Indonesian Journal of Agricultural Research Vol. 02, No. 03, 2019 | 89 – 96

talenta PUBLISHER

InJAR
Indonesian Journal of Agricultural Research

Streptomyces sp.: Characterization, Identification and Its Potential as a Ralstonia solanacearum Biological Control Agent in vitro

Rachmad Saputra[1], Triwidodo Arwiyanto[2], and Arif Wibowo[2]

[1]*Department of Agrotechnology, Faculty of Agriculture, Universitas Riau, Indonesia*
[2]*Department of Plant Pests and Diseases, Faculty of Agriculture, Gadjah Mada University, Indonesia*

Abstract. *Streptomyces* sp. bacteria have the potential to produce antibiotic compounds, which are one of the mechanisms that are widely used in biological control. However, in general, biological control mechanisms also occur through competition, cell wall degradation and induced resistance. This study was aimed to determine the physiological, biochemical and molecular characteristics of two isolates of *Streptomyces* sp. (S-4 and S16 isolates) isolated from the tomatoes roots, and to find out their ability to control *Ralstonia solanacearum*, which causes bacterial wilt disease on a wide range of hosts. The results showed both *Streptomyces* sp. isolates had several different physiological and biochemical characteristics and had a different ability to inhibit *R. solanacearum in vitro*. *Streptomyces* sp. S-16 isolate had a high similarity with *Streptomyces diastaticus* subsp. *ardesiacus* strain NRRL B-1773T based on the molecular identification results. Further research needs to be done to see the potential inhibition of the two *Streptomyces* isolates in inhibiting the development of bacterial wilt disease in tomato plants caused by *R. solanacearum*.

Keywords: biological control agent, *Ralstonia solanacearum*, *Streptomyces* sp.

Received 06 September 2019 | Revised 14 October 2019 | Accepted 14 December 2019

1. Introduction

Streptomyces is a genus of Gram-positive bacteria that belongs to the phylum Actinobacteria. In many ways, these bacteria resemble filamentous fungi, which form vegetative mycelium and spread through spores formed in special reproductive structures called air hyphae, which emerge from the surface and point upwards. Most *Streptomyces* live as saprophytes in the soil [1].

Streptomyces are aerobic, saprophytic and mesophilic in their natural habitat in the soil. Some Actinomycetes members produce important secondary metabolites, including antibiotics, siderophores, enzymes and plant growth triggers that can contribute to their host plants by helping to accelerate growth and enhance the ability of plants under environmental stress conditions [2]. About 80% of useful antibiotics are derived from antibiotics produced by the

*Corresponding author at: Agrotechnology Department, Faculty of Agriculture, Universitas Riau. Jl. H.R. Soebrantras, Pekanbaru, Riau, Indonesia

E-mail address: rachmadsaputra@lecturer.unri.ac.id

 p-ISSN: 2622-7681 | e-ISSN: 2615-5842
Journal Homepage: https://talenta.usu.ac.id/InJAR DOI 10.32734/injar.v2i3.1389

species *Streptomyces* [3] which are also potentially antagonistic and are toxic to several pathogens [4].

Several studies have reported that *in vitro*, *Streptomyces* has shown good results in suppressing some root pathogens [5]. *Streptomyces pluricolorescens* which are endophytic actinomycetes isolated from tomato plants in Brazil showed 86.6% of antimicrobial activity that could fight pathogens in tomato plants [6]. Another research report also stated that the antagonistic mechanism of *Streptomyces* as a biological control agent for bacterial wilt disease is the mechanism of antibiosis by producing antibiotics [7].

Ralstonia solanacearum is a soil-borne plant pathogenic bacteria that is commonly found in subtropical and tropical regions which naturally infects roots, reproduces itself in xylem tissue and has a very broad host range [8] [9]. Its ability to survive in the soil causes many control efforts that show limited success, such as cultivation techniques, resistant varieties, and soil fumigation [8] [10]. Therefore, the use of biological agents such as *Streptomyces* sp. needs to be studied further because this bacteria has the potential to produce antibiotic compounds. However, in general, biological control mechanisms also occur through competition, cell wall degradation and induced resistance.

