B. phenazinium (LMG2247)T            aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. phenoliruptrix (AC1100)T          aatggtcgga acagagggtc gccaagccgc gaggtggagc caatcccaga
B. phymatum (STM815)T                aatggtcgga acagagggtt gccaagccgc gaggcggagc caatcccaga
B. phytofirmans (PsJN)T              aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. pickettii (ATCC27511)T            aatggtgcat acagagggtt gccaagccgc gaggtggagc taatcccaga
B. pseudomallei (ATCC23343)T         aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. pyrrocinia (LMG14191)T            aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. pyrrocinia ATCC15958)T            aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. sacchari (AF263278)T              aatggtcgga acagagggtt gcgaagccgc gaggtggagc caatcccaga
B. silvatlantica (SRMrh-20)T         aatggtcgga acagagggta gccaagccgc gaggtggagc caatcccgga
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B. solanacearum (ATCC11696)T    aatggtgcat acagagggtt gccaagccgc gaggtggagc taatcccaga
B. sordicola (S5-B)T            aatggtcgga acagagggct gccaacccgt gagggggagc taatcccaga
B. stabilis (LMG14294)T         aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. terrae (KMY02)T              aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
B. terriccola (LMG20594)T       aatggtcgga acagagggtc gccaacccgc gaggtggagc caatcccaga
B. thailandensis (E264)T        aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. tropicalis (Ppe8)T           aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
B. tuberum (STM678)T            aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
B. unamae (MT1-641)T            aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
B. vandii (LMG16020)T           aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
B. vietnamiensis (AMMD)T        aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. vietnamiensis (TVV75)T       aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. vietnamiensis (TVV70)T       aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. plantarii (LMG9035)T         aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
B. xenovorans (LB400)T          aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. cenocepacia (KN9)T           aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
B. ferrariae (FeG101)T          aatggtcgga acagagggtt gcgaagccgc gaggtggagc caatcccaga
B. ambifaria (LMG19182)T        aatggtcgga acagagggtt gccaacccgc gagggggagc taatcccaga
B. oklahomensis (C6786)T        aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
B. ubonensis (GTC-P3-415)T      aatggtcgga acagagggtc gccaacccgc gagggggagc taatcccaga
WC5.4c L. ambigua SA            aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
WC5.4d L. ambigua SA            aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
WC5.4e L. ambigua SA            aatggtcgga acagagggtc gccaacccgc gagggggagc caatcccaga
WC21.1i L. pauciflora SA        aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccgga
WC21.1b L. pauciflora SA        aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccgga
WC21.1j L. pauciflora SA        aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
WC26.1d L. meyerianna SA        aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
WC26.1e L. meyerianna SA        aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
WC26.1f L. meyerianna SA        aatggtcgga acagagggtt gccaagccgc gaggtggagc caatcccaga
P. lemoignei (LMG2207)          aatggtacat acagagggct gccaacccgc gagggggagc taatcccaga
R. detusculanense (APF11)       aatggtgcat acagagggtt gccaagccgc gaggtggagc taatcccaga
R. solanacearum (R221)          aatggtgcat acagagggtt gccaagccgc gaggtggagc taatcccaga
R. pickettii (ATCC27512)        aatggtgcat acagagggtt gccaagccgc gaggtggagc taatcccaga
Az. caulinodans (LMG6465)       aatggcggtg acaatgggat gcgagcctgc gagggtgagc aaatctccaa
P. aeruginosa (ATCC10145)       aatggtcggt acaaagggtt gccaagccgc gaggtggagc taatcccata
K. pneumoniae (AU45)            aatggcatat acaaagagaa gcgacctcgc gagagcaagc ggacctcata


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B. andropogonis (ATCC23061)T    aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagt
B. fungorum (LMG19076)T         aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. caribiensi (MWAP64)T         aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagc
B. caryophylii (ATCC25418)T     aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. cepacia (ATCC25416)T         aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. cocovenenas (ATCC33664)T     aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. cocovenenas (LMG11626)T      aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. fungorum (LMG16225)          aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. gladioli (ATCC10248)T        aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. glathei (LMG14190)T          aaaccgat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
B. glathei (ATCC29195)T         aaaccgat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
B. glathei (ATCC29195)T         aaaccgat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
B. glumae (LMG2196)T            aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. graminis (C4D1M)T            aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. hospita (LMG20598)T          aaaccgat-c gtagt-ccgg atcgcactct gcaactcgac tgcgtgaagc
B. mallei (ATCC23344)T          aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. multivorans (LMG13010)T      aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. norimbergensis (Y09879)T     aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagt
B. phenazinium (LMG2247)T       aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. phenoliruptrix (AC1100)T     aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. phymatum (STM815)T           aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagc
B. phytofirmans (PsJN)T         aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. pickettii (ATCC27511)T       aaatgcat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
B. pseudomallei (ATCC23343)T    aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. pyrrocinia (LMG14191)T       aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. pyrrocinia ATCC15958)T       aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. sacchari (AF263278)T         aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagc
B. silvatlantica (SRMrh-20)T    aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagc
B. solanacearum (ATCC11696)T    aaatgcat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
B. sordicola (S5-B)T            aaaccgat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
B. stabilis (LMG14294)T         aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. terrae (KMY02)T              aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgag tgcgtgaagc
B. terriccola (LMG20594)T       aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
B. thailandensis (E264)T        aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. tropicalis (Ppe8)T           aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagc
B. tuberum (STM678)T            aaaccgat-c gtagt-ccgg atcgcagtct gcaactcggg tgcgtgaagc
B. unamae (MT1-641)T            aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgag tgcgtgaagc
B. vandii (LMG16020)T           aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. vietnamiensis (AMMD)T        aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
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B. vietnamiensis (TVV75)T        aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. vietnamiensis (TVV70)T        aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. plantarii (LMG9035)T          aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. xenovorans (LB400)T           aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
B. cenocepacia (KN9)T            aaaccgatgc gtagtgccgg attgca---- ---------- ----------
B. ferrariae (FeG101)T           aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagc
B. ambifaria (LMG19182)T         aaaccgat-c gtagt-ccgg attgcactct gcaactcgac tgcgtgaagc
B. oklahomensis (C6786)T         aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
B. ubonensis (GTC-P3-415)T       aaaccgat-c gtagt-ccgg attgcactct gcaactcgag tgcatgaagc
WC5.4c L. ambigua SA             aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
WC5.4d L. ambigua SA             aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
WC5.4e L. ambigua SA             aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
WC21.1i L. pauciflora SA         aaaccgat-c gtagt-ccgg atcgcactct gcaactcgag tgcgtgaagc
WC21.1b L. pauciflora SA         aaaccgat-c gtggt-ccgg atcgcactct gcaactcgag tgcgtgaagc
WC21.1j L. pauciflora SA         aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
WC26.1d L. meyerianna SA         aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
WC26.1e L. meyerianna SA         aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
WC26.1f L. meyerianna SA         aaaccgat-c gtagt-ccgg atcgcactct gcaactcggg tgcgtgaagc
P. lemoignei (LMG2207)           aagtgtat-c gtagt-ccgg attgtagtct gcaactcgac tacatgaagt
R. detusculanense (APF11)        aaatgcat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
R. solanacearum (R221)           aaatgcat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
R. pickettii (ATCC27512)         aaatgcat-c gtagt-ccgg atcgtagtct gcaactcgac tacgtgaagc
Az. caulinodans (LMG6465)        aagccg-t-c tcagt-tcgg attgcactct gcaactcgag tgcatgaagt
P. aeruginosa (ATCC10145)        aaaccgat-c gtagt-ccgg atcgcagtct gcaactcgac tgcgtgaagt
K. pneumoniae (AU45)             aagtatgt-c gtagt-ccgg attggagtct gcaactcgac tccatgaagt


                                    1310       1320       1330       1340       1350
B. andropogonis (ATCC23061)T     tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. fungorum (LMG19076)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. caribiensi (MWAP64)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. caryophylii (ATCC25418)T      tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. cepacia (ATCC25416)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. cocovenenas (ATCC33664)T      tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. cocovenenas (LMG11626)T       tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. fungorum (LMG16225)           tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. gladioli (ATCC10248)T         tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. glathei (LMG14190)T           tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. glathei (ATCC29195)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. glathei (ATCC29195)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. glumae (LMG2196)T             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. graminis (C4D1M)T             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. hospita (LMG20598)T           tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa taccgttccc
B. mallei (ATCC23344)T           tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. multivorans (LMG13010)T       tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. norimbergensis (Y09879)T      tggaatcgct agtaatcgcg gatcagcatg tcgcggtgaa ta-cgttccc
B. phenazinium (LMG2247)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. phenoliruptrix (AC1100)T      tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. phymatum (STM815)T            tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. phytofirmans (PsJN)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. pickettii (ATCC27511)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. pseudomallei (ATCC23343)T     tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. pyrrocinia (LMG14191)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. pyrrocinia ATCC15958)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. sacchari (AF263278)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. silvatlantica (SRMrh-20)T     tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. solanacearum (ATCC11696)T     tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. sordicola (S5-B)T             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. stabilis (LMG14294)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. terrae (KMY02)T               tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. terriccola (LMG20594)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. thailandensis (E264)T         tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. tropicalis (Ppe8)T            tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. tuberum (STM678)T             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. unamae (MT1-641)T             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. vandii (LMG16020)T            tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. vietnamiensis (AMMD)T         tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. vietnamiensis (TVV75)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. vietnamiensis (TVV70)T        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. plantarii (LMG9035)T          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. xenovorans (LB400)T           tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. cenocepacia (KN9)T            ---------- ---------- ---------- ---------- ----------
B. ferrariae (FeG101)T           tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. ambifaria (LMG19182)T         tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. oklahomensis (C6786)T         tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
B. ubonensis (GTC-P3-415)T       tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
WC5.4c L. ambigua SA             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
WC5.4d L. ambigua SA             tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
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WC5.4e L. ambigua SA            tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
WC21.1i L. pauciflora SA        tggaatcgct agtaatcgcg gatcagcatg ccgcgg-aga ta-cgttccc
WC21.1b L. pauciflora SA        tggaatcgct agtaatcgcg gatcagcatg ccgcat-gaa ta-cgttccc
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WC26.1d L. meyerianna SA        tggaatcgct agtaatcgcg gatcagcatg cggcggtgaa ta-cgttccc
WC26.1e L. meyerianna SA        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
WC26.1f L. meyerianna SA        tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
P. lemoignei (LMG2207)          tggaatcgct agtaatcgcg gatcagcatg tcgcggtgaa ta-cgttccc
R. detusculanense (APF11)       tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
R. solanacearum (R221)          tggaatcgct agtaatcgcg gatcagcatg ccgcggtgaa ta-cgttccc
R. pickettii (ATCC27512)        tggaatcgct agtaatcgcg gatcagcatg ccgcggt--- ----------
Az. caulinodans (LMG6465)       tggaatcgct agtaatcgtg gatcagcatg ccacggtgaa ta-cgttccc
P. aeruginosa (ATCC10145)       cggaatcgct agtaatcgtg aatcagaatg tcacggtgaa ta-cgttccc
K. pneumoniae (AU45)            cggaatcgct agtaatcgta gatcagaatg ctacggtgaa ta-cgttccc


                                1360       1370       1380       1390       1400
B. andropogonis (ATCC23061)T    gggtcttgta cacaccgccc gtcacaccat gggagcgggt ttcaccagaa
B. fungorum (LMG19076)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt ttcaccagaa
B. caribiensi (MWAP64)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. caryophylii (ATCC25418)T     gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. cepacia (ATCC25416)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. cocovenenas (ATCC33664)T     gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. cocovenenas (LMG11626)T      gggt-ttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. fungorum (LMG16225)          gggtcttgta cacaccgccc gtcacaccat gggagtgggt ttcaccagaa
B. gladioli (ATCC10248)T        gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. glathei (LMG14190)T          gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. glathei (ATCC29195)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. glathei (ATCC29195)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. glumae (LMG2196)T            gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. graminis (C4D1M)T            gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. hospita (LMG20598)T          gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. mallei (ATCC23344)T          gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. multivorans (LMG13010)T      gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. norimbergensis (Y09879)T     gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. phenazinium (LMG2247)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. phenoliruptrix (AC1100)T     gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. phymatum (STM815)T           gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. phytofirmans (PsJN)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. pickettii (ATCC27511)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggc tttaccagaa
B. pseudomallei (ATCC23343)T    gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. pyrrocinia (LMG14191)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. pyrrocinia ATCC15958)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. sacchari (AF263278)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. silvatlantica (SRMrh-20)T    gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. solanacearum (ATCC11696)T    gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. sordicola (S5-B)T            gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. stabilis (LMG14294)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. terrae (KMY02)T              gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. terriccola (LMG20594)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. thailandensis (E264)T        gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. tropicalis (Ppe8)T           gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. tuberum (STM678)T            gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. unamae (MT1-641)T            gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. vandii (LMG16020)T           gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. vietnamiensis (AMMD)T        gggtcatgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. vietnamiensis (TVV75)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. vietnamiensis (TVV70)T       gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. plantarii (LMG9035)T         gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. xenovorans (LB400)T          gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. cenocepacia (KN9)T           ---------- ---------- ---------- ---------- ----------
B. ferrariae (FeG101)T          gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttgccagaa
B. ambifaria (LMG19182)T        gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. oklahomensis (C6786)T        gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
B. ubonensis (GTC-P3-415)T      gggtcttgta cacaccgccc gtcacaccat gggagtgggt tttaccagaa
WC5.4c L. ambigua SA            gggtcttgta cacaccgccc gtca-agcat gggagtgggt tttaccagaa
WC5.4d L. ambigua SA            gggtcttgta cacaccgccc gtcacaccat gggagtgggt ttcaccagaa
WC5.4e L. ambigua SA            gggtcttgta cacaccgccc gtcacaccat gggagtgggt ttcaccagaa
WC21.1i L. pauciflora SA        gggtcttgta cacaccgccc gtt---cacg tggagtgggt tttaccagaa
WC21.1b L. pauciflora SA        gggtcttgta cacaccgccc gtcactccat gggagtgggt tttaccagaa
WC21.1j L. pauciflora SA        gggtcttgta cacaccgccc gtcacaccat gggagtgggt ttcaccagaa
WC26.1d L. meyerianna SA        gggtcttgta cacaccgccc a-aagaccat gggagtgggt tttaccagaa
WC26.1e L. meyerianna SA        gggtcttgta cacaccgccc gtctctccat gggagtgggt tttaccagaa
WC26.1f L. meyerianna SA        gggtcttgta cacaccgccc gacacaccat gggagtgggt tttaccagaa
P. lemoignei (LMG2207)          gggtcttgta cacaccgccc gtcacaccat gggagcgggt tttaccagaa
R. detusculanense (APF11)       gggtcttgta cacaccgccc gtcacaccat gggagtgggc tttaccagaa
R. solanacearum (R221)          gggtcttgta cacaccgccc gtcacaccat gggagtgggc tttaccagaa
R. pickettii (ATCC27512)        ---------- ---------- ---------- ---------- ----------
```

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Az. caulinodans (LMG6465)      gggccttcta cacaccgccc gtcacaccat gggagttggc tttacccgaa
P. aeruginosa (ATCC10145)      gggccttgta cacaccgccc gtcacaccat gggagtgggt tgctccagaa
K. pneumoniae (AU45)           gggccttgta cacaccgccc gtcacaccat gggagtgggt tgcaaaagaa


                                         1410
B. andropogonis (ATCC23061)T   gtaggtagct taacc
B. fungorum (LMG19076)T        gtaggtagcc taacc
B. caribiensi (MWAP64)T        gtggctagtc taacc
B. caryophylii (ATCC25418)T    gtggcagtct taacc
B. cepacia (ATCC25416)T        gtggctagtc taacc
B. cocovenenas (ATCC33664)T    gtggctagtc taacc
B. cocovenenas (LMG11626)T     gtggctagtc taacc
B. fungorum (LMG16225)         gtggctagtc taacc
B. gladioli (ATCC10248)T       gtggctagtc taacc
B. glathei (LMG14190)T         gtggctagtc taacc
B. glathei (ATCC29195)T        gtggctagtc taacc
B. glathei (ATCC29195)T        gtggctagtc taacc
B. glumae (LMG2196)T           gtggctagtc taacc
B. graminis (C4D1M)T           gtggctagtc taacc
B. hospita (LMG20598)T         gtggctagtc taacc
B. mallei (ATCC23344)T         gtggctagtc taacc
B. multivorans (LMG13010)T     gtggctagtc taacc
B. norimbergensis (Y09879)T    gtaggtagcc ttaac
B. phenazinium (LMG2247)T      gtggctagtc taacc
B. phenoliruptrix (AC1100)T    gtggctagtc taacc
B. phymatum (STM815)T          gtggctagtc taacc
B. phytofirmans (PsJN)T        gtggctagtc taacc
B. pickettii (ATCC27511)T      gtagttagcc taacc
B. pseudomallei (ATCC23343)T   gtggctagtc taacc
B. pyrrocinia (LMG14191)T      gtggctagtc taacc
B. pyrrocinia ATCC15958)T      gtggctagtc taacc
B. sacchari (AF263278)T        gtggctagtc taacc
B. silvatlantica (SRMrh-20)T   gtggctagtc taacc
B. solanacearum (ATCC11696)T   gtagttagcc ttaac
B. sordicola (S5-B)T           gtggctagtc taacc
B. stabilis (LMG14294)T        gtggctagtc taacc
B. terrae (KMY02)T             gtggctagtc taacc
B. terriccola (LMG20594)T      gtggctagtc taacc
B. thailandensis (E264)T       gtggctagtc taacc
B. tropicalis (Ppe8)T          gtggctagtc t----
B. tuberum (STM678)T           gtggctagtc taacc
B. unamae (MT1-641)T           gtggctagtc taacc
B. vandii (LMG16020)T          gtggctagtc taacc
B. vietnamiensis (AMMD)T       gtggctagtc taacc
B. vietnamiensis (TVV75)T      gtggctagtc taacc
B. vietnamiensis (TVV70)T      gtggctagtc taacc
B. plantarii (LMG9035)T        gtggctagtc taacc
B. xenovorans (LB400)T         gtggctagtc taacc
B. cenocepacia (KN9)T          ---------- -----
B. ferrariae (FeG101)T         gtggctagtc taacc
B. ambifaria (LMG19182)T       gtggctagtc taacc
B. oklahomensis (C6786)T       gtggctagtc taacc
B. ubonensis (GTC-P3-415)T     gtggctagtc taacc
WC5.4c L. ambigua SA           gtggctagtc taacc
WC5.4d L. ambigua SA           gtaggtagcc taacc
WC5.4e L. ambigua SA           gtaggtagcc taacc
WC21.1i L. pauciflora SA       gtggctagtc taacc
WC21.1b L. pauciflora SA       gtggctagtc taacc
WC21.1j L. pauciflora SA       gtggctagtc taacc
WC26.1d L. meyerianna SA       gtggctagtc taacc
WC26.1e L. meyerianna SA       gtggctagtc taacc
WC26.1f L. meyerianna SA       gtggctagtc taacc
P. lemoignei (LMG2207)         gtaggtagct taacc
R. detusculanense (APF11)      gtagttagcc taacc
R. solanacearum (R221)         gtagttagcc taacc
R. pickettii (ATCC27512)       -----tagcc taacc
Az. caulinodans (LMG6465)      ggcgttgcgc taacc
P. aeruginosa (ATCC10145)      gtagctagtc taacc
K. pneumoniae (AU45)           gtaggtagct taacc
```

APPENDIX C: α-RHIZOBIA 16S rRNA alignment

```
M. mediterraneum (LMG17148)T         tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
M. septentrionale (SDW014)T          tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
M. temperatum (SDW018)T              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
M. thiogangeticum (SJT)T             tgcaagtcga gc----gcc- ---------- -ccgca--gg gggagcgtgc
M.tianshanense (A-1BS)T              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC12.1a L. sepiaria SA               tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC19.1b L. cytisoides SA             --gcagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC19.1c L. cytisoides SA             tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC23.1b L. multiflora SA             -----gtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC23.1c L. multiflora SA             tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC23.1d L. multiflora SA             tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC28.1F L. sericea SA                tgc-agtcga gc----gcc- ---------- -ccgga--tg gggagcg-gc
WC28.1g L. sericea SA                tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC28.1h L. sericea SA                tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC28.1i L. sericea SA                tgc-agtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC33b L. sericea SA                  tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC33c L. sericea SA                  tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC33e L. sericea SA                  tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
WC33h L. sericea SA                  ---------- gc----gcc- ---------- -ccgga--ag gggagcg-gc
S. americanum (CFNEI156)T            tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
S. arboris (LMG14919)T               tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
S. fredii (LMG6217)T                 tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
S. kostiense (LMG19227)              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
S. kummerowiae (CCBAU71714)T         tgcaagtcga ac----gcc- ---------- -ccgca--ag gggagcg-gc
S. meliloti (LMG6133)T               tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BH1LSc L. spinescens SA              tgcgagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BH2LSa L. spinescens SA              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BH3LSd L. spinescens SA              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BW1LSab L. spinescens SA             tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BW2LSa L. spinescens SA              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BW2LSb L. spinescens SA              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BW2LSc L. spinescens SA              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
BW3LSd L. spinescens SA              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
B. canariense (MSFC2-M3-R-3)T        ----agtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
B. japonicum (LMG6138)T              tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
B. liaoningense (LMG18230)T          tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
Betal106d L. pungens SA              tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
Betal106a L. pungens SA              -gcgagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
NK22 L. sericea SA                   tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
WC21.1L L. pauciflora SA             ------tcga gcgg--gca- ---------- -tagcaatat gtcagcg-gc
XHR1a L. simsiana SA                 ----agtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
Betal106f L. pungens SA              tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
R. palustris (ATCC17001)             tgcaagtcga acgg--gcg- ---------- -tagcaatac gtcagtg-gc
M. nodulans (ORS2060)                tgcaagtcga gcgg--gcc- ---------- -ctgcg--gg gtcagcgc-gc
Az. caulinodans (LMG6465)            tgcaagtcga gc----gcc- ---------- -cagca--at gggagcg-gc
R. rubrum (LMGATCC11170)             tgcaagtcga acgc--atc- ---------- -cttcg--gg atgagtg-gc
A. magnetotacticum (M58171)          tgcaagtcga acga--agt- ---------- -cttcg--ga cttagtg-gc
Az. brasilense (NCIMB11860)          tgcaagtcga acga--agg- ---------- -cttcg--gc cttagtg-gc
Az. lipoferum (NCIMB11861)           tgcaagtcga acga--ggg- -----tttcc ttcgggggc cttagtg-gc
R. acidophila (M34128)               tgcaagtcga acgg--gca- ---------- -tagcaatat gtcagtg-gc
A. clevelandensis (M69186)           tgcaagtcga acgg--gcg- ---------- -tagcaatac gtcagtg-gc
S. morelense (LMG21331)T             tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
M. amorphae (LMG18977)T              tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
M. ciceri (LMG14989)T                tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
M. huakuii (LMG14107)T               tgcaagtcga gc----g-c- ---------- -ccgca--ag gggagcg-gc
M. loti (LMG6125)T                   tgcaagtcga gc----gcc- ---------- -tcgca--a- --gagcg-gc
M. plurifarium (LMG11892)T           tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc
B. elkanii (LMG6134)T                tgcaagtcga gcgg--gca- ---------- -tagcaatat gtcagcg-gc
B. beta (LMG21987)T                  tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
B. yuanmingense (CCBAU10071)T        tgcaagtcga gcgg--gcg- ---------- -tagcaatac gtcagcg-gc
S. medicae (A321)T                   tgcaagtcga gc----gcc- ---------- -ccgca--ag gggagcg-gc

                                            60         70         80         90        100
M. mediterraneum (LMG17148)T         agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. septentrionale (SDW014)T          agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. temperatum (SDW018)T              agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. thiogangeticum (SJT)T             agacgggtga gtaacgcgtg ggaatctacc cagctctacg gaataaccca
M.tianshanense (A-1BS)T              agacgggtga gtaacgcgtg ggaatctacc catctctacg gagcaactcc
WC12.1a L. sepiaria SA               agacgggtga gtaacgcttg -gaatctacc catctctacg gaacaactcc
WC19.1b L. cytisoides SA             agacgggtga gtaaccctt- -aaagctacc catctctacg gaacaactcc
WC19.1c L. cytisoides SA             agacgggtga gtaa--cggg ggaatctacc catctctacg gaacaactcc
WC23.1b L. multiflora SA             agacgggtga gtaacgccgg ggaatctacc catctctacg gaacaactcc
WC23.1c L. multiflora SA             agacgggtga gtaacgctt- -ggatctccc catctctacg gaacaactcc
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WC23.1d L. multiflora SA          agacgggtga gtaacgcgtg tgaatctacc catctctacg gaacaactcc
WC28.1F L. sericea SA             agacgggtga gtaagtttt- -aaaggtacc catctctacg gaacaactcc
WC28.1g L. sericea SA             agacgggtga gtaacgcgtg tgaatctacc catctctacg gaacaactcc
WC28.1h L. sericea SA             agacgggtga gtaa---ggg ggaatctacc catctctacg gaacaactcc
WC28.1i L. sericea SA             agacgggtga gtaacggccg tgaatctacc catctctacg gaacaactcc
WC33b L. sericea SA               agacgggtga gtaacgcgtg tgaatctacc catctctacg gaacaactcc
WC33c L. sericea SA               agacgggtga gtaacgcgt- --tatctacc catctctacg gaacaactcc
WC33e L. sericea SA               agacgggtga gtaa--cggg ggaatctacc catctctacg gaacaactcc
WC33h L. sericea SA               agacgggtga gtaaggcctg aaaggctacc catctctacg gaacaactcc
S. americanum (CFNEI156)T         agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
S. arboris (LMG14919)T            agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
S. fredii (LMG6217)T              agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
S. kostiense (LMG19227)           agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
S. kummerowiae (CCBAU71714)T      agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
S. meliloti (LMG6133)T            agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
BH1LSc L. spinescens SA           agacgggtga gtaacgctt- --gatctacc cttttctacg gaataacgca
BH2LSa L. spinescens SA           agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
BH3LSd L. spinescens SA           agacgggtga gtaacgcgtt ggaatctacc cttttctacg gaataacgca
BW1LSab L. spinescens SA          agacgggtga gtaacgcgcg ggaatctacc cttttctacg gaataacgca
BW2LSa L. spinescens SA           agacgggtga gtaacgcgt- -gaatctacc cttttctacg gaataacgca
BW2LSb L. spinescens SA           agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
BW2LSc L. spinescens SA           agacgggtga gtaacgctt- ggaatctacc cttttctacg gaataacgca
BW3LSd L. spinescens SA           agacgggtga gtaacgcct- ggaatctacc cttttctacg gaataacgca
B. canariense (MSFC2-M3-R-3)T     agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaacaca
B. japonicum (LMG6138)T           agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaacaca
B. liaoningense (LMG18230)T       agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaacaca
Betal106d L. pungens SA           agacgggtga gtaa-gcgtg ggaacgtacc ttttggttcg gaacaaccca
Betal106a L. pungens SA           agacgggtga gc-ctgcatg ggaacgtacc ttttggttcg gaacaaccga
NK22 L. sericea SA                agacgggtga gtaat-cgag ggaacgtacc ttttggttcg gaacaacaca
WC21.1L L. pauciflora SA          agacgggtga gt-acgtgag ggaacgtacc ttttggttcg gaacaactga
XHR1a L. simsiana SA              agacgggtga gcaatgagtg ggaacatacc ttttggttcg gaacaacaca
Betal106f L. pungens SA           agacgggtga gtaac-catg agaacgtacc ttttggttcg gaacaacaca
R. palustris (ATCC17001)          agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaacaca
M. nodulans (ORS2060)             agacgggtga gtaacgcgtg ggaacgtgcc cttcggttcg gaacaaccct
Az. caulinodans (LMG6465)         agacgggtga gtaacgcgtg gggatgtgcc caatggtgcg gaataaccca
R. rubrum (LMGATCC11170)          gcacgggtga gtaacacgtg ggaacgtacc ttggagtgcg gaataatctt
A. magnetotacticum (M58171)       gcacgggtga gtaacacgtg ggaatatacc tcttggtggg gaataacgtc
Az. brasilense (NCIMB11860)       gcacgggtga gtaacacgtg ggaacctgcc tttcggttcg ggataacgtc
Az. lipoferum (NCIMB11861)        gcacgggtga gtaacacgtg ggaacctgcc tttcggttcg gaataacgtc
R. acidophila (M34128)            agacgggtga ggaacacgtg ggaacatgcc ctttggttcg gaacaactca
A. clevelandensis (M69186)        agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaacaca
S. morelense (LMG21331)T          agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca
M. amorphae (LMG18977)T           agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. ciceri (LMG14989)T             agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. huakuii (LMG14107)T            agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. loti (LMG6125)T                agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
M. plurifarium (LMG11892)T        agacgggtga gtaacgcgtg ggaatctacc catctctacg gaacaactcc
B. elkanii (LMG6134)T             agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaactga
B. beta (LMG21987)T               agacgggtga gtaacgcgtg ggaacatacc ttttggttcg gaacaacaca
B. yuanmingense (CCBAU10071)T     agacgggtga gtaacgcgtg ggaacgtacc ttttggttcg gaacaacaca
S. medicae (A321)T                agacgggtga gtaacgcgtg ggaatctacc cttttctacg gaataacgca


                                         110        120        130        140        150
M. mediterraneum (LMG17148)T      gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
M. septentrionale (SDW014)T       gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
M. temperatum (SDW018)T           gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
M. thiogangeticum (SJT)T          gggaaacttg gactaatacc gtatacgtcc tattgga--- --------g
M.tianshanense (A-1BS)T           gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
WC12.1a L. sepiaria SA            gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC19.1b L. cytisoides SA          ggg-aactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC19.1c L. cytisoides SA          gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC23.1b L. multiflora SA          gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC23.1c L. multiflora SA          gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC23.1d L. multiflora SA          gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC28.1F L. sericea SA             ggg-aactgg aggtaatacc gtatacgtcc ttttgga--- --------g
WC28.1g L. sericea SA             gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC28.1h L. sericea SA             gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC28.1i L. sericea SA             gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC33b L. sericea SA               gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC33c L. sericea SA               gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC33e L. sericea SA               gggaaactgg agctaatacc gtatacgtcc ttttgga--- --------g
WC33h L. sericea SA               ggg-aacagg agctaatacc gtatacgtcc ttttgga--- --------g
S. americanum (CFNEI156)T         gggaaacttg tgctaatacc gtatgagccc ttcgggg--- --------g
S. arboris (LMG14919)T            gggaaacttg tgctaatacc gtataagccc ttcgggg--- --------g
S. fredii (LMG6217)T              gggaaacttg tgctaatacc gtatgagccc ttcgggg--- --------g
S. kostiense (LMG19227)           gggaaacttg tgctaatacc gtatacgccc ttcgggg--- --------g
S. kummerowiae (CCBAU71714)T      gggaaacttg tgctaatacc gtatgagccc ttcgggg--- --------g
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S. meliloti (LMG6133)T              gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BH1LSc L. spinescens SA             gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BH2LSa L. spinescens SA             gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BH3LSd L. spinescens SA             gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BW1LSab L. spinescens SA            gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BW2LSa L. spinescens SA             gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BW2LSb L. spinescens SA             gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
BW2LSc L. spinescens SA             gggaaatttg tgctaatacc gtatgagccc ttcggggg--- --------g
BW3LSd L. spinescens SA             gggaaacttg tgctaatacc gtatgagccc ttcggggg--- --------g
B. canariense (MSFC2-M3-R-3)T       gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
B. japonicum (LMG6138)T             gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
B. liaoningense (LMG18230)T         gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
Betal106d L. pungens SA             gggaaacttg ggctaatacc ggataagccc ttacggg--- --------g
Betal106a L. pungens SA             ggtaaacttg ggctaatacc ggataagccc ttacggg--- --------g
NK22 L. sericea SA                  gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
WC21.1L L. pauciflora SA            ggcaaacttc agctaatacc ggataagccc ttacggg--- --------g
XHR1a L. simsiana SA                gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
Betal106f L. pungens SA             gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
R. palustris (ATCC17001)            gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
M. nodulans (ORS2060)               gggaaactag ggctaatacc ggatacgtcc gcaagga--- --------g
Az. caulinodans (LMG6465)           gggaaacttg gattaatacc gcatgtgccc ttaggggg--- --------g
R. rubrum (LMGATCC11170)            tggaaacgag gactaatacc gcatacgccc ttagggg--- --------g
A. magnetotacticum (M58171)         gggaaactga cgctaatacc gcatacgccc ttcggggg--- --------g
Az. brasilense (NCIMB11860)         tggaaacgga cgctaacacc ggatacgtcc cccagagaga tttgg-----
Az. lipoferum (NCIMB11861)          tggaaacgga cgctaacacc ggatacgtcc tatgggg--- --------g
R. acidophila (M34128)              gggaaacttg agctaatacc ggataagcct ttcgagg--- --------g
A. clevelandensis (M69186)          gggaaacttg tgctaatacc ggataag-cc ttacggg--- --------g
S. morelense (LMG21331)T            tggaaacgtg tgctaatacc gtatgagccc ttcggggg--- --------g
M. amorphae (LMG18977)T             gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
M. ciceri (LMG14989)T               gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
M. huakuii (LMG14107)T              gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
M. loti (LMG6125)T                  gggaaactgg agctaatacc gtatacgt-c ttcggga--- --------g
M. plurifarium (LMG11892)T          gggaaactgg agctaatacc gtatacgtcc ttcggga--- --------g
B. elkanii (LMG6134)T               gggaaacttc agctaatacc ggataagccc ttacggg--- --------g
B. beta (LMG21987)T                 gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
B. yuanmingense (CCBAU10071)T       gggaaacttg tgctaatacc ggataagccc ttacggg--- --------g
S. medicae (A321)T                  gggaaacttg tgctaatacc gtataagccc ttcggggg--- --------g

                                               160        170        180        190        200
M. mediterraneum (LMG17148)T        aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. septentrionale (SDW014)T         aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. temperatum (SDW018)T             aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. thiogangeticum (SJT)T            aaa------- -------gat tt--atcgga gttggatgag cccgcgttgg
M.tianshanense (A-1BS)T             aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC12.1a L. sepiaria SA              aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC19.1b L. cytisoides SA            aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC19.1c L. cytisoides SA            aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC23.1b L. multiflora SA            aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC23.1c L. multiflora SA            aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC23.1d L. multiflora SA            aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC28.1F L. sericea SA               aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC28.1g L. sericea SA               aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC28.1h L. sericea SA               aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC28.1i L. sericea SA               aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC33b L. sericea SA                 aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC33c L. sericea SA                 aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC33e L. sericea SA                 aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
WC33h L. sericea SA                 aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
S. americanum (CFNEI156)T           aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
S. arboris (LMG14919)T              aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
S. fredii (LMG6217)T                aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
S. kostiense (LMG19227)             aaa------- -------gat tt--atcgga gaaggatgag cccgcgttgg
S. kummerowiae (CCBAU71714)T        aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
S. meliloti (LMG6133)T              aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BH1LSc L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BH2LSa L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BH3LSd L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BW1LSab L. spinescens SA            aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BW2LSa L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BW2LSb L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BW2LSc L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
BW3LSd L. spinescens SA             aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
B. canariense (MSFC2-M3-R-3)T       aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
B. japonicum (LMG6138)T             aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
B. liaoningense (LMG18230)T         aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
Betal106d L. pungens SA             aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
Betal106a L. pungens SA             aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
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NK22 L. sericea SA                  aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
WC21.1L L. pauciflora SA            aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
XHR1a L. simsiana SA                aaa------- -------gat tt--atcgcc gaaagattgg cccgcgtctg
Betal106f L. pungens SA             aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
R. palustris (ATCC17001)            aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
M. nodulans (ORS2060)               aaa------- -------ggc ttg-actgcc gaaggatcgg cccgcgtctg
Az. caulinodans (LMG6465)           aaa------- -------gat tt--atcgcc attggatcaa cccgcgtctg
R. rubrum (LMGATCC11170)            aaa------- -------gat tt--atcgct ccaagatcgg cccgcgtccg
A. magnetotacticum (M58171)         aaa------- -------gat tt--atcgcc gagagattag cccgcgtccg
Az. brasilense (NCIMB11860)         aga------- -------aag tw--tacgcc rwgagagggg cccgcgtccg
Az. lipoferum (NCIMB11861)          aaa------- ---------g tt--tacgcc gagagaggg  cccgcgtcgg
R. acidophila (M34128)              aaa------- -------gat tt--atcgcc gaaggagtgg cccgcgtccg
A. clevelandensis (M69186)          aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
S. morelense (LMG21331)T            aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg
M. amorphae (LMG18977)T             aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. ciceri (LMG14989)T               aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. huakuii (LMG14107)T              aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. loti (LMG6125)T                  aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
M. plurifarium (LMG11892)T          aaa------- -------gat tt--atcgga gatggatgag cccgcgttgg
B. elkanii (LMG6134)T               aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
B. beta (LMG21987)T                 aaa------- -------gat tt--atcgcc gaaagattgg cccgcgtctg
B. yuanmingense (CCBAU10071)T       aaa------- -------gat tt--atcgcc gaaagatcgg cccgcgtctg
S. medicae (A321)T                  aaa------- -------gat tt--atcggg aaaggatgag cccgcgttgg


                                         210        220        230        240        250
M. mediterraneum (LMG17148)T        attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. septentrionale (SDW014)T         attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. temperatum (SDW018)T             attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. thiogangeticum (SJT)T            attagctagt tggtgaggta atggctcacc aaggcgacga tccatagctg
M.tianshanense (A-1BS)T             attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC12.1a L. sepiaria SA              attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC19.1b L. cytisoides SA            attcgctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC19.1c L. cytisoides SA            attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC23.1b L. multiflora SA            attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC23.1c L. multiflora SA            attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC23.1d L. multiflora SA            attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC28.1F L. sericea SA               attttctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC28.1g L. sericea SA               attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC28.1h L. sericea SA               attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC28.1i L. sericea SA               attagcttgt tggtggggta atggcctacc aaggcgacga tccatagctg
WC33b L. sericea SA                 attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC33c L. sericea SA                 attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC33e L. sericea SA                 attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
WC33h L. sericea SA                 attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
S. americanum (CFNEI156)T           attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
S. arboris (LMG14919)T              attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
S. fredii (LMG6217)T                attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
S. kostiense (LMG19227)             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
S. kummerowiae (CCBAU71714)T        attagctagt tggtggggta aaggcctacc aaggcgacca tccatagctg
S. meliloti (LMG6133)T              attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BH1LSc L. spinescens SA             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BH2LSa L. spinescens SA             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BH3LSd L. spinescens SA             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BW1LSab L. spinescens SA            attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BW2LSa L. spinescens SA             atta-ctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BW2LSb L. spinescens SA             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BW2LSc L. spinescens SA             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
BW3LSd L. spinescens SA             attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
B. canariense (MSFC2-M3-R-3)T       attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
B. japonicum (LMG6138)T             attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
B. liaoningense (LMG18230)T         attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
Betal106d L. pungens SA             attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
Betal106a L. pungens SA             attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
NK22 L. sericea SA                  attagctagt tggtagggta atggcctacc aaggcgacga tcagtagctg
WC21.1L L. pauciflora SA            attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
XHR1a L. simsiana SA                attagctagt tggtagggta atggcctacc aaggcgacga tcagtagctg
Betal106f L. pungens SA             attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
R. palustris (ATCC17001)            attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
M. nodulans (ORS2060)               attagctggt tggtggggta acggctcacc aaggcgacga tcagtagctg
Az. caulinodans (LMG6465)           attagctagt tggtgaggta aaggctcacc aaggcgacga tcagtagctg
R. rubrum (LMGATCC11170)            attagctagt tggcggggta atggcccacc aaggcgacga tcggtagctg
A. magnetotacticum (M58171)         attagctagt tggtgaggta atggctcacc aaggcgacga tcggtagctg
Az. brasilense (NCIMB11860)         attagtagt  tggtggggta atggcccacc aagccgacga tcggtagctg
Az. lipoferum (NCIMB11861)          attaggtagt tggtgaggta atggctcacc aagcctgcga tccgtagctg
R. acidophila (M34128)              attagctagt tggtgaggta atggctcacc aaggcgacga tcgg-agct-
A. clevelandensis (M69186)          attagctagt tggtgaggta acggctcacc aaggcgacga tcagtagctg
S. morelense (LMG21331)T            attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg
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M. amorphae (LMG18977)T             attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. ciceri (LMG14989)T               attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. huakuii (LMG14107)T              attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. loti (LMG6125)T                  attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
M. plurifarium (LMG11892)T          attagctagt tggtggggta atggcctacc aaggcgacga tccatagctg
B. elkanii (LMG6134)T               attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
B. beta (LMG21987)T                 attagctagt tggtagggta atggcctacc aaggcgacga tcagtagctg
B. yuanmingense (CCBAU10071)T       attagctagt tggtgaggta atggctcacc aaggcgacga tcagtagctg
S. medicae (A321)T                  attagctagt tggtggggta aaggcctacc aaggcgacga tccatagctg


                                           260        270        280        290        300
M. mediterraneum (LMG17148)T        gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
M. septentrionale (SDW014)T         gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
M. temperatum (SDW018)T             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
M. thiogangeticum (SJT)T            gtctgagagg atgatcagcc acactgggac tgagacacgg tccaaactcc
M.tianshanense (A-1BS)T             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
WC12.1a L. sepiaria SA              gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC19.1b L. cytisoides SA            gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
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WC23.1b L. multiflora SA            gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
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WC23.1d L. multiflora SA            gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC28.1F L. sericea SA               gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC28.1g L. sericea SA               gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC28.1h L. sericea SA               gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC28.1i L. sericea SA               gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC33b L. sericea SA                 gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
WC33c L. sericea SA                 gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
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WC33h L. sericea SA                 gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
S. americanum (CFNEI156)T           gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
S. arboris (LMG14919)T              gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
S. fredii (LMG6217)T                gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
S. kostiense (LMG19227)             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
S. kummerowiae (CCBAU71714)T        gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
S. meliloti (LMG6133)T              gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BH1LSc L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BH2LSa L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BH3LSd L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BW1LSab L. spinescens SA            gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BW2LSa L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BW2LSb L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BW2LSc L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
BW3LSd L. spinescens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
B. canariense (MSFC2-M3-R-3)T       gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
B. japonicum (LMG6138)T             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
B. liaoningense (LMG18230)T         gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
Betal106d L. pungens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
Betal106a L. pungens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
NK22 L. sericea SA                  gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
WC21.1L L. pauciflora SA            gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
XHR1a L. simsiana SA                gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
Betal106f L. pungens SA             gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
R. palustris (ATCC17001)            gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
M. nodulans (ORS2060)               gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
Az. caulinodans (LMG6465)           gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
R. rubrum (LMGATCC11170)            gtctgagagg atggccagcc acactgggac tgagacacgg cccagactcc
A. magnetotacticum (M58171)         gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
Az. brasilense (NCIMB11860)         gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
Az. lipoferum (NCIMB11861)          gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
R. acidophila (M34128)              gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
A. clevelandensis (M69186)          gtctgagagg atgatcagcc acattgggac tgagacacgg cc--aactcc
S. morelense (LMG21331)T            gtctgagagg atgatcagcc acactgggac tgagacacgg cccaaactcc
M. amorphae (LMG18977)T             gtctgagagg atgatcagcc acactgggac tgagacacgg cccaaactcc
M. ciceri (LMG14989)T               gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
M. huakuii (LMG14107)T              gtctgagagg atgatcagcc acactgggac tgagacacgg cccagactcc
M. loti (LMG6125)T                  gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
M. plurifarium (LMG11892)T          gtctgagagg atgatcagcc acactgggac tgagacacgg cccaaactcc
B. elkanii (LMG6134)T               gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
B. beta (LMG21987)T                 gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
B. yuanmingense (CCBAU10071)T       gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc
S. medicae (A321)T                  gtctgagagg atgatcagcc acattgggac tgagacacgg cccaaactcc


                                           310        320        330        340        350
M. mediterraneum (LMG17148)T        tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
M. septentrionale (SDW014)T         tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
M. temperatum (SDW018)T             tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
```