2. Materials and Methods

The study was conducted at the Plant Bacteriology Laboratory, Faculty of Agriculture, Gadjah Mada University. Antagonistic bacteria used were *Streptomyces* sp. S-4 and S-16 isolated from the rhizosphere of tomato plants and isolate of *Ralstonia solanacearum* isolated from roots of eggplant. The data obtained were analyzed descriptively and displayed in tables and figures.

2.1. Physiological and Biochemical Characterization of *Streptomyces* sp.

Characterization and identification of the bacteria *Streptomyces* sp. consisted of molecular physiological and biochemical tests referring to Lelliot and Stead [11], Williams *et al.* [4] and Chun and Vidaver [12]. Physiological and biochemical characterization included Gram staining, catalase, oxidase, oxygen demand (oxidative-fermentation), gelatin hydrolysis, starch, levan formation, Voges Proskauer test, arginine dehydrolase, motility, tolerance of bacterial growth at several temperatures, pH and HCl concentration, the use and amendment of carbon, citrate and nitrogen compounds.

2.2. Molecular Identification of *Streptomyces* sp.

Bacterial DNA was extracted and isolated using the method according to Berlian [13]. The DNA was identified molecularly by PCR technique using16S rDNA universal pair primers, i.e. 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1378R (5'-GGG CGG WGT GTA CAA GGC-3') with product targets measuring 1300 bp. The PCR products were then sequenced using

BLAST (Basic Local Alignment Search Tool) in the National Center for Biotechnology Information (NCBI) website to determine the closest kinship level with bacteria in the GenBank database.

2.3. *In vitro* Antagonism Test of *Ralstonia solanacearum*

Antagonism test was carried out *in vitro* with a technique developed by Arwiyanto [14]. *Streptomyces* sp. cultures were incubated for 96 hours at 29ºC in Yeast Peptone Glucose Agar (YPGA) medium. The petri dish reversed and 1 ml of chloroform was added, then left for 2 hours at room temperature. After the chloroform has evaporated, the petri dish was turned in original position, then a 200 µl *R. solanacearum* suspension in 4 ml 0.6% water agar poured. Culture was incubated for 24 hours at 29ºC to observe the inhibition zone. The isolates that produced inhibiting compounds were characterized by the presence of inhibition zones around the colonies of the antagonistic bacteria (*Streptomyces* sp.). Inhibition zones were measured and expressed in millimeters.

Detection mechanism of inhibition was done by taking clear agar in the inhibition zone and put in a test tube containing 5 ml of 0.5% peptone solution and incubated aerobically for 24 hours at room temperature. Clear peptone solution shows that the inhibition of growth was bactericidal, while puddly peptone solution indicates inhibition was bacteriostatic [14].

3. Results and Discussion

3.1. Physiological and Biochemical Characterization of *Streptomyces* sp.

The physiological and biochemical characteristics of both isolates of *Streptomyces* sp. were almost similar (Table 1). However from several assays, the results showed differences. *Streptomyces* sp. S-4 isolate were able to hydrolyze gelatin, could grow at low temperature (4ºC) and pH 4, whereas *Streptomyses* sp. S-16 isolates did not show the same ability. This indicates that *Streptomyces* sp. S-4 isolate can produce proteolytic enzymes. Gelatin hydrolysis test shows the presence of proteolytic enzymes that can hydrolyze proteins in gelatinous fluid [15].

The data in Table 2 shows that there are differences between the two isolates of *Streptomyces* sp. on the use of several carbon sources, citrate and nitrates assays. *Streptomyses* sp. S-4 isolate was able to remodel all carbon sources and citrate. In contrast,*Streptomyses* sp. S-16 isolate was not able to remodel the Trehalosa, Dulcitol and Sorbitol as carbon source, but could reduce nitrates. This reaction indicates that the presence of the enzyme oxidoreductase play a role in converting nitrates into nitrites, ammonia and nitrogen gas [15].

Tabel 1. Physiological and biochemical properties of *Streptomyces* sp.