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M. thiogangeticum (SJT)T              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
M.tianshanense (A-1BS)T               tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
WC12.1a L. sepiaria SA                tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC19.1b L. cytisoides SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC19.1c L. cytisoides SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC23.1b L. multiflora SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC23.1c L. multiflora SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC23.1d L. multiflora SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC28.1F L. sericea SA                 tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC28.1g L. sericea SA                 tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC28.1h L. sericea SA                 tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC28.1i L. sericea SA                 tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC33b L. sericea SA                   tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC33c L. sericea SA                   tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC33e L. sericea SA                   tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
WC33h L. sericea SA                   tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
S. americanum (CFNEI156)T             tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
S. arboris (LMG14919)T                tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
S. fredii (LMG6217)T                  tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
S. kostiense (LMG19227)               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
S. kummerowiae (CCBAU71714)T          tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
S. meliloti (LMG6133)T                tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BH1LSc L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BH2LSa L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BH3LSd L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BW1LSab L. spinescens SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BW2LSa L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BW2LSb L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BW2LSc L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
BW3LSd L. spinescens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
B. canariense (MSFC2-M3-R-3)T         tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
B. japonicum (LMG6138)T               tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
B. liaoningense (LMG18230)T           tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
Betal106d L. pungens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
Betal106a L. pungens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
NK22 L. sericea SA                    tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
WC21.1L L. pauciflora SA              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
XHR1a L. simsiana SA                  tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
Betal106f L. pungens SA               tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
R. palustris (ATCC17001)              tacgggaggc agcagtgggg aatattggac aatgggggaa accctgatcc
M. nodulans (ORS2060)                 tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
Az. caulinodans (LMG6465)             tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
R. rubrum (LMGATCC11170)              tacgggaggc agcagtgggg aatattgcgc aatggggggca accctgacgc
A. magnetotacticum (M58171)           tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
Az. brasilense (NCIMB11860)           tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
Az. lipoferum (NCIMB11861)            tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
R. acidophila (M34128)                tacgggaggc agcagtgggg aatattggac aatgggcgca agcc-gatcc
A. clevelandensis (M69186)            tacgggaggc agcagtgggg aatattggac aatggggggca accct-atcc
S. morelense (LMG21331)T              tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
M. amorphae (LMG18977)T               tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
M. ciceri (LMG14989)T                 tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
M. huakuii (LMG14107)T                tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
M. loti (LMG6125)T                    tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
M. plurifarium (LMG11892)T            tacgggaggc agcagtgggg aatattggac aatgggcgaa agcctgatcc
B. elkanii (LMG6134)T                 tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc
B. beta (LMG21987)T                   tacgggaggc agcagtgggg aatattggac aatggggggca accctgatcc
B. yuanmingense (CCBAU10071)T         tacgggaggc agcagtgggg aatattggac aatgggcgca accctgatcc
S. medicae (A321)T                    tacgggaggc agcagtgggg aatattggac aatgggcgca agcctgatcc


                                      360        370        380        390        400
M. mediterraneum (LMG17148)T          agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
M. septentrionale (SDW014)T           agccatgccg cgtgagtgat gaag-ccta gggttgtaaa gctctttcaa
M. temperatum (SDW018)T               agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
M. thiogangeticum (SJT)T              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
M.tianshanense (A-1BS)T               agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC12.1a L. sepiaria SA                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC19.1b L. cytisoides SA              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC19.1c L. cytisoides SA              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC23.1b L. multiflora SA              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC23.1c L. multiflora SA              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC23.1d L. multiflora SA              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC28.1F L. sericea SA                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC28.1g L. sericea SA                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC28.1h L. sericea SA                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC28.1i L. sericea SA                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC33b L. sericea SA                   agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC33c L. sericea SA                   agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
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WC33e  L. sericea SA                    agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
WC33h  L. sericea SA                    agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
S. americanum (CFNEI156)T               agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
S. arboris (LMG14919)T                  agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
S. fredii (LMG6217)T                    agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
S. kostiense (LMG19227)                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
S. kummerowiae (CCBAU71714)T            agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
S. meliloti (LMG6133)T                  agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
BH1LSc  L. spinescens SA                agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
BH2LSa  L. spinescens SA                agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
BH3LSd  L. spinescens SA                agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
BW1LSab  L. spinescens SA               agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
BW2LSa  L. spinescens SA                agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
BW2LSb  L. spinescens SA                agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
BW2LSc  L. spinescens SA                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
BW3LSd  L. spinescens SA                agccatgccg cgtgagtgaa gaaggcccta gggttgtaaa gctctttcac
B. canariense (MSFC2-M3-R-3)T           agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
B. japonicum (LMG6138)T                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
B. liaoningense (LMG18230)T             agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
Betal106d  L. pungens SA                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
Betal106a  L. pungens SA                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
NK22  L. sericea SA                      agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
WC21.1L  L. pauciflora SA               agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
XHR1a  L. simsiana SA                    agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
Betal106f  L. pungens SA                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
R. palustris (ATCC17001)                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gct-ttttgt
M. nodulans (ORS2060)                   agccatgccg cgtgagtgat gacggcctta gggttgtaaa gctctttct
Az. caulinodans (LMG6465)               agccatgccg cgtgagtgat gaaggcctta gggttgtaaa gctctttcgc
R. rubrum (LMGATCC11170)                agccatgccg cgtgagtgaa gaaggccttc gggttgtaaa gctctttcgg
A. magnetotacticum (M58171)             agccatgccg cgtgagtgat gaaggcctta gggttgtaaa gctctttcac
Az. brasilense (NCIMB11860)             agcaatgccg cgtgagtgat gaaggcctta gggttgtaaa gctctttcgc
Az. lipoferum (NCIMB11861)              agcaatgccg cgtgagtgat gaaggcctta gggttgtaaa gctctttcgc
R. acidophila (M34128)                  agccatgccg cgtgagtgat gacggcccta gggttgtaaa gctctttcac
A. clevelandensis (M69186)              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
S. morelense (LMG21331)T                agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac
M. amorphae (LMG18977)T                 agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
M. ciceri (LMG14989)T                   agccatgccg cgtgagtgat gaaggtccta gggttgtaaa gctctttcaa
M. huakuii (LMG14107)T                  agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
M. loti (LMG6125)T                      agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
M. plurifarium (LMG11892)T              agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcaa
B. elkanii (LMG6134)T                   agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
B. beta (LMG21987)T                     agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
B. yuanmingense (CCBAU10071)T           agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctcttttgt
S. medicae (A321)T                      agccatgccg cgtgagtgat gaaggcccta gggttgtaaa gctctttcac


                                                 410        420        430        440        450
M. mediterraneum (LMG17148)T            cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. septentrionale (SDW014)T             cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. temperatum (SDW018)T                 cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. thiogangeticum (SJT)T                cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M.tianshanense (A-1BS)T                 cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC12.1a  L. sepiaria SA                 cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC19.1b  L. cytisoides SA               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC19.1c  L. cytisoides SA               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC23.1b  L. multiflora SA               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC23.1c  L. multiflora SA               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC23.1d  L. multiflora SA               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC28.1F  L. sericea SA                  cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC28.1g  L. sericea SA                  cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC28.1h  L. sericea SA                  cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC28.1i  L. sericea SA                  cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC33b  L. sericea SA                    cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC33c  L. sericea SA                    cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC33e  L. sericea SA                    cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
WC33h  L. sericea SA                    cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
S. americanum (CFNEI156)T               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
S. arboris (LMG14919)T                  cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
S. fredii (LMG6217)T                    cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
S. kostiense (LMG19227)                 cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
S. kummerowiae (CCBAU71714)T            cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
S. meliloti (LMG6133)T                  cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BH1LSc  L. spinescens SA                cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BH2LSa  L. spinescens SA                cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BH3LSd  L. spinescens SA                cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BW1LSab  L. spinescens SA               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BW2LSa  L. spinescens SA                cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BW2LSb  L. spinescens SA                cggtgaagat aa-------- ---------- -------tga cggtaaccccg
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BW2LSc L. spinescens SA          cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
BW3LSd L. spinescens SA          cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
B. canariense (MSFC2-M3-R-3)T    gcgggaagat aa-------- ---------- -------tga cggtac-cgc
B. japonicum (LMG6138)T          gcgggaagat aa-------- ---------- -------tga cggtac-cgc
B. liaoningense (LMG18230)T      gcgggaagat aa-------- ---------- -------tga cggtac-cgc
Betal106d L. pungens SA          gcgggaagat aa-------- ---------- -------tga cggtac-cgc
Betal106a L. pungens SA          gcgggaagat aa-------- ---------- -------tga cggtac-cgc
NK22 L. sericea SA               gcgggaagat aa-------- ---------- -------tga cggtac-cgc
WC21.1L L. pauciflora SA         gcgggaagat aa-------- ---------- -------tga cggtac-cgc
XHR1a L. simsiana SA             gcgggaagat aa-------- ---------- -------tga cggtac-cgc
Betal106f L. pungens SA          gcgggaagat aa-------- ---------- -------tga cggtac-cgc
R. palustris (ATCC17001)         gcgggaagat aa-------- ---------- -------tga cggtac-cgc
M. nodulans (ORS2060)            tcgggacgat aa-------- ---------- -------tga cggtac-cgg
Az. caulinodans (LMG6465)        cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
R. rubrum (LMGATCC11170)         gtgtgaagat ga-------- ---------- -------tga cggtaa-cac
A. magnetotacticum (M58171)      ccacgacgat ga-------- ---------- -------tga cggtag-tgg
Az. brasilense (NCIMB11860)      acgcgacgat ga-------- ---------- -------tga cggtag-cgt
Az. lipoferum (NCIMB11861)       acgcgacgat ga-------- ---------- -------tga cggtag-cgt
R. acidophila (M34128)           ccacgacgat aa-------- ---------- -------tga cggtag-tgg
A. clevelandensis (M69186)       gcgggaagat aa-------- ---------- -------tga cggtac-cgg
S. morelense (LMG21331)T         cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. amorphae (LMG18977)T          cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. ciceri (LMG14989)T            cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. huakuii (LMG14107)T           cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. loti (LMG6125)T               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
M. plurifarium (LMG11892)T       cggtgaagat aa-------- ---------- -------tga cggtaa-ccg
B. elkanii (LMG6134)T            gcgggaagat aa-------- ---------- -------tga cggtac-cgc
B. beta (LMG21987)T              gcgggaagat aa-------- ---------- -------tga cggtac-cgc
B. yuanmingense (CCBAU10071)T    gcgggaagat aa-------- ---------- -------tga cggtac-cgc
S. medicae (A321)T               cggtgaagat aa-------- ---------- -------tga cggtaa-ccg

                                     460        470        480        490        500
M. mediterraneum (LMG17148)T     tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. septentrionale (SDW014)T      tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. temperatum (SDW018)T          tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. thiogangeticum (SJT)T         gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M.tianshanense (A-1BS)T          tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC12.1a L. sepiaria SA           tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC19.1b L. cytisoides SA         tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC19.1c L. cytisoides SA         tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC23.1b L. multiflora SA         tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacgaaa-
WC23.1c L. multiflora SA         tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC23.1d L. multiflora SA         tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC28.1F L. sericea SA            tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC28.1g L. sericea SA            tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC28.1h L. sericea SA            tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC28.1i L. sericea SA            tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC33b L. sericea SA              tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC33c L. sericea SA              tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC33e L. sericea SA              tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC33h L. sericea SA              tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. americanum (CFNEI156)T        gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. arboris (LMG14919)T           gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. fredii (LMG6217)T             gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. kostiense (LMG19227)          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. kummerowiae (CCBAU71714)T     gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. meliloti (LMG6133)T           gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BH1LSc L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aag
BH2LSa L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BH3LSd L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BW1LSab L. spinescens SA         gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BW2LSa L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BW2LSb L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BW2LSc L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
BW3LSd L. spinescens SA          gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
B. canariense (MSFC2-M3-R-3)T    aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
B. japonicum (LMG6138)T          aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
B. liaoningense (LMG18230)T      aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
Betal106d L. pungens SA          aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
Betal106a L. pungens SA          aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
NK22 L. sericea SA               aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
WC21.1L L. pauciflora SA         aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
XHR1a L. simsiana SA             aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
Betal106f L. pungens SA          aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
R. palustris (ATCC17001)         aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. nodulans (ORS2060)            aggaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
Az. caulinodans (LMG6465)        gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
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R. rubrum (LMGATCC11170)              cagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
A. magnetotacticum (M58171)           gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
Az. brasilense (NCIMB11860)           gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
Az. lipoferum (NCIMB11861)            gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
R. acidophila (M34128)                gcgaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
A. clevelandensis (M69186)            aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. morelense (LMG21331)T              gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. amorphae (LMG18977)T               tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. ciceri (LMG14989)T                 tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. huakuii (LMG14107)T                tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. loti (LMG6125)T                    tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
M. plurifarium (LMG11892)T            tagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
B. elkanii (LMG6134)T                 aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
B. beta (LMG21987)T                   aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
B. yuanmingense (CCBAU10071)T         aagaataagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-
S. medicae (A321)T                    gagaagaagc cccggctaac ttcgtgccag cagccgcggt aatacg-aa-

                                      510        520        530        540        550
M. mediterraneum (LMG17148)T          gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. septentrionale (SDW014)T           gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. temperatum (SDW018)T               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. thiogangeticum (SJT)T              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M.tianshanense (A-1BS)T               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC12.1a L. sepiaria SA                gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC19.1b L. cytisoides SA              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC19.1c L. cytisoides SA              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC23.1b L. multiflora SA              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC23.1c L. multiflora SA              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC23.1d L. multiflora SA              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC28.1F L. sericea SA                 gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC28.1g L. sericea SA                 gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC28.1h L. sericea SA                 gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC28.1i L. sericea SA                 gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC33b L. sericea SA                   gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC33c L. sericea SA                   gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC33e L. sericea SA                   gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
WC33h L. sericea SA                   gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
S. americanum (CFNEI156)T             gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
S. arboris (LMG14919)T                gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
S. fredii (LMG6217)T                  gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
S. kostiense (LMG19227)               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
S. kummerowiae (CCBAU71714)T          gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
S. meliloti (LMG6133)T                gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BH1LSc L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BH2LSa L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BH3LSd L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BW1LSab L. spinescens SA              gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BW2LSa L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BW2LSb L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BW2LSc L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
BW3LSd L. spinescens SA               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
B. canariense (MSFC2-M3-R-3)T         gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
B. japonicum (LMG6138)T               gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
B. liaoningense (LMG18230)T           gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
Betal106d L. pungens SA               gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
Betal106a L. pungens SA               gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
NK22 L. sericea SA                    gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
WC21.1L L. pauciflora SA              gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
XHR1a L. simsiana SA                  gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
Betal106f L. pungens SA               gggggctagc gttgctcgga atcactgggc gtaaagggtt gcgtaaggcg
R. palustris (ATCC17001)              gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
M. nodulans (ORS2060)                 gggggctagc gttgctcgga atcactgggc gtaaagggc- --gcgtaggc
Az. caulinodans (LMG6465)             gggggctagc gttgctcgga atcactgggc gtaaagcgc- --acgtaggc
R. rubrum (LMGATCC11170)              gggggcaagc gttgttcgga attactgggc gtaaagagc- --gcgtaggc
A. magnetotacticum (M58171)           gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgcaggc
Az. brasilense (NCIMB11860)           gggggcgagc gttgttcgga attactgggc gtaaagggc- --gcgtaggc
Az. lipoferum (NCIMB11861)            gggggctagc gttgttcgga attactgggc gtaaagggc- --gcgtaggc
R. acidophila (M34128)                gggggc-agc gttgttcgga tttactgggc gtaaagggc- --acgtaggc
A. clevelandensis (M69186)            gggggctagc gttgctcgga atctctgggc gtaaagggt- --gcgtaggc
S. morelense (LMG21331)T              gggggctagc gttgttcgga attactgggc gtaaagcgt- --acgtaggc
M. amorphae (LMG18977)T               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. ciceri (LMG14989)T                 gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. huakuii (LMG14107)T                gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. loti (LMG6125)T                    gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
M. plurifarium (LMG11892)T            gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc
B. elkanii (LMG6134)T                 gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
B. beta (LMG21987)T                   gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
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B. yuanmingense (CCBAU10071)T    gggggctagc gttgctcgga atcactgggc gtaaagggt- --gcgtaggc
S. medicae (A321)T               gggggctagc gttgttcgga attactgggc gtaaagcgc- --acgtaggc


                                    560        570        580        590        600
M. mediterraneum (LMG17148)T     ggatatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
M. septentrionale (SDW014)T      ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
M. temperatum (SDW018)T          ggatatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
M. thiogangeticum (SJT)T         ggactattaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
M.tianshanense (A-1BS)T          ggatatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC12.1a L. sepiaria SA           ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC19.1b L. cytisoides SA         ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC19.1c L. cytisoides SA         ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC23.1b L. multiflora SA         ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC23.1c L. multiflora SA         ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC23.1d L. multiflora SA         ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC28.1F L. sericea SA            ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC28.1g L. sericea SA            ggattgttaa gttagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC28.1h L. sericea SA            ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC28.1i L. sericea SA            ggatacttaa gtcaggggtt gaaa-tcccg gggctcaac- cccgg-aact
WC33b L. sericea SA              ggattgttaa gttagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC33c L. sericea SA              ggattgttaa gttagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC33e L. sericea SA              ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
WC33h L. sericea SA              ggattgttaa gttaggggtt gaaa-tcccg gggctcaac- cccgg-aact
S. americanum (CFNEI156)T        ggacatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
S. arboris (LMG14919)T           ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
S. fredii (LMG6217)T             ggacatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
S. kostiense (LMG19227)          ggacatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
S. kummerowiae (CCBAU71714)T     ggacatttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
S. meliloti (LMG6133)T           ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BH1LSc L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BH2LSa L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BH3LSd L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BW1LSab L. spinescens SA         ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BW2LSa L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BW2LSb L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BW2LSc L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
BW3LSd L. spinescens SA          ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact
B. canariense (MSFC2-M3-R-3)T    gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccagaaact
B. japonicum (LMG6138)T          gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
B. liaoningense (LMG18230)T      gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
Betal106d L. pungens SA          gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
Betal106a L. pungens SA          gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
NK22 L. sericea SA               gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
WC21.1L L. pauciflora SA         gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
XHR1a L. simsiana SA             gggttttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
Betal106f L. pungens SA          ggtctttaa gtcagggg-t gaaattcctg gagctcaact tccag-aact
R. palustris (ATCC17001)         gggtttcaa gtcagagg-t gaaa-gcctg gagctcaac- tccag-aact
M. nodulans (ORS2060)            ggctcgccaa gtcggggg-t gaaa-gcccg tggctcaac- cacgg-aatg
Az. caulinodans (LMG6465)        ggatcgttaa gtcagggg-t gaaa-gcctg gagctcaac- tccag-aact
R. rubrum (LMGATCC11170)         ggtctgatta gtcagagg-t gaaa-tccca gagctcaac- tttgg-aact
A. magnetotacticum (M58171)      ggtggtcata gtcagaag-t gaaa-gccct gggctcaac- ccggg-aatt
Az. brasilense (NCIMB11860)      ggcccgatca gtcagatg-t gaaa-gcccc gggctcaac- ctggg-aact
Az. lipoferum (NCIMB11861)       ggcccgttta gtcagaag-t gaaa-gcccc gggctcaac- ctggg-aaca
R. acidophila (M34128)           -gatctttaa gtcagggg-t gaaa-tgccg gagctcaac- ttcgg-aact
A. clevelandensis (M69186)       gggtctttaa gtcagagg-t gaaa-gcctg gagctcaac- tccag-aact
S. morelense (LMG21331)T         ggacatttaa gtcagggg-t gaaa-tccca gagctcaac- tctgg-aact
M. amorphae (LMG18977)T          ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
M. ciceri (LMG14989)T            ggattgttaa gttagggg-t gaaa-tccca gggctcaac- cctgg-aact
M. huakuii (LMG14107)T           ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
M. loti (LMG6125)T               ggattgttaa gttagggg-t gaaa-tccca gggctcaac- cctgg-aact
M. plurifarium (LMG11892)T       ggatacttaa gtcagggg-t gaaa-tcccg gggctcaac- cccgg-aact
B. elkanii (LMG6134)T            gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
B. beta (LMG21987)T              gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
B. yuanmingense (CCBAU10071)T    gggtctttaa gtcagggg-t gaaa-tcctg gagctcaac- tccag-aact
S. medicae (A321)T               ggattgttaa gtgagggg-t gaaa-tccca gggctcaac- cctgg-aact