Assay Parameters	***Streptomyces* sp. isolates**	
	S-4	**S-16**
Gram staining	+	+
Catalase test	+	+
Oxidase Test	+	+
Oxidative/Fermentative	Fermentative	Fermentative
Gelatin hydrolysis	+	-
Starch hydrolysis	-	-
Levan form	-	-
*Voges*test	-	-
Arginine Dehydrolase	-	-
Growth on:		
NaCl 1.0%	+	+
NaCl 2.0%	+	+
NaCl 4.0%	+	+
NaCl 6.0%	+	+
NaCl 8.0%	+	+
NaCl 10.0%	+	+
NaCl 12.0%	+	+
Temperature of 4oC	+	-
Temperature of 15oC	+	+
Temperature of 26oC	+	+
Temperature of 30oC	+	+
Temperature of 40oC	-	-
pH 4	+	-
pH 5.5	+	+
pH 7	+	+
pH 8.5	+	+
pH 10	+	+

Note: -: negative reaction, +: positive reaction

Tabel 2. Utilization of carbon, citrate and nitrogen compounds byc*Streptomyces* sp.

Assay Parameters	***Streptomyces* sp. isolates**	
	S-4	**S-16**
Carbon sources utilization and remodel:		
Arabinose	+	+
Dextrose	+	+
Sucrose	+	+
Maltose	+	+
Fructose	+	+
Glucose	+	+
Galactose	+	+
Lactose	+	+
Trehalosa	+	+
Dulcitol	+	-
Sorbitol	+	-
Citrate Utilization	+	-
Nitrogen compounds remodel:		
Nitrate reduction	-	+

Note: -: negative reaction, +: positive reaction

3.2. Molecular Identification of *Streptomyces* sp.

Visualization of the amplification of all *Streptomyces* sp. isolates using universal primers 63F and 1378R showed the presence of parallel DNA bands at size of ±1300bp (Figure 1). Homology of S-16 isolates based on 16S rDNA genes was related to *Streptomyces diastaticus* subsp. *ardesiacus* strain NRRL B-1773, *Streptomyces coelicoflavus* strain NBRC 15399 with homology reaching 99% (Table 3).

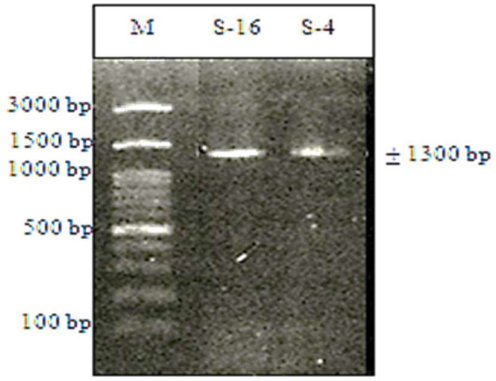

Figure 1. PCR products of *Streptomyces* sp. DNA. on agarose gels with a target size of ± 1,300 bp.

Table 3. *Streptomyces* sp. S-16 isolate similarity based on the sequence analysis derived from BLAST in NCBI website

Description	Max Score	Total Score	Query Cover	Percent Identity
Streptomyces diastaticus subsp. *ardesiacus* strain NRRL B-1773	2266	2266	100%	99.21%
Streptomyces coelicoflavus strain NBRC 15399	2263	2263	100%	99.13%
Streptomyces rubrogriseus strain NBRC 15455	2224	2224	100%	98.57%
Streptomyces hyderabadensis strain OU-40 16S	2222	2222	100%	98.57%
Streptomyces fragilis strain NBRC 12862	2222	2222	100%	98.57%
Streptomyces tricolor strain NBRC 15461	2218	2218	100%	98.50%
Streptomyces lienomycini strain NBRC 15425	2218	2218	100%	98.50%
Streptomyces anthocyanicus strain NBRC 14892	2218	2218	100%	98.50%
Streptomyces violaceolatus strain NBRC 13101	2218	2218	100%	98.50%
Streptomyces humiferus strain NBRC 12244	2218	2218	100%	98.50%
Streptomyces coelescens strain AS 4.1594	2218	2218	100%	98.50%
Streptomyces violaceoruber strain DSM 40049	2218	2218	100%	98.50%
Streptomyces aurantiogriseus strain CSSP525	2218	2218	100%	98.50%
Streptomyces albogriseolus strain DSM 40003	2218	2218	100%	98.50%

3.3. *In vitro* antagonism test of *Streptomyces* sp. against *Ralstonia solanacearum*

The results of *in vitro* antagonism test showed that *Streptomyces* sp. S-4 isolate could produce a inhibition zone (clear zone) of 4.53 mm, while there was no inhibition zone was formed with S-16 isolate. The formation of this inhibition zone indicates the mechanism of antibiosis against *R. solanacearum* (Table 4, Figure 2).