                                    610        620        630        640        650
M. mediterraneum (LMG17148)T     gcctttgata ctggg-tatc tcgagtccga g--aga-ggt gagtgg-aat
M. septentrionale (SDW014)T      gcctttgata ctggg-tatc tcgagtccga a--aga-ggt gagtgg-aat
M. temperatum (SDW018)T          gcctttgata ctggg-tatc tcgagtccga g--aga-ggt gagtgg-aat
M. thiogangeticum (SJT)T         gcctttgata ctggt-gatc tagagtccgg a--aga-ggt gagtgg-aat
M.tianshanense (A-1BS)T          gcctttgata ctggg-tatc tcgagtccga g--aga-ggt gagtgg-aat
WC12.1a L. sepiaria SA           gcctttgata ctggg-tatc tcgagtccga a--aga-ggt gagtgg-aat
WC19.1b L. cytisoides SA         gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC19.1c L. cytisoides SA         gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC23.1b L. multiflora SA         gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC23.1c L. multiflora SA         gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
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WC23.1d L. multiflora SA            gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC28.1F L. sericea SA               gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC28.1g L. sericea SA               gcctttaata ctggc-aatc tcgagtccga g--aga-ggt gagtgg-aat
WC28.1h L. sericea SA               gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC28.1i L. sericea SA               gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
WC33b L. sericea SA                 gcctttaata ctggc-aatc tcgagtccga g--aga-ggt gagtgg-aat
WC33c L. sericea SA                 gcctttaata ctggc-aatc tcgagtccga g--aga-ggt gagtgg-aat
WC33e L. sericea SA                 gcctttgata ctggg-tatc tcgagtccgg a--agagggt gagtgg-aat
WC33h L. sericea SA                 gcctttaata ctggc-aatc tcgagtccga g--aga-ggt gagtggaaat
S. americanum (CFNEI156)T           gcctttgata ctggg-tgtc tagagtccgg a--aga-ggt gagtgg-aat
S. arboris (LMG14919)T              gcctttcata ctggg-aatc tagagtccag a--aga-ggt gagtgg-aat
S. fredii (LMG6217)T                gcctttgata ctggg-tgtc tagagtccgg a--aga-ggt gagtgg-aat
S. kostiense (LMG19227)             gcctttgata ctggg-tgtc tagagtccgg a--aga-ggt gagtgg-aat
S. kummerowiae (CCBAU71714)T        gcctttgata ctggg-tgtc tagagtccgg a--aga-ggt gagtgg-aat
S. meliloti (LMG6133)T              gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BH1LSc L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BH2LSa L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BH3LSd L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BW1LSab L. spinescens SA            gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BW2LSa L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BW2LSb L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BW2LSc L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
BW3LSd L. spinescens SA             gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat
B. canariense (MSFC2-M3-R-3)T       gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
B. japonicum (LMG6138)T             gcctttgata ctgag-gatc ttgagttcgg g--aga-ggt gagtgg-aac
B. liaoningense (LMG18230)T         gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
Betal106d L. pungens SA             gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
Betal106a L. pungens SA             gcctttgata ctgag-gatc ttgagttcgg g--aga-ggt gagtgg-aac
NK22 L. sericea SA                  gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
WC21.1L L. pauciflora SA            gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
XHR1a L. simsiana SA                gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
Betal106f L. pungens SA             gcctttgata ctgaaggatc ttgagttcgg ggagaa-ggt gagtgggaac
R. palustris (ATCC17001)            gcctttgata ctgga-agtc ttgagtatgg c--aga-ggt gagtgg-aac
M. nodulans (ORS2060)               gccttcgata ctggc-gggc ttgagaccgg a--aga-gga cagcgg-aac
Az. caulinodans (LMG6465)           gcccttgata ctggc-gatc ttgagttcga g--aga-ggt tggtgg-aac
R. rubrum (LMGATCC11170)            gcctttgata ctgtt-agac tagaatccgt g--aga-ggg tggtgg-aat
A. magnetotacticum (M58171)         gcttttgata ctgga-ccgc tagaatcacg g--aga-ggg tagtgg-aat
Az. brasilense (NCIMB11860)         gcatttgata ctgtc-gggc ttgagttccg g--aga-gga tggtgg-aat
Az. lipoferum (NCIMB11861)          gcttttgata ctggc-gggc ttgagttccg g--aga-gga tggtgg-aat
R. acidophila (M34128)              gcctttgata ctgga-gatc ttgagttcga g--aga-ggt gagtgg-aac
A. clevelandensis (M69186)          gcctttgata ctgag-gatc ttgagttcgg g--aga-ggt gagtgg-aac
S. morelense (LMG21331)T            gcctttgata ctggg-tgtc tagagtatgg a--aga-ggt gagtgg-aat
M. amorphae (LMG18977)T             gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
M. ciceri (LMG14989)T               gcctttaata ctggc-aatc tcgagtccga g--aga-ggt gagtgg-aat
M. huakuii (LMG14107)T              gcctttgata ctggg-tatc tcgagtccgg a--aga-ggt gagtgg-aat
M. loti (LMG6125)T                  gcctttaata ctggc-aatc tcgagtccga g--aga-ggt gagtgg-aat
M. plurifarium (LMG11892)T          gcctttgata ctggg-tatc tggagtccgg a--aga-ggt gagtgg-aat
B. elkanii (LMG6134)T               gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
B. beta (LMG21987)T                 gcctttgata ctgaa-gatc ttgagttcgg g--aga-ggt gagtgg-aac
B. yuanmingense (CCBAU10071)T       gcctttgata ctgaa-gatc ttgagtccgg g--aga-ggt gagtgg-aac
S. medicae (A321)T                  gcctttcata ctggc-aatc tagagtccag a--aga-ggt gagtgg-aat


                                    660        670        680        690        700
M. mediterraneum (LMG17148)T        tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. septentrionale (SDW014)T         tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. temperatum (SDW018)T             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. thiogangeticum (SJT)T            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M.tianshanense (A-1BS)T             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC12.1a L. sepiaria SA              tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC19.1b L. cytisoides SA            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC19.1c L. cytisoides SA            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC23.1b L. multiflora SA            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC23.1c L. multiflora SA            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC23.1d L. multiflora SA            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC28.1F L. sericea SA               tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC28.1g L. sericea SA               tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC28.1h L. sericea SA               tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC28.1i L. sericea SA               tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC33b L. sericea SA                 tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC33c L. sericea SA                 tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
WC33e L. sericea SA                 tccgagtgta gaggtgaaat tcgtagatat tcgggaggaa acaccagtgg
WC33h L. sericea SA                 tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
S. americanum (CFNEI156)T           tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
S. arboris (LMG14919)T              tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
S. fredii (LMG6217)T                tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
S. kostiense (LMG19227)             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
S. kummerowiae (CCBAU71714)T        tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
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S. meliloti (LMG6133)T              tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BH1LSc L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BH2LSa L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BH3LSd L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BW1LSab L. spinescens SA            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BW2LSa L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BW2LSb L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BW2LSc L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
BW3LSd L. spinescens SA             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
B. canariense (MSFC2-M3-R-3)T       tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
B. japonicum (LMG6138)T             tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
B. liaoningense (LMG18230)T         tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
Betal106d L. pungens SA             tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
Betal106a L. punges SA              tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
NK22 L. sericea SA                  tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
WC21.1L L. pauciflora SA            tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
XHR1a L. simsiana SA                tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
Betal106f L. pungens SA             kgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
R. palustris (ATCC17001)            tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
M. nodulans (ORS2060)               tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
Az. caulinodans (LMG6465)           tccgagtgta gaggtgaaat tcgtagatat tcgg--aaga acaccagtgg
R. rubrum (LMGATCC11170)            tcccagtgta gaggtgaaat tcgtagatat tggg--agga acaccagtgg
A. magnetotacticum (M58171)         tcccagtgta gaggtgaaat tcgtagatat tcgg--aaga acaccagtgg
Az. brasilense (NCIMB11860)         tcccagtgta gaggtgaaat tcgtagatat tggg--aaga acaccggtgg
Az. lipoferum (NCIMB11861)          tcccagtgta gaggtgaaat tcgtagatat tggg--aaga acaccggtgg
R. acidophila (M34128)              tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
A. clevelandensis (M69186)          tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
S. morelense (LMG21331)T            tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. amorphae (LMG18977)T             tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. ciceri (LMG14989)T               tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. huakuii (LMG14107)T              tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. loti (LMG6125)T                  tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
M. plurifarium (LMG11892)T          tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg
B. elkanii (LMG6134)T               tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
B. beta (LMG21987)T                 tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
B. yuanmingense (CCBAU10071)T       tgcgagtgta gaggtgaaat tcgtagatat tcgc--aaga acaccagtgg
S. medicae (A321)T                  tccgagtgta gaggtgaaat tcgtagatat tcgg--agga acaccagtgg


                                    710        720        730        740        750
M. mediterraneum (LMG17148)T        cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
M. septentrionale (SDW014)T         cgaagc--ag gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
M. temperatum (SDW018)T             cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
M. thiogangeticum (SJT)T            cgaagg--cg gctcactggc ccggtactga cgctg-aggt gcg-aaagcg
M.tianshanense (A-1BS)T             cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
WC12.1a L. sepiaria SA              cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
WC19.1b L. cytisoides SA            cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
WC19.1c L. cytisoides SA            cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
WC23.1b L. multiflora SA            cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
WC23.1c L. multiflora SA            cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
WC23.1d L. multiflora SA            cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
WC28.1F L. sericea SA               cgaagg--cg gctcactggc ccggtactga cgctg-aggt gcg-aaagcg
WC28.1g L. sericea SA               cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
WC28.1h L. sericea SA               cgaagg--cg gctcactggc ccggtactga cgctg-aggt gcg-aaagcg
WC28.1i L. sericea SA               cgaagg--cg gctcactggc ccggtactga cgctg-aggt gcg-aaagcg
WC33b L. sericea SA                 cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
WC33c L. sericea SA                 cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
WC33e L. sericea SA                 cgaagg--cg gctcactggc ccggtactga cgctg-aggt gcg-aaagcg
WC33h L. sericea SA                 cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcgaaaagcg
S. americanum (CFNEI156)T           cgaagg--cg gctcactggc ccggtactga cgctg-aggt gcg-aaagcg
S. arboris (LMG14919)T              cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
S. fredii (LMG6217)T                cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
S. kostiense (LMG19227)             cgaagg--cg gctcactggt ccggaactga cgctg-aggt gcg-aaagcg
S. kummerowiae (CCBAU71714)T        cgaagg--cg gctcactggt ctggaactga cgctg-aggt gcg-aaagcg
S. meliloti (LMG6133)T              cgaagg--cg gctcactggt ctggaactga cgctg-aggt gcg-aaagcg
BH1LSc L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
BH2LSa L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctgaaggt gcg-aaagcg
BH3LSd L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
BW1LSab L. spinescens SA            cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
BW2LSa L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
BW2LSb L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
BW2LSc L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
BW3LSd L. spinescens SA             cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg
B. canariense (MSFC2-M3-R-3)T       cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
B. japonicum (LMG6138)T             cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
B. liaoningense (LMG18230)T         cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
Betal106d L. pungens SA             cgaaggcgg ctccactggc ccgatactga cgctg-aggc acg-aaagcg
Betal106a L. pungens SA             cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
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NK22 L. sericea SA                     cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
WC21.1L L. pauciflora SA               cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
XHR1a L. simsiana SA                   cgaagg--cg gctcactggc ccggtactga cgctg-aggc acg-aaagcg
Betal106f L. pungens SA                cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
R. palustris (ATCC17001)               cgaagg--cg gctcactggg ccattactga cgctg-aggc acg-aaagcg
M. nodulans (ORS2060)                  cgaagg--cg gctgtctggt ccggttctga cgctg-aggc gcg-aaagcg
Az. caulinodans (LMG6465)              cgaagg--cg gccaactggc tcggtactga cgctg-aggt gcg-aaagcg
R. rubrum (LMGATCC11170)               cgaagg--cg gccacctggc gcggtattga cgctg-aggc gcg-aaagcg
A. magnetotacticum (M58171)            cgaagg--cg actactggc cgtsgattga ssctc-atgt gcg-aaagc-
Az. brasilense (NCIMB11860)            cgaagg--cg gccatctgga cggacactga cgctg-aggc gcg-aaagcg
Az. lipoferum (NCIMB11861)             cgaagg--cg gccatctgga cggacactga cgctg-aggc gcg-aaagcg
R. acidophila (M34128)                 cgaagg--cg gctcactggc tcgatactga cgctg-aggt gc---aagcg
A. clevelandensis (M69186)             cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
S. morelense (LMG21331)T               cgaagg--cg gctcactggt ccattactga cgctg-aggt gcg-aaagcg
M. amorphae (LMG18977)T                cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
M. ciceri (LMG14989)T                  cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
M. huakuii (LMG14107)T                 cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
M. loti (LMG6125)T                     cgaagg--cg gctcactggc tcggtactga cgctg-aggt gcg-aaagcg
M. plurifarium (LMG11892)T             cgaagg--cg gctcactggt ccggtactga cgctg-aggt gcg-aaagcg
B. elkanii (LMG6134)T                  cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
B. beta (LMG21987)T                    cgaagg--cg gctcactggc ccgatactga cgctg-aggc acg-aaagcg
B. yuanmingense (CCBAU10071)T          cgaagg--cg gctcactggc ccggtactga cgctg-aggc acg-aaagcg
S. medicae (A321)T                     cgaagg--cg gctcactggt ctggtactga cgctg-aggt gcg-aaagcg


                                              760        770        780        790        800
M. mediterraneum (LMG17148)T           tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. septentrionale (SDW014)T            tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. temperatum (SDW018)T                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. thiogangeticum (SJT)T               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M.tianshanense (A-1BS)T                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC12.1a L. sepiaria SA                 tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC19.1b L. cytisoides SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC19.1c L. cytisoides SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC23.1b L. multiflora SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC23.1c L. multiflora SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC23.1d L. multiflora SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC28.1F L. sericea SA                  tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC28.1g L. sericea SA                  tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC28.1h L. sericea SA                  tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC28.1i L. sericea SA                  tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC33b L. sericea SA                    tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC33c L. sericea SA                    tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC33e L. sericea SA                    tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC33h L. sericea SA                    tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. americanum (CFNEI156)T              tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. arboris (LMG14919)T                 tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. fredii (LMG6217)T                   tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. kostiense (LMG19227)                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. kummerowiae (CCBAU71714)T           tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. meliloti (LMG6133)T                 tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BH1LSc L. spinescens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BH2LSa L. spinescens SA                tgggg--gaa gcaaaacagg attaggatac cctgggtagt cccacgcccg
BH3LSd L. spinescens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BW1LSab L. spinescens SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BW2LSa L. spinescens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BW2LSb L. spinescens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BW2LSc L. spinescens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
BW3LSd L. spinescens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
B. canariense (MSFC2-M3-R-3)T          tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
B. japonicum (LMG6138)T                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
B. liaoningense (LMG18230)T            tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
Betal106d L. pungens SA                tgggggagg- --caaacagg atta-gatac cctggtagtc cacgccgt--
Betal106a L. pungens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
NK22 L. sericea SA                     tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
WC21.1L L. pauciflora SA               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
XHR1a L. simsiana SA                   tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
Betal106f L. pungens SA                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
R. palustris (ATCC17001)               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. nodulans (ORS2060)                  tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgctgt--
Az. caulinodans (LMG6465)              tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
R. rubrum (LMGATCC11170)               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
A. magnetotacticum (M58171)            tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
Az. brasilense (NCIMB11860)            tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
Az. lipoferum (NCIMB11861)             tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
R. acidophila (M34128)                 tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
A. clevelandensis (M69186)             tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. morelense (LMG21331)T               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
```

```
M. amorphae (LMG18977)T              tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. ciceri (LMG14989)T                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. huakuii (LMG14107)T               tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. loti (LMG6125)T                   tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
M. plurifarium (LMG11892)T           tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
B. elkanii (LMG6134)T                tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgctgt--
B. beta (LMG21987)T                  tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
B. yuanmingense (CCBAU10071)T        tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--
S. medicae (A321)T                   tgggg--ag- --caaacagg atta-gatac cctggtagtc cacgccgt--


                                     810        820        830        840        850
M. mediterraneum (LMG17148)T         --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
M. septentrionale (SDW014)T          --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
M. temperatum (SDW018)T              --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
M. thiogangeticum (SJT)T             --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
M.tianshanense (A-1BS)T              --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC12.1a L. sepiaria SA               --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC19.1b L. cytisoides SA             --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC19.1c L. cytisoides SA             --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC23.1b L. multiflora SA             --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC23.1c L. multiflora SA             --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC23.1d L. multiflora SA             --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC28.1F L. sericea SA                --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC28.1g L. sericea SA                --aaactatg ag-agctagc cgtcggc-aa gtttt-acttg tcggtggcgc
WC28.1h L. sericea SA                --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC28.1i L. sericea SA                --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC33b L. sericea SA                  --aaactatg ag-agctagc cgtcggc-aa gtttt-acttg tcggtggcgc
WC33c L. sericea SA                  --aaactatg ag-agctagc cgtcggc-aa gtttt-acttg tcggtggcgc
WC33e L. sericea SA                  --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
WC33h L. sericea SA                  --aaactatg ag-agctagc cgtcggc-aa gtttt-acttg tcggtggcgc
S. americanum (CFNEI156)T            --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
S. arboris (LMG14919)T               --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
S. fredii (LMG6217)T                 --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
S. kostiense (LMG19227)              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
S. kummerowiae (CCBAU71714)T         --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
S. meliloti (LMG6133)T               --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BH1LSc L. spinescens SA              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BH2LSa L. spinescens SA              taaaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BH3LSd L. spinescens SA              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BW1LSab L. spinescens SA             --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BW2LSa L. spinescens SA              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BW2LSb L. spinescens SA              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BW2LSc L. spinescens SA              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
BW3LSd L. spinescens SA              --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
B. canariense (MSFC2-M3-R-3)T        --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
B. japonicum (LMG6138)T              --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
B. liaoningense (LMG18230)T          --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
Betal106d L. pungens SA              --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
Betal106a L. pungens SA              --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
NK22 L. sericea SA                   --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
WC21.1L L. pauciflora SA             --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
XHR1a L. simsiana SA                 --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
Betal106f L. pungens SA              --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
R. palustris (ATCC17001)             --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
M. nodulans (ORS2060)                --aaacgatg aa-tgctagc cgttggg-gt gcat-gcacc tcagtggcgc
Az. caulinodans (LMG6465)            --aaacgatg ga-tgctagc cgttggg-ga gctt-gctct tcagtggcgc
R. rubrum (LMGATCC11170)             --aaacgatg ag-tgctaga tgtcggg-gt acat-gtacc tcggtgtcgc
A. magnetotacticum (M58171)          --aaacgatg ag-tgctaag ttgttgg-gt gcat-gcacc tcggtgacgc
Az. brasilense (NCIMB11860)          --aaacgatg aa-tgctaga cgctggg-gt gcat-gcact tcggtgtcgc
Az. lipoferum (NCIMB11861)           --aaacgatg aa-tgctaga cgtcggg-gt gcat-gcact tcggtgtcgc
R. acidophila (M34128)               --aaacgatg ga-tgctagc cgttagg-ca gctt-gctgc ttagtggcgc
A. clevelandensis (M69186)           --aaacgatg aa-tgccagc cgttgga-aa gtttt-acttt tcagtggcgc
S. morelense (LMG21331)T             --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc
M. amorphae (LMG18977)T              --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
M. ciceri (LMG14989)T                --atactatg aagagctagc cgtcggctaa gtttt-acttg tcggtggcgc
M. huakuii (LMG14107)T               --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
M. loti (LMG6125)T                   --aaacgatg ag-agctagc cgtcggc-aa gtttt-acttg tcggtggcgc
M. plurifarium (LMG11892)T           --aaacgatg ga-agctagc cgttggc-aa gtttt-acttg tcggtggcgc
B. elkanii (LMG6134)T                --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
B. beta (LMG21987)T                  --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
B. yuanmingense (CCBAU10071)T        --aaacgatg aa-tgccagc cgttagt-gg gtttt-actca ctagtggcgc
S. medicae (A321)T                   --aaacgatg aa-tgttagc cgtcggg-ca gtttt-actgt tcggtggcgc