Table 4. Formation of inhibition zones of *Streptomyces* sp. against *R.solanacearum in vitro*

***Streptomyces* sp. isolate**	**Inhibition Zones (mm)**	**Mechanism of Inhibition**
S-4	4.53	Bacteriostatic
S-16	nd	nd

Note: nd = inhibiton zone not detected

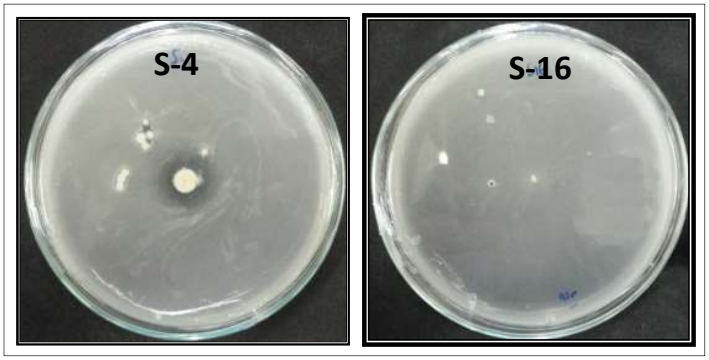

Figure 2. Results of the antagonism of *Streptomyces* sp. against *Ralstonia solanacearum* on YPGA medium

Different ability between the *Streptomyces* sp. isolates in producing inhibitory compounds is possible because of the differences in strains of each isolate. This can be seen from the results of utilization and remodel of carbon sources, citrates and nitrates compounds (Table 2), where the isolates from the two groups of bacteria have differences in several tests. Pindi *et al.* obtained similar results, in which 7 isolates of *Streptomyces* spp. used in the study showed different antimicrobial activity against the 9 microbes tested [3].

Based on the molecular identification, *Streptomyces* sp. S-16 isolate has a similarity with *Streptomyces diastaticus* subsp. *ardesiacus* strain NRRL B-1773. Not much information that states the use of *Streptomyces diastaticus* subsp. *ardesiacus* as a biological control agent. Therefore further research is needed in controlling *R. solanacearum* in plants. This is because *Streptomyces* is one of the producing antibiotic compounds bacteria. A lot of useful antibiotics are derived from antibiotics produced by *Streptomyces* species which are also potentially antagonistic and are toxic to several pathogens [3] [4] [7].

The absence of inhibition zones in *Streptomyces* sp. S-16 isolate cannot indicate that this isolate cannot be used as biological control agents. Similarly, Saputra *et al.* [16] reported that only one isolate of *Bacillus* sp., i.e. Ba-1 isolate of five tested isolates showed inhibition zone *in vitro*, and it did not show a good ability to inhibit *R. solanacearum* in tomato plants.

4. Conclusions and Recommendation

Both of *Streptomyces* sp. isolates had several different physiological and biochemical characteristics and had a different ability to inhibit *R. solanacearum in vitro*. *Streptomyces* sp. S-16 isolate has a similarity with *Streptomyces diastaticus* subsp. *ardesiacus* strain NRRL B-

1773 based on the molecular identification results. Further research needs to be done to see the potential inhibition of the two *Streptomyces* isolates in inhibiting the development of bacterial wilt disease in tomato plants caused by *R. solanacearum*.

Acknowledgments

This research was supported by the Directorate General Higher Education, Ministry of Education and Culture the Republic of Indonesia by agreement Number 108/SP2H/PL/Dit.litabmas/IV/2004 dated 30 April 2014. The authors say many thanks.

REFERENCES

[1] K. Flardh and M. J. Buttner, "*Streptomyces* Morphogenetics: Dissecting Differentiation in a Filamentous Bacterium", *Nature Review Microbiology* 7: 36-50, 2009.