                                     860        870        880        890        900
M. mediterraneum (LMG17148)T         agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
M. septentrionale (SDW014)T          agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
M. temperatum (SDW018)T              agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
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M. thiogangeticum (SJT)T          agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
M.tianshanense (A-1BS)T           agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC12.1a L. sepiaria SA            agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC19.1b L. cytisoides SA          agctaacgca ttaagcttcc cgcctggggg agtacggtcg caagattaaa
WC19.1c L. cytisoides SA          agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC23.1b L. multiflora SA          agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC23.1c L. multiflora SA          agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC23.1d L. multiflora SA          agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC28.1F L. sericea SA             agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
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WC28.1h L. sericea SA             agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
WC28.1i L. sericea SA             agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
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WC33c L. sericea SA               agctaacgca ttaagctctc cgcct-gggg agtacggtcg caagattaaa
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WC33h L. sericea SA               agctaacgca ttaagctctc cgcct-gggg agtacggtcg caagattaaa
S. americanum (CFNEI156)T         agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
S. arboris (LMG14919)T            agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
S. fredii (LMG6217)T              agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
S. kostiense (LMG19227)           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
S. kummerowiae (CCBAU71714)T      agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
S. meliloti (LMG6133)T            agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BH1LSc L. spinescens SA           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BH2LSa L. spinescens SA           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BH3LSd L. spinescens SA           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BW1LSab L. spinescens SA          agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BW2LSa L. spinescens SA           agctaacgca ttaaacattc cgcctggggg agtacggtcg caagattaaa
BW2LSb L. spinescens SA           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BW2LSc L. spinescens SA           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
BW3LSd L. spinescens SA           agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
B. canariense (MSFC2-M3-R-3)T     agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
B. japonicum (LMG6138)T           agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
B. liaoningense (LMG18230)T       agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
Betal106d L. pungens SA           agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
Betal106a L. pungens SA           agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
NK22 L. sericea SA                agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
WC21.1L L. pauciflora SA          agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
XHR1a L. simsiana SA              agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
Betal106f L. pungens SA           agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
R. palustris (ATCC17001)          a--taacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
M. nodulans (ORS2060)             cgctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
Az. caulinodans (LMG6465)         agctaacgcc ttaagcatcc cgcct-gggg agtacggtcg caagattaaa
R. rubrum (LMGATCC11170)          agctaacgca ttaagcactc cgcct-gggg agtacggccg caaggttaaa
A. magnetotacticum (M58171)       acgtaacgcg ttaagcactc cgcct-gggg agtacggccg caaggttaaa
Az. brasilense (NCIMB11860)       -cctaacgca ttaagcattc cgcct-gggg agtacggccg caaggttaaa
Az. lipoferum (NCIMB11861)        ccgtaacgca ttaagcattc cgcct-gggg agtacggccg caaggttaaa
R. acidophila (M34128)            agctaacgct ttaagcatcc cgcct-gggg agtacggtcg caagattaaa
A. clevelandensis (M69186)        agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
S. morelense (LMG21331)T          agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa
M. amorphae (LMG18977)T           agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
M. ciceri (LMG14989)T             agctaacgca ttaagctctc cgcct-gggg agtacggtcg caagattaaa
M. huakuii (LMG14107)T            acgtaacgca ttaagcttgc cgcct-gggg agtacggtcg caagattaaa
M. loti (LMG6125)T                acgtaacgca ttaagctctc cgcct-gggg agtacggtcg caagattaaa
M. plurifarium (LMG11892)T        agctaacgca ttaagcttcc cgcct-gggg agtacggtcg caagattaaa
B. elkanii (LMG6134)T             agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
B. beta (LMG21987)T               agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
B. yuanmingense (CCBAU10071)T     agctaacgct ttaagcattc cgcct-gggg agtacggtcg caagattaaa
S. medicae (A321)T                agctaacgca ttaaacattc cgcct-gggg agtacggtcg caagattaaa

                                  910        920        930        940        950
M. mediterraneum (LMG17148)T      actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. septentrionale (SDW014)T       actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. temperatum (SDW018)T           actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. thiogangeticum (SJT)T          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M.tianshanense (A-1BS)T           actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC12.1a L. sepiaria SA            actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC19.1b L. cytisoides SA          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC19.1c L. cytisoides SA          actcaaagga a-ttgacggg gggcccgcac -aagcggt-g gagcatgtgg
WC23.1b L. multiflora SA          actcaaagga a-ttgacggg gggcccgcac -aagcggt-g gagcatgtgg
WC23.1c L. multiflora SA          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC23.1d L. multiflora SA          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC28.1F L. sericea SA             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC28.1g L. sericea SA             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC28.1h L. sericea SA             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC28.1i L. sericea SA             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC33b L. sericea SA               actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC33c L. sericea SA               actcaaagga atttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
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WC33e L. sericea SA            actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC33h L. sericea SA            actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. americanum (CFNEI156)T      actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. arboris (LMG14919)T         actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. fredii (LMG6217)T           actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. kostiense (LMG19227)        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. kummerowiae (CCBAU71714)T   actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. meliloti (LMG6133)T         actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BH1LSc L. spinescens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BH2LSa L. spinescens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BH3LSd L. spinescens SA        actcaaagga a-ttgacggg gggcccgcac -aagcggt-g gagcatgtgg
BW1LSab L. spinescens SA       actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BW2LSa L. spinescens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BW2LSb L. spinescens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BW2LSc L. spinescens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
BW3LSd L. spinescens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
B. canariense (MSFC2-M3-R-3)T  actcaaagga a-ttgac-gg gggcccgcac aaagcggt-g gagcatgtgg
B. japonicum (LMG6138)T        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
B. liaoningense (LMG18230)T    actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
Betal106d L. pungens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
Betal106a L. pungens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
NK22 L. sericea SA             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
WC21.1L L. pauciflora SA       actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
XHR1a L. simsiana SA           actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
Betal106f L. pungens SA        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
R. palustris (ATCC17001)       actcaaagga a-tt----gg ggg-ccgcac -aagcggt-g gagcatgtgg
M. nodulans (ORS2060)          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
Az. caulinodans (LMG6465)      actcaaagga a-ttgac-gg ggg-ccgcac -aagcggt-g gagcatgtgg
R. rubrum (LMGATCC11170)       actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
A. magnetotacticum (M58171)    actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
Az. brasilense (NCIMB11860)    actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
Az. lipoferum (NCIMB11861)     actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
R. acidophila (M34128)         actcaaagga a-ttgac-gg gggc-cgcac -aagcggt-g gagcatgtgg
A. clevelandensis (M69186)     actcaaagga a-ttgac-gg ggg-ccgcac -aagcggt-g gagcatgtgg
S. morelense (LMG21331)T       acttaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. amorphae (LMG18977)T        actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. ciceri (LMG14989)T          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. huakuii (LMG14107)T         actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. loti (LMG6125)T             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
M. plurifarium (LMG11892)T     actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
B. elkanii (LMG6134)T          actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
B. beta (LMG21987)T            actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
B. yuanmingense (CCBAU10071)T  actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg
S. medicae (A321)T             actcaaagga a-ttgac-gg gggcccgcac -aagcggt-g gagcatgtgg


                                 960        970        980        990        1000
M. mediterraneum (LMG17148)T   tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. septentrionale (SDW014)T    tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. temperatum (SDW018)T        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. thiogangeticum (SJT)T       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M.tianshanense (A-1BS)T        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC12.1a L. sepiaria SA         tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC19.1b L. cytisoides SA       tttaa-ttcg aagcaacgcg cagaaccttt accagccctt gacat-cccg
WC19.1c L. cytisoides SA       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC23.1b L. multiflora SA       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC23.1c L. multiflora SA       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC23.1d L. multiflora SA       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC28.1F L. sericea SA          tttaatttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC28.1g L. sericea SA          tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC28.1h L. sericea SA          tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC28.1i L. sericea SA          tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC33b L. sericea SA            tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC33c L. sericea SA            tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC33e L. sericea SA            tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
WC33h L. sericea SA            tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
S. americanum (CFNEI156)T      tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
S. arboris (LMG14919)T         tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
S. fredii (LMG6217)T           tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
S. kostiense (LMG19227)        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
S. kummerowiae (CCBAU71714)T   tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacatccccg
S. meliloti (LMG6133)T         tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BH1LSc L. spinescens SA        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BH2LSa L. spinescens SA        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BH3LSd L. spinescens SA        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BW1LSab L. spinescens SA       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BW2LSa L. spinescens SA        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BW2LSb L. spinescens SA        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
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BW2LSc L. spinescens SA           tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
BW3LSd L. spinescens SA           tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
B. canariense (MSFC2-M3-R-3)T     tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
B. japonicum (LMG6138)T           tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
B. liaoningense (LMG18230)T       tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
Betal106d L. pungens SA           tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-cccg
Betal106a L. punges SA            tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-cccg
NK22 L. sericea SA                tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
WC21.1L L. pauciflora SA          tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-cccg
XHR1a L. simsiana SA              tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
Betal106f L. pungens SA           tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-cccg
R. palustris (ATCC17001)          tttaa-tt-- ------cgcg cagaacc-tt accagccctt gacat-gtcc
M. nodulans (ORS2060)             tttaa-ttcg aagcaacgcg cagaacc-tt accatccctt gacat-ggca
Az. caulinodans (LMG6465)         tttaa-ttcg aagcaacgcg cagaacc-tt accagccttt gacat-ggca
R. rubrum (LMGATCC11170)          tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
A. magnetotacticum (M58171)       tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-ggga
Az. brasilense (NCIMB11860)       tttaa-ttcg aagcaacgcg cagaacc-tt accaaccctt gacat-gtcc
Az. lipoferum (NCIMB11861)        tttaa-ttcg aagcaacgcg cagaacc-tt accaaccctt gacat-gtcc
R. acidophila (M34128)            tttaa-ttcg aagcaacgcg cagaacc-tt accagctttt gacat-gtct
A. clevelandensis (M69186)        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-gtcc
S. morelense (LMG21331)T          tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. amorphae (LMG18977)T           tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. ciceri (LMG14989)T             tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. huakuii (LMG14107)T            tttaa-ttcg aagcaacgcg cagaacc-tt acctgccctt gacat-cccg
M. loti (LMG6125)T                tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
M. plurifarium (LMG11892)T        tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg
B. elkanii (LMG6134)T             tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-cccg
B. beta (LMG21987)T               tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
B. yuanmingense (CCBAU10071)T     tttaa-ttcg acgcaacgcg cagaacc-tt accagccctt gacat-gtcc
S. medicae (A321)T                tttaa-ttcg aagcaacgcg cagaacc-tt accagccctt gacat-cccg

                                       1010       1020       1030       1040       1050
M. mediterraneum (LMG17148)T      gtcgc-ggtc tccagagatg gagacc-ttc agttcggctg gaccggtg-a
M. septentrionale (SDW014)T       gtcgc-ggtt tccagagatg gattcc-ttc agttcggctg gaccggtg-a
M. temperatum (SDW018)T           gtcgc-ggtc tccagagatg gagacc-ttc agttcggctg gaccggtg-a
M. thiogangeticum (SJT)T          gtcgc-ggtt accagagatg gtatcc-ttc agttcggctg gaccggtg-a
M.tianshanense (A-1BS)T           gtcgc-ggtt tccagagatg gattcc-ttc agttcggctg gaccggtg-a
WC12.1a L. sepiaria SA            gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC19.1b L. cytisoides SA          gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC19.1c L. cytisoides SA          gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC23.1b L. multiflora SA          gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC23.1c L. multiflora SA          gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC23.1d L. multiflora SA          gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC28.1F L. sericea SA             gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC28.1g L. sericea SA             gtcgcgggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC28.1h L. sericea SA             gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC28.1i L. sericea SA             gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC33b L. sericea SA               gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC33c L. sericea SA               gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC33e L. sericea SA               gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
WC33h L. sericea SA               gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
S. americanum (CFNEI156)T         atcgc-ggat acgagagatc gtatcc-ttc agttcggctg gatcggag-a
S. arboris (LMG14919)T            gtcgc-ggat acgagagatc gtatcc-ttc agttcggctg gaccggag-a
S. fredii (LMG6217)T              atcgc-ggat acgagagatc gtatcc-ttc agttcggctg gatcggag-a
S. kostiense (LMG19227)           gtcgc-ggaa acgagagatc gtttcc-ttc agttcggctg gaccggag-a
S. kummerowiae (CCBAU71714)T      atcgc-ggat acgagagatc g-tatc-tcc agttcggctg gatcggag-a
S. meliloti (LMG6133)             atcgc-ggat acgagagatc gtatcc-ttc agttcggctg gatcggag-a
BH1LSc L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BH2LSa L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BH3LSd L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BW1LSab L. spinescens SA          atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BW2LSa L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BW2LSb L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BW2LSc L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
BW3LSd L. spinescens SA           atcgc-ggat tacagagatg ttttcc-ttc agttcggctg gatcggag-a
B. canariense (MSFC2-M3-R-3)T     aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggagc-a
B. japonicum (LMG6138)T           aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggagc-a
B. liaoningense (LMG18230)T       aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggaac-a
Betal106d L. pungens SA           gtcgc-ggac tccagagatg gagttc-ttc agttcggctg gaccggag-a
Betal106a L. pungens SA           gtcgc-ggac tccagagacg gagttc-ttc agttcggctg gaccggagaa
NK22 L. sericea SA                aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggagc-a
WC21.1L L. pauciflora SA          gtcgc-ggac tccagagacg gagttc-ttc agttcggctg gaccggag-a
XHR1a L. simsiana SA              aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggaac-a
Betal106f L. pungens SA           gtcgc-ggac tccagagatg gagttc-ttc agttcggctg gaccggag-a
R. palustris (ATCC17001)          agga--acgt cgcagagacg cgacct-tct --cttcggag cctggagc-a
M. nodulans (ORS2060)             ggc---tacg tggagagatt cacggt-tcc --cttcgggg acctgcac-a
Az. caulinodans (LMG6465)         g-gac-gact tccggagacg gatttc-ttc ca-gcaatgg acctgcac-a
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R. rubrum (LMGATCC11170)          tga---cact tccagagatg gaaggt-tcc --cttcgggg acacggtg-a
A. magnetotacticum (M58171)       cgtmt-gttt gccagagatg gtgact-tgt --cttcggga cgcgtcac-a
Az. brasilense (NCIMB11860)       actac-cggc tcgagagatc gggct--ttc agttcggctg ggtggaac-a
Az. lipoferum (NCIMB11861)        actatggtcg ctcagagatg gcgtccttca ggttcggctg ggtggaag-a
R. acidophila (M34128)            tggac-ggat agcagagatg ttatct-tct --cttcggag ccgagaac-a
A. clevelandensis (M69186)        aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggagc-a
S. morelense (LMG21331)T          atcgc-ggat tacagagatg tattcc-ttc agttcggctg gatcggag-a
M. amorphae (LMG18977)T           gtcgc-ggtt tccagagatg gattcc-ttc agttcggctg gaccggtg-a
M. ciceri (LMG14989)T             gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
M. huakuii (LMG14107)T            gtcgc-ggtt tccagagatg gattcctttc agttcggctg gaccggtg-a
M. loti (LMG6125)T                gtcgc-ggtt tccagagatg gatacc-ttc agttcggctg gaccggtg-a
M. plurifarium (LMG11892)T        gtcgc-ggtt accagaaatg gtttcc-ttc agttcggctg gaccggtg-a
B. elkanii (LMG6134)T             gtcgcgggac tccagagacg gagttc-ttc agttcggctg gaccggag-a
B. beta (LMG21987)T               aggac-cggt cgcagagatg tgacct-tct --cttcggag cctggaac-a
B. yuanmingense (CCBAU10071)T     aggac-cggt cgcagagatg tgaccc-tct --cttcggag cctggagc-a
S. medicae (A321)T                atcgc-ggat aggagagatc ctatcc-ttc agttcggctg gatcggag-a


                                            1060       1070       1080       1090       1100
M. mediterraneum (LMG17148)T      caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. septentrionale (SDW014)T       caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. temperatum (SDW018)T           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. thiogangeticum (SJT)T          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M.tianshanense (A-1BS)T           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC12.1a L. sepiaria SA            caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC19.1b L. cytisoides SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC19.1c L. cytisoides SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC23.1b L. multiflora SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC23.1c L. multiflora SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC23.1d L. multiflora SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC28.1F L. sericea SA             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC28.1g L. sericea SA             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC28.1h L. sericea SA             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC28.1i L. sericea SA             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC33b L. sericea SA               caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC33c L. sericea SA               caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC33e L. sericea SA               caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC33h L. sericea SA               caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. americanum (CFNEI156)T         caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. arboris (LMG14919)T            caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. fredii (LMG6217)T              caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. kostiense (LMG19227)           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. kummerowiae (CCBAU71714)T      caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttgag
S. meliloti (LMG6133)T            caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BH1LSc L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BH2LSa L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BH3LSd L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BW1LSab L. spinescens SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BW2LSa L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BW2LSb L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BW2LSc L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
BW3LSd L. spinescens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
B. canariense (MSFC2-M3-R-3)T     caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
B. japonicum (LMG6138)T           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
B. liaoningense (LMG18230)T       caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
Betal106d L. pungens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
Betal106a L. punges SA            caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
NK22 L. sericea SA                caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
WC21.1L L. pauciflora SA          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
XHR1a L. simsiana SA              caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
Betal106f L. pungens SA           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
R. palustris (ATCC17001)          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. nodulans (ORS2060)             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
Az. caulinodans (LMG6465)         caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
R. rubrum (LMGATCC11170)          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
A. magnetotacticum (M58171)       caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
Az. brasilense (NCIMB11860)       caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
Az. lipoferum (NCIMB11861)        gaggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
R. acidophila (M34128)            caggtgctgc atggctgtcg tc-gctcgtg tcgtgagatg ttgggttaag
A. clevelandensis (M69186)        caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. morelense (LMG21331)T          caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. amorphae (LMG18977)T           caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. ciceri (LMG14989)T             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. huakuii (LMG14107)T            caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. loti (LMG6125)T                caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
M. plurifarium (LMG11892)T        caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
B. elkanii (LMG6134)T             caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
B. beta (LMG21987)T               caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
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B. yuanmingense (CCBAU10071)T    caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag
S. medicae (A321)T               caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg ttgggttaag


                                   1110       1120       1130       1140       1150
M. mediterraneum (LMG17148)T     tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
M. septentrionale (SDW014)T      tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
M. temperatum (SDW018)T          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
M. thiogangeticum (SJT)T         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- ct--tca--g
M.tianshanense (A-1BS)T          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
WC12.1a L. sepiaria SA           tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC19.1b L. cytisoides SA         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC19.1c L. cytisoides SA         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC23.1b L. multiflora SA         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC23.1c L. multiflora SA         tcccgcaacg agcgcaaccc tcgcccttta gttgccagc- at--tca--g
WC23.1d L. multiflora SA         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC28.1F L. sericea SA            tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC28.1g L. sericea SA            tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
WC28.1h L. sericea SA            tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC28.1i L. sericea SA            tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC33b L. sericea SA              tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
WC33c L. sericea SA              tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
WC33e L. sericea SA              tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
WC33h L. sericea SA              tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
S. americanum (CFNEI156)T        tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tta--g
S. arboris (LMG14919)T           tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tta--g
S. fredii (LMG6217)T             tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tta--g
S. kostiense (LMG19227)          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
S. kummerowiae (CCBAU71714)T     tcccgcaacg agcgcaa-cc tcgccc-tta gttgccagc- at--tca--g
S. meliloti (LMG6133)T           tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
BH1LSc L. spinescens SA          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
BH2LSa L. spinescens SA          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
BH3LSd L. spinescens SA          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
BW1LSab L. spinescens SA         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
BW2LSa L. spinescens SA          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
BW2LSb L. spinescens SA          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
BW2LSc L. spinescens SA          tcccgcaacg agcgcaaccc tcgcccttta gttgccagc- at--taa--g
BW3LSd L. spinescens SA          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
B. canariense (MSFC2-M3-R-3)T    tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
B. japonicum (LMG6138)T          tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
B. liaoningense (LMG18230)T      tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
Betal106d L. pungens SA          tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
Betal106a L. pungens SA          tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
NK22 L. sericea SA               tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
WC21.1L L. pauciflora SA         tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
XHR1a L. simsiana SA             tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
Betal106f L. pungens SA          tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
R. palustris (ATCC17001)         tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
M. nodulans (ORS2060)            tcccgcaacg agcgcaaccc tcgccc-tta gttgccatc- at--tga--g
Az. caulinodans (LMG6465)        tcccgcaacg agcgcaaccc tcgcct-tta gttgccatc- at--tca--g
R. rubrum (LMGATCC11170)         tcccgcaacg agcgcaaccc tcatct-tca gttgccagc- aa--gtaacg
A. magnetotacticum (M58171)      tcccgcaacg agcgcaaccc tcatct-tca gttgccatc- at--tta--g
Az. brasilense (NCIMB11860)      tcccgcaacg agcgcaaccc ctaccg-cca gttgccatc- at--tca--g
Az. lipoferum (NCIMB11861)       tcccgcaacg agcgcaaccc ctaccg-tca gttgccatc- at--tca--g
R. acidophila (M34128)           tcccgcaacg agcgcaaccc acgtcc-tta gttgccatc- at--taa--g
A. clevelandensis (M69186)       tcccgcaacg agcgcaaccc t-gtcc-tta gttgctacc- at--tta--g
S. morelense (LMG21331)T         tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
M. amorphae (LMG18977)T          tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--taa--g
M. ciceri (LMG14989)T            tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
M. huakuii (LMG14107)T           tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
M. loti (LMG6125)T               tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g
M. plurifarium (LMG11892)T       tcccgcaacg agcgcaaccc tcgccc-tta gttgccatc- at--tca--g
B. elkanii (LMG6134)T            tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
B. beta (LMG21987)T              tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
B. yuanmingense (CCBAU10071)T    tcccgcaacg agcgcaaccc ccgtcc-tta gttgctacc- at--tta--g
S. medicae (A321)T               tcccgcaacg agcgcaaccc tcgccc-tta gttgccagc- at--tca--g


                                   1160       1170       1180       1190       1200
M. mediterraneum (LMG17148)T     ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
M. septentrionale (SDW014)T      ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
M. temperatum (SDW018)T          ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
M. thiogangeticum (SJT)T         ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
M.tianshanense (A-1BS)T          ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
WC12.1a L. sepiaria SA           ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
WC19.1b L. cytisoides SA         ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
WC19.1c L. cytisoides SA         ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
WC23.1b L. multiflora SA         ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
WC23.1c L. multiflora SA         ttgggcactc taaggggact gccggtgata agccgagagg aaggtggggga
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WC23.1d L. multiflora SA          ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC28.1F L. sericea SA             ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC28.1g L. sericea SA             ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC28.1h L. sericea SA             ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC28.1i L. sericea SA             ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC33b L. sericea SA               ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC33c L. sericea SA               ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC33e L. sericea SA               ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
WC33h L. sericea SA               ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
S. americanum (CFNEI156)T         ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
S. arboris (LMG14919)T            ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
S. fredii (LMG6217)T              ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
S. kostiense (LMG19227)           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
S. kummerowiae (CCBAU71714)T      ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
S. meliloti (LMG6133)T            ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BH1LSc L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BH2LSa L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BH3LSd L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BW1LSab L. spinescens SA          ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BW2LSa L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BW2LSb L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BW2LSc L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
BW3LSd L. spinescens SA           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
B. canariense (MSFC2-M3-R-3)T     ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
B. japonicum (LMG6138)T           ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
B. liaoningense (LMG18230)T       ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
Betal106d L. pungens SA           ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
Betal106a L. pungens SA           ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
NK22 L. sericea SA                ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
WC21.1L L. pauciflora SA          ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
XHR1a L. simsiana SA              ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
Betal106f L. pungens SA           ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
R. palustris (ATCC17001)          ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
M. nodulans (ORS2060)             ttgggcactc taggggggact gccggtgata agccgcgagg aaggtggggga
Az. caulinodans (LMG6465)         ttgggcactc taaagggact gccggtgata agccgcgagg aaggtggggga
R. rubrum (LMGATCC11170)          ttgggcactc tgaagagact gccggtgaca agccg-gagg aaggtgggga
A. magnetotacticum (M58171)       ttgggcacgc tgaagaaact gccggtgaca agcc-ggagg aaggtgggga
Az. brasilense (NCIMB11860)       ttgggcactc tggtggaact gccggtgaca agccg-gagg aaggcgggga
Az. lipoferum (NCIMB11861)        ttgggcactc tggtggaacc gccggtgaca agccg-gagg aaggcgggga
R. acidophila (M34128)            ttgggcactc tagggagact gccggtgata agccgagagg aaggtgtgga
A. clevelandensis (M69186)        ttgggcactc taaggagact gccggtgata agccgcgagg aaggtgggga
S. morelense (LMG21331)T          ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
M. amorphae (LMG18977)T           ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
M. ciceri (LMG14989)T             ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
M. huakuii (LMG14107)T            ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
M. loti (LMG6125)T                ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
M. plurifarium (LMG11892)T        ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga
B. elkanii (LMG6134)T             ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
B. beta (LMG21987)T               ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
B. yuanmingense (CCBAU10071)T     ttgagcactc taaggagact gccggtgata agccgcgagg aaggtggggga
S. medicae (A321)T                ttgggcactc taagggggact gccggtgata agccgagagg aaggtggggga


                                  1210       1220       1230       1240       1250
M. mediterraneum (LMG17148)T      tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. septentrionale (SDW014)T       tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. temperatum (SDW018)T           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. thiogangeticum (SJT)T          tgacgtcaag tcctcatggc ccttacggga tgggcttaca cacgtgctac
M.tianshanense (A-1BS)T           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC12.1a L. sepiaria SA            tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC19.1b L. cytisoides SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC19.1c L. cytisoides SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC23.1b L. multiflora SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC23.1c L. multiflora SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC23.1d L. multiflora SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC28.1F L. sericea SA             tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
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WC28.1i L. sericea SA             tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC33b L. sericea SA               tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC33c L. sericea SA               tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC33e L. sericea SA               tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC33h L. sericea SA               tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. americanum (CFNEI156)T         tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. arboris (LMG14919)T            tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. fredii (LMG6217)T              tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. kostiense (LMG19227)           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. kummerowiae (CCBAU71714)T      tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
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S. meliloti (LMG6133)T            tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BH1LSc L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BH2LSa L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BH3LSd L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BW1LSab L. spinescens SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BW2LSa L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BW2LSb L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BW2LSc L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
BW3LSd L. spinescens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
B. canariense (MSFC2-M3-R-3)T     tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
B. japonicum (LMG6138)T           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
B. liaoningense (LMG18230)T       tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
Betal106d L. pungens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
Betal106a L. pungens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
NK22 L. sericea SA                tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
WC21.1L L. pauciflora SA          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
XHR1a L. simsiana SA              tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
Betal106f L. pungens SA           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
R. palustris (ATCC17001)          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. nodulans (ORS2060)             tgacgtcaag tcctcatggc ccttacggga tgggc-taca cacgtgctac
Az. caulinodans (LMG6465)         tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
R. rubrum (LMGATCC11170)          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
A. magnetotacticum (M58171)       tgacgtcaag tcctcatgcg --ttacgggc tgggc-taca cacgtgctac
Az. brasilense (NCIMB11860)       tgacgtcaag tcctcatggc ccttatgggt tgggc-taca cacgtgctac
Az. lipoferum (NCIMB11861)        tgacgtcaag tcctcatggc ccttatgggt tgggc-taca cacgtgctac
R. acidophila (M34128)            tgacgtcaag tcctcatggc ccttacaggc tgggc-taca cacgtgctac
A. clevelandensis (M69186)        tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. morelense (LMG21331)T          tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. amorphae (LMG18977)T           tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. ciceri (LMG14989)T             tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. huakuii (LMG14107)T            tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. loti (LMG6125)T                tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
M. plurifarium (LMG11892)T        tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
B. elkanii (LMG6134)T             tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
B. beta (LMG21987)T               tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
B. yuanmingense (CCBAU10071)T     tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac
S. medicae (A321)T                tgacgtcaag tcctcatggc ccttacgggc tgggc-taca cacgtgctac

                                   1260       1270       1280       1290       1300
M. mediterraneum (LMG17148)T      aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. septentrionale (SDW014)T       aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. temperatum (SDW018)T           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. thiogangeticum (SJT)T          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M.tianshanense (A-1BS)T           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC12.1a L. sepiaria SA            aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC19.1b L. cytisoides SA          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC19.1c L. cytisoides SA          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC23.1b L. multiflora SA          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC23.1c L. multiflora SA          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC23.1d L. multiflora SA          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC28.1F L. sericea SA             aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC28.1g L. sericea SA             aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
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WC33b L. sericea SA               aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
WC33c L. sericea SA               aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
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WC33h L. sericea SA               aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
S. americanum (CFNEI156)T         aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
S. arboris (LMG14919)T            aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
S. fredii (LMG6217)T              aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
S. kostiense (LMG19227)           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
S. kummerowiae (CCBAU71714)T      aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
S. meliloti (LMG6133)T            aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
BH1LSc L. spinescens SA           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
BH2LSa L. spinescens SA           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
BH3LSd L. spinescens SA           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
BW1LSab L. spinescens SA          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
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BW3LSd L. spinescens SA           aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
B. canariense (MSFC2-M3-R-3)T     aatggcggtg acaatgggat gcgaagacgc aagtcctggc aaatctcaaa
B. japonicum (LMG6138)T           aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
B. liaoningense (LMG18230)T       aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
Betal106d L. pungens SA           aatggcggtg acaatgggat gctaaggggt gacccttcgc aaatctcaaa
Betal106a L. pungens SA           aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
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NK22 L. sericea SA             aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
WC21.1L L. pauciflora SA       aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
XHR1a L. simsiana SA           aatggcggtg acaatgggac gctaaggggc aacccttcgc aaatctcaaa
Betal106f L. pungens SA        aatggcggtg acaatgggat gctaaggggt gacccttcgc aaatctcaaa
R. palustris (ATCC17001)       aatggcggtg acaatgggaa gctaaggggc gacccttcgc aaatctcaaa
M. nodulans (ORS2060)          aatggcggtg acaatgggaa gcgaaggggc gacctggagc aaatccccaa
Az. caulinodans (LMG6465)      aatggcggtg acaatgggat gcgagcctgc gagggtgagc aaatctccaa
R. rubrum (LMGATCC11170)       aatggcgcct acaatgggca gcgacctcgc gaggggaagc taatctccaa
A. magnetotacticum (M58171)    aatggtggtg acagtgggts sctaactcgc gaggtatg-c taatccctaa
Az. brasilense (NCIMB11860)    aatggcggtg acagtgggat gcgaagtcgc aagatggagc caatcccaa
Az. lipoferum (NCIMB11861)     aatggcggtg acagtgggaa gcgaagtcgc gagatggagc caatccccaa
R. acidophila (M34128)         aatggcggtg acaatgggaa gcgaaggggc gacctctagc aaatctcaaa
A. clevelandensis (M69186)     aatggcggtg acaatggga- gcaaaggggc gacccctagc aaatctcaaa
S. morelense (LMG21331)T       aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. amorphae (LMG18977)T        aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. ciceri (LMG14989)T          aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. huakuii (LMG14107)T         aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. loti (LMG6125)T             aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
M. plurifarium (LMG11892)T     aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa
B. elkanii (LMG6134)T          aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
B. beta (LMG21987)T            aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
B. yuanmingense (CCBAU10071)T  aatggcggtg acaatgggat gctaaggggc gacccttcgc aaatctcaaa
S. medicae (A321)T             aatggtggtg acagtgggca gcgagaccgc gaggtcgagc taatctccaa