[2] R. D. Hastuti, Y. Lestari, R. Saraswati, A. Suswanto and Chaerani, "Capability of *Streptomycess* spp. in Controling Bacterial Leaf Blight Disease in Rice Plant", *American Journal of Agricultural and Biological Sciences* 7: 217-223, 2012.

[3] P. K. Pindi, G. Balakrishna and A. S. Shanker, "Isolation and Characterization of Actinobacteria from Forest Soil of Mahabubnagar District of Andhra Pradesh, India", Int. *J. Pharm &/ Ind. Res* 2: 63-67, 2012.

[4] T. S. Williams, M. Goodfellow and G. Alderson, *Genus Streptomyces* Warksman and Henrici, dalam *Bergeys Manual of Systematic Bacteriology*, Diedit oleh M.E. Williams & J.G. Holt, London: Williams and Wilkins, hal. 2452-2492, 1989.

[5] G. H. S. Bonjar, S. Zamanian, S. Aghighi, P. R. Farrokhi, M. J. Mahdavi and I. Saadoun, "Antibacterial Activity of Iranian *Streptomyces coralus* Strain 63 Against *Ralstonia solanacearum*", *Journal of Biological Sciences* 6: 127-129. 2006.

[6] M. F. de-Oliveira, M. G. da-Silva and S. T. V. Sand, "Anti Phytopathogen Potential of Endophytic Actinobacteria Isolated from Tomato Plants in Southern Brazil and Characterization of *Streptomyces* sp. R18(6), a Potential Biocontrol Agent", *Microbiology* 161: 565-572, 2010.

[7] T. Arwiyanto, Supriadi and M. Januwati, Pengendalian Biologi Penyakit Layu Bakteri Jahe dengan Streptomyces spp., Laporan Hasil Kegiatan, Lembaga Penelitian dan Pengabdian kepada Masyarakat UGM bekerjasama dengan Badan Penelitian dan Pengembangan Pertanian. 2009,

[8] A. C. Hayward, Biology and Epidemiology of Bacterial Wilt Caused by *Pseudomonas solanacearum*", *Annual Review of Phytopathology* 29: 65-87, 1991.

[9] E. Yabuuchi, Y. Kosako, I. Yano, H. Hotta and Y. Nishiuchi, "Transfer of Two *Burkholderia* and an *Alcaligenes* Species to Ralstonia Genus nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Douderoff 1973) comb.nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov.", *Microbiology and Immunology* 39: 897, 1995.

[10] S. Maji and P. K. Chakrabartty, "Biocontrol of bacterial wilt of tomato caused by *Ralstonia solanacearum* by isolates of plant growth promoting rhizobacteria", *Australian Journal of Crop Science* 8: 2008-214, 2014.\

[11] R. A. Lelliot and D. E. Stead, *Methodes for The Diagnosis of Bacterial Diseases of Plant.* Melbourne: British Society for Plant Pathology by Blackwel Sscientific Publication, 1987.

[12] W. Chun and A. K. Vidaver, *Gram Positive Bacteria: Bacillus, dalam Plant Pathogenic*

Bacteria 3nd ed, Diedit oleh N.W. Schaad, J.B. Jones & W. Chun (Eds), Minnesota: APS Press, Hal. 250-260, 2001.

[13] I. Berlian, Analisis Petogenesitas dan Karakterisasi Keragaman Genetik *Banana Blood Disease Bacterium* (BDB), Tesis: Universitas Gadjah Mada, 2012.

[14] T. Arwiyanto, "Pengendalian Hayati Penyakit Layu Bakteri Tembakau", *Jurnal Perlindungan Tanaman Indonesia* 5: 54−60, 1997.

[15] A. J. Salle, *Fundamental Principle of Bacteriology,*7thed. New Delhi: Tata McGraw-hill Publishing Company LTD, 1979.

[16] R. Saputra, T. Arwiyanto and A. Wibowo, "Test Antagonistic Activity of Some Isolates *Bacillus* spp. Against Bacterial Wilt (*Ralstonia solanacearum*) in Some Tomato Varieties and their Identification", *Pros of Indon Biodiv Model*, vo. 1, no, 5, p. 1116-1122. DOI: 10.13057 / psnmbi / m010525, 2015.