                                     1310      1320      1330      1340      1350
M. mediterraneum (LMG17148)T   aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
M. septentrionale (SDW014)T    aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
M. temperatum (SDW018)T        aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
M. thiogangeticum (SJT)T       gagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
M. tianshanense (A-1BS)T       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
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WC19.1b L. cytisoides SA       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
WC19.1c L. cytisoides SA       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
WC23.1b L. multiflora SA       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
WC23.1c L. multiflora SA       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
WC23.1d L. multiflora SA       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
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WC28.1g L. sericea SA          aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
WC28.1h L. sericea SA          aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
WC28.1i L. sericea SA          aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
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S. arboris (LMG14919)T         aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
S. fredii (LMG6217)T           aagcc-atct cagttcggat tgcactctgc aactcggtg catgaagttg
S. kostiense (LMG19227)        aagcc-atct cagttcggat tgcactctgc aactcgggtg catgaagttg
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BH1LSc L. spinescens SA        aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
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BW3LSd L. spinescens SA        aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
B. canariense (MSFC2-M3-R-3)T  aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaa----
B. japonicum (LMG6138)T        aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
B. liaoningense (LMG18230)T    aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
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XHR1a L. simsiana SA           aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
Betal106f L. pungens SA        aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
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M. nodulans (ORS2060)          aagcc-gtct cagttcagat tgcactctgc aactcgagtg catgaaggcg
Az. caulinodans (LMG6465)      aagcc-gtct cagttcggat tgcactctgc aactcgagtg catgaagttg
R. rubrum (LMGATCC11170)       aaggc-gtct cagttcggat tgcactctgc aactcgggtg catgaagtcg
A. magnetotacticum (M58171)    aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagtcg
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R. acidophila (M34128)         aaacc-gtct cagttcggat tgtactctgc aactcgagtg catgaaggtg
A. clevelandensis (M69186)     aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
S. morelense (LMG21331)T       aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
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M. huakuii (LMG14107)T             aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
M. loti (LMG6125)T                 aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
M. plurifarium (LMG11892)T         aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg
B. elkanii (LMG6134)T              aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
B. beta (LMG21987)T                aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
B. yuanmingense (CCBAU10071)T      aagcc-gtct cagttcggat tgggctctgc aactcgagcc catgaagttg
S. medicae (A321)T                 aagcc-atct cagttcggat tgcactctgc aactcgagtg catgaagttg

                                   1360       1370       1380       1390       1400
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M. septentrionale (SDW014)T        gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
M. temperatum (SDW018)T            gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
M. thiogangeticum (SJT)T           gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
M.tianshanense (A-1BS)T            gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
WC12.1a L. sepiaria SA             gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
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WC19.1c L. cytisoides SA           gaatcgctag taatcgcgga tcagcatccc gcagtgaata cgttcccggg
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WC23.1c L. multiflora SA           gaatcgctag taatcgcgga tcagcatgcc gcagtgaata cgttcccggg
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WC28.1i L. sericea SA              gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
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WC33c L. sericea SA                gaatcgctag taatcgcgga tcagcatgcc gcagtgaata cgttcccggg
WC33e L. sericea SA                gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
WC33h L. sericea SA                gaatcgctag taatcgcgga tg-------- ---------- ----------
S. americanum (CFNEI156)T          gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
S. arboris (LMG14919)T             gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
S. fredii (LMG6217)T               gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
S. kostiense (LMG19227)            gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
S. kummerowiae (CCBAU71714)T       gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
S. meliloti (LMG6133)T             gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
BH1LSc L. spinescens SA            gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
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BW1LSab L. spinescens SA           gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
BW2LSa L. spinescens SA            gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
BW2LSb L. spinescens SA            gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
BW2LSc L. spinescens SA            gaatcgctag taatcgcaga tcagcatg-t gaggtgaata cgttcccggg
BW3LSd L. spinescens SA            gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg
B. canariense (MSFC2-M3-R-3)T      ---------- ---------- ---------- ---------- ----------
B. japonicum (LMG6138)T            gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
B. liaoningense (LMG18230)T        gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
Betal106d L. pungens SA            gaatcgctag taatcgtgga tcagcacgcc acaatgaata cgttcccggg
Betal106a L. pungens SA            gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
NK22 L. sericea SA                 gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
WC21.1L L. pauciflora SA           gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
XHR1a L. simsiana SA               gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
Betal106f L. pungens SA            gaatcgctag taatcgtgga tcagcacgcc actgtgaata cgttcccggg
R. palustris (ATCC17001)           gaatcgctag taatcgtgga tcagcatgcc acggtgaata cgt-------
M. nodulans (ORS2060)              gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
Az. caulinodans (LMG6465)          gaatcgctag taatcgtgga tcagcatgcc acggtgaata cgttcccggg
R. rubrum (LMGATCC11170)           gaatcgctag taatcgtgga tcagcatgcc acggtgaata cgttcccggg
A. magnetotacticum (M58171)        gaatcgctag taatcgtgga tcagcatgcc acggtgaata cgttcccggg
Az. brasilense (NCIMB11860)        gaatcgctag taatcgcgga tcagcacgcc gcggtgaata cgttcccggg
Az. lipoferum (NCIMB11861)         gaatcgctag taatcgcgga tcagcacgcc gcggtgaata cgttcccggg
R. acidophila (M34128)             gaatcgctag taatcgcaga tcagaacgct gcggtgaata cgttcccggg
A. clevelandensis (M69186)         gaatcgctag taatcgtgga tcagcatgcc acggtgaata cgttcccggg
S. morelense (LMG21331)T           gaatcgctag taatcgtgga tcagcatgct gcggtgaata cgttcccggg
M. amorphae (LMG18977)T            gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
M. ciceri (LMG14989)T              gaatcgcta- ---------- ---------- ---------- ----------
M. huakuii (LMG14107)T             gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
M. loti (LMG6125)T                 gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
M. plurifarium (LMG11892)T         gaatcgctag taatcgcgga tcagcatgcc gcggtgaata cgttcccggg
B. elkanii (LMG6134)T              gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
B. beta (LMG21987)T                gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
B. yuanmingense (CCBAU10071)T      gaatcgctag taatcgtgga tcagcacgcc acggtgaata cgttcccggg
S. medicae (A321)T                 gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccggg

                                   1410       1420       1430       1440       1450
M. mediterraneum (LMG17148)T       ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
M. septentrionale (SDW014)T        ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
M. temperatum (SDW018)T            ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
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M. thiogangeticum (SJT)T             ccttgtacac accgcccgtc acaccatggg agttggctttt acccgaaggc
M.tianshanense (A-1BS)T              ccttgtacac accgcccgtc acaccatggg agttggttttt acccgaaggc
WC12.1a L. sepiaria SA               ccttgtacac accgcccgtc acaccatggg agttggttttt acccgaaggc
WC19.1b L. cytisoides SA             ccttgtacac accgcccctt ---taagag agttggtctt -cccgaaggc
WC19.1c L. cytisoides SA             ccttgtacac accgcccgtc a-agcatggg agttggttttt acccgaaggc
WC23.1b L. multiflora SA             ccttgtacac accgcccgtc tacccatggg agttggttttt acccgaaggc
WC23.1c L. multiflora SA             ccttgtacac accgcccgtc aaggcatggg agttggttttt acccgaaggc
WC23.1d L. multiflora SA             ccttgtacac accgcccgtc acaccatggg agttggttttt acccgaaggc
WC28.1F L. sericea SA                ccttgtacac accgcccgtc acaccgtggg agttggttttt acccgaaggc
WC28.1g L. sericea SA                ccttgtacac accgcccgtc --cgcatggg agttggttttt acccgaaggc
WC28.1h L. sericea SA                ccttgtacac accgcccgtc a-cccatggg agttggttttt acccgaaggc
WC28.1i L. sericea SA                ccttgtacac accgcccgtc acaccatggg agttggttttt acccgaaggc
WC33b L. sericea SA                  ccttgtacac accgcccgtc acaccatggg agttggttttt acccgaaggc
WC33c L. sericea SA                  ccttgtacac accgcccgtc a-agcatggg agttggttttt acccgaaggc
WC33e L. sericea SA                  ccttgtacac accgcccgcc --agcatggg agttggttttt acccgaaggc
WC33h L. sericea SA                  ---------- ---------- ---------- ---------- ----------
S. americanum (CFNEI156)T            ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
S. arboris (LMG14919)T               ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
S. fredii (LMG6217)T                 ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
S. kostiense (LMG19227)              ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
S. kummerowiae (CCBAU71714)T         ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
S. meliloti (LMG6133)T               ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
BH1LSc L. spinescens SA              ccttgtacac accgcccgcc --cccatggg agttggttct acccgaaggt
BH2LSa L. spinescens SA              ccttgtacac accgcccgtc acagcatggg agttggttct acccgaaggt
BH3LSd L. spinescens SA              ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
BW1LSab L. spinescens SA             ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
BW2LSa L. spinescens SA              ccttgtacac accgcccgtc --cccatggg agttggttct acccgaaggt
BW2LSb L. spinescens SA              ccttgtacac accgcccgtc a-accatggg agttggttct acccgaaggt
BW2LSc L. spinescens SA              ccttgtacac accgccc--t gacgcatggg agttggttct acccgaaggt
BW3LSd L. spinescens SA              ccttgtacac accgcccgtc -taccatggg agttggttct acccgaaggt
B. canariense (MSFC2-M3-R-3)T        ---------- ---------- ---------- ---------- ----------
B. japonicum (LMG6138)T              ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaagac
B. liaoningense (LMG18230)T          ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaagac
Betal106d L. pungens SA              ccttgtacac accgcccgtt a-agcatggg agttggtttt acctgaagac
Betal106a L. pungens SA              ccttgtacac accgcccccc ---------- ---------- ----------
NK22 L. sericea SA                   ccttgtacac accgcccgtc acaacgt-gg agttggtttt acctgaagac
WC21.1L L. pauciflora SA             ccttgtacac accgcccg-- aacccatggg agttggtttt acctgaagac
XHR1a L. simsiana SA                 ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaagac
Betal106f L. pungens SA              ccttgtacac accggccgcc ---ccatggg agttggtttt acctgaagac
R. palustris (ATCC17001)             ---------- ---------- ---------- ---------- ----------
M. nodulans (ORS2060)                ccttgtacac accgcccgtc acaccatggg agttggtctt acccgacggc
Az. caulinodans (LMG6465)            ccttctacac accgcccgtc acaccatggg agttggcttt acccgaaggc
R. rubrum (LMGATCC11170)             ccttgtacac accgcccgtc acaccatggg agttggttct acccgaagac
A. magnetotacticum (M58171)          ccttgtacac accgcccgtc acaccatggg agttggcttt acccgaagcc
Az. brasilense (NCIMB11860)          ccttgtacac accgcccgtc acaccatggg agttggcttt acccgaaggt
Az. lipoferum (NCIMB11861)           ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggt
R. acidophila (M34128)               ccttgtacac accgcccgtc acaccatggg agttggcttt acccgaaggc
A. clevelandensis (M69186)           ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaagac
S. morelense (LMG21331)T             ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt
M. amorphae (LMG18977)T              ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
M. ciceri (LMG14989)T                ---------- ---------- ---------- ---------- ----------
M. huakuii (LMG14107)T               ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
M. loti (LMG6125)T                   ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
M. plurifarium (LMG11892)T           ccttgtacac accgcccgtc acaccatggg agttggtttt acccgaaggc
B. elkanii (LMG6134)T                ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaagac
B. beta (LMG21987)T                  ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaaggt
B. yuanmingense (CCBAU10071)T        ccttgtacac accgcccgtc acaccatggg agttggtttt acctgaagac
S. medicae (A321)T                   ccttgtacac accgcccgtc acaccatggg agttggttct acccgaaggt


                                      1460       1470
M. mediterraneum (LMG17148)T         gctgtgctaa ccg--caagg ag--gca
M. septentrionale (SDW014)T          gctgtgctaa ccg--caagg ag--gca
M. temperatum (SDW018)T              gctgtgctaa ccg--caaga ag--gca
M. thiogangeticum (SJT)T             gctgtgctaa ccg--ca-gt ag--gca
M.tianshanense (A-1BS)T              gctgtgctaa ccg--caagg ag--gca
WC12.1a L. sepiaria SA               gctgtgctaa ccg—c----- ------
WC19.1b L. cytisoides SA             gctgtgctaa ccg--caagg ag--gca
WC19.1c L. cytisoides SA             gctgtgctaa ccg--caagg ag--gca
WC23.1b L. multiflora SA             gctgtgctaa ccg--caagg ag--gca
WC23.1c L. multiflora SA             gctgtgctaa ccg--caagg ag—gc--
WC23.1d L. multiflora SA             gctgtgctaa ccg--caagg ag--gca
WC28.1F L. sericea SA                gctgtgctaa ccg--caagg ag--gca
WC28.1g L. sericea SA                gctgtgctaa ccg--caagg ag--gca
WC28.1h L. sericea SA                gctgtgctaa ccg--caagg ag--gca
WC28.1i L. sericea SA                gctgtgctaa ccg--caagg ag--gca
WC33b L. sericea SA                  gctgtgctaa ccg--caagg ag--gca
WC33c L. sericea SA                  gctgtgctaa ccg--caagg ag--gca
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WC33e L. sericea SA                gctgtgctaa ccg--caagg ag—gc--
WC33h L. sericea SA                ---------- ---------- -------
S. americanum (CFNEI156)T          agtgcgctaa ccg--caagg ag--gca
S. arboris (LMG14919)T             agtgcgctaa ccg--caagg ag--gca
S. fredii (LMG6217)T               agtgcgctaa ccg--caagg ag--gca
S. kostiense (LMG19227)            agtgcgctaa ccg--caagg ag--gca
S. kummerowiae (CCBAU71714)T       agtgcgctaa ccg--caagg ag--gca
S. meliloti (LMG6133)T             agtgcgctaa ccg--caagg ag--gca
BH1LSc L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
BH2LSa L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
BH3LSd L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
BW1LSab L. spinescens SA           agtgtgctaa ccg--caagg ag--gca
BW2LSa L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
BW2LSb L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
BW2LSc L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
BW3LSd L. spinescens SA            agtgtgctaa ccg--caagg ag--gca
B. canariense (MSFC2-M3-R-3)T      ---------- ---------- -------
B. japonicum (LMG6138)T            ggtgcgctaa ccc--gcaag ggaggca
B. liaoningense (LMG18230)T        ggtgcgctaa ccc--gcaag ggaggca
Betal106d L. pungens SA            ggtgcgctaa ccg--aaagg ggcag--
Betal106a L. pungens SA            ---------- ---------- -------
NK22 L. sericea SA                 ggtgcgctaa ccc--gcaag ggaggca
WC21.1L L. pauciflora SA           ggtgcgctaa ccg--aaagg gg--gca
XHR1a L. simsiana SA               ggtgcgctaa cca--gcaat ggaggca
Betal106f L. pungens SA            ggtgcgctaa ccg--aaagg gg--gca
R. palustris (ATCC17001)           ---------- ---------- -------
M. nodulans (ORS2060)              gctgcgccaa ccg--cgagg gg--gca
Az. caulinodans (LMG6465)          gttgcgctaa ccg--caagg ag--gca
R. rubrum (LMGATCC11170)           ggtacgctaa ccg--caagg ag--gca
A. magnetotacticum (M58171)        ggtgcgctaa ccg--caagg ag--gca
Az. brasilense (NCIMB11860)        ggtgcgctaa ccggcaacgg ag--gca
Az. lipoferum (NCIMB11861)         ggtgcgctaa ccg--caagg ag--gca
R. acidophila (M34128)             gtttcgctaa ccg--caagg ag--gca
A. clevelandensis (M69186)         ggtgcgctaa ccc--gcaag ga-ggca
S. morelense (LMG21331)T           agtgcgctaa ccg--caagg ag--gca
M. amorphae (LMG18977)T            gctgcgctaa ccg--caagg ag--gca
M. ciceri (LMG14989)T              ---------- ---------- -------
M. huakuii (LMG14107)T             gctgtgctaa ccg--caagg ag--gca
M. loti (LMG6125)T                 gctgtgctaa ccg--caagg ag--gca
M. plurifarium (LMG11892)T         gctgtgctaa ccg--caagg ag--gca
B. elkanii (LMG6134)T              ggtgcgctaa ccg--aaagg gg--gca
B. beta (LMG21987)T                agtgcgctaa ccc--gcaag ggaggca
B. yuanmingense (CCBAU10071)T      ggtgcgctaa ccc--gcaag ggaggca
S. medicae (A321)T                 agtgcgctaa ccg--caagg ag--gca
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