International Journal of Mycobacteriology 3 (2014) 286–289

HOSTED BY

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Short Communication

Detection of *Mycobacterium gilvum* first time from the bathing water of leprosy patient from Purulia, West Bengal

Mallika Lavania *, *Ravindra Turankar*, *Itu Singh*, *Astha Nigam*, *Utpal Sengupta*

Stanley Browne Laboratory, TLM Community Hospital, Nand Nagari, Delhi 110093, India

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
14 October 2014
Accepted 14 October 2014
Available online 23 October 2014

Keywords:
M. gilvum
Acanthamoeba
16S rRNA sequencing
Leprosy
NTM
Purulia

ABSTRACT

In this present study for the first time the authors are reporting the isolation of *Mycobacterium gilvum* from the accumulated water in the drain connected to the bathing place of leprosy patients residing in an endemic region. The identification and characterization of this isolate was carried out by various conventional and molecular tests, including 16S rDNA sequencing. These findings might shed further light and association with amoeba in the leprosy endemic area of this rare *Mycobacterium* species.

Introduction

Amongst the reported list of bacteria, there are 169 species and 13 subspecies known to be present in the genus *Mycobacterium* (2014). Genus *Mycobacterium* has been further categorized into strict pathogens (*Mycobacterium tuberculosis* complex) and potential pathogens, non-tuberculous mycobacteria (NTM) (http://www.bacterio.net/mycobacterium.html accessed on 23 September 2014) [1]. Mycobacteria are aerobic, non-motile and acid fast bacteria. Members of the genus *Mycobacterium* are widespread in nature. NTM have been designated as "atypical" or anonymous mycobacterial species or mycobacteria other than tuberculosis (MOTT). Environmental mycobacteria consist of both rapid and slow-growing bacteria. These microbes share many common properties, such as acid fastness and the ability to cause pulmonary and extrapulmonary granulomatous disorders. As a group, they are comprised of diverse organisms with dissimilarities in their cultural characteristics and pathogenicity to humans compared with *Mycobacterium tuberculosis* (MTB).

NTM are widely distributed in the environment and are passed on by ingestion, inhalation, and inoculation from such

* Corresponding author. Tel.: +91 9717739608, +91 11 22594295.
E-mail address: mallikalavania@gmail.com (M. Lavania).
http://dx.doi.org/10.1016/j.ijmyco.2014.10.005

sources. Studies estimate that 30% of mycobacterial species isolated from water [2,3], soil [4], air and patients do not belong to any identified species [5]. The large number of isolated environment *Mycobacterium* remains non-identified by phenotypic and molecular techniques, which are usually employed for identification of clinical samples. However, all the previous studies suggest that there is the existence of diversity in this group of bacteria and such techniques are not discriminating enough for identification of mycobacterial species in environment samples.

Methods

Water samples

Water samples were collected from the drain connected to the bathing place of patients of leprosy in an endemic region and has been investigated for this study. Decontamination was done by the protocol published by Parashar et al. [6] by treating water samples with 3% SDS, 4% NaOH and 2% cetrimide. Water samples were centrifuged for $8000 \times g$ for 15 min at 4 °C. Pellets were re-suspended in 20 ml of treatment solution (3% SDS + 4% NaOH) and divided into two parts: A and B. Part A was incubated for 15 min and B was incubated for 30 min at room temperature to obtain the growth of rapid and slow growers, respectively. Both the suspensions were centrifuged for 15 min after incubation at $8000 \times g$. Pellets were treated with 2% cetrimide and incubated for 5 and 15 min for rapid and slow growers, respectively. After incubation sediments were washed twice with sterile water and 100 μl of decontaminated suspension was inoculated on Lowenstein Jenson (LJ medium), slants were incubated at 37 °C and primary colonies were subcultured on LJ medium again. Each sample was processed two times. Identification of growth was done by growth rate and morphology of colonies. The colonies were pale yellow in color and were smooth and pleomorphic with rapid growth in 7 days.

Identification of mycobacterial isolates at species level was done by growth rate, colony morphology and pigment production.

Identification by 16S rRNA sequencing

DNA from the growth from LJ slants were extracted by a procedure established earlier using lysozyme and proteinase K.

Sequencing of the 16S rRNA gene

Sequencing was performed by 16S rRNA gene region [7]. Each reaction mixture contained approximately 10 ng of DNA, with 1× PCR master mix from Qiagen, USA; 0.3 μg/reaction of each forward and reverse primer was added in a final volume of 25 μl. The PCR was performed using the Corbett PCR system (USA) 95 °C for 5 min for initial denaturation followed by 37 cycles, each cycle consisting of denaturation at 95 °C for 30 s, annealing at 60 °C for 2 min and extension at 72 °C for 4 min with a final extension at 72 °C for 10 min. 10 μl of PCR product was subjected to electrophoresis to ensure that successful amplification had occurred and that the correct fragment was obtained which was purified by Qiagen PCR purification kit (Qiagen, USA). PCR product was sent for commercial sequencing (an Xplorigen Pvt., Ltd., Delhi, India). Sequences were blasted on the NCBI Blast http://blast.ncbi.nlm.nih.gov/Blast.cgi to compare the sequences of the study strains with the ones in the databases.

Results and discussion

In this study the presence of *M. gilvum* was found for the first time from a water sample collected from an endemic area wherein a study on the transmission of *Mycobacterium leprae* in the population was being conducted. Other species which were found in the environment were *Mycobacterium asiaticum* and *Mycobacterium parascrofulaceum*.

Sequencing of the nearly complete 16S rRNA gene revealed a unique organism *M. glivum* distantly related to

Score	Expect	Identities	Gaps	Strand
307 bits(166)	1e-85	166/166(100%)	0/166(0%)	Plus/Minus

```
Query  1    TATTCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAAAGTGCAGGGCAGATCACCCACG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  173  TATTCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAAAGTGCAGGGCAGATCACCCACG  114

Query  61   TGTTACTCACCCGTTCGCCACTCGAGTACCCCGAAGGGCCTTTCCGTTCGACTTGCATGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  113  TGTTACTCACCCGTTCGCCACTCGAGTACCCCGAAGGGCCTTTCCGTTCGACTTGCATGT  54

Query  121  GTTAAGCACGCCGCCAGCGTTCGTCCTGAGCCAGAATCAAACTCTC  166
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  53   GTTAAGCACGCCGCCAGCGTTCGTCCTGAGCCAGAATCAAACTCTC  8
```

Fig. 1 – Nucleotide sequence of the 16S rRNA gene region of isolate identified as *M. gilvum* by rRNA sequencing.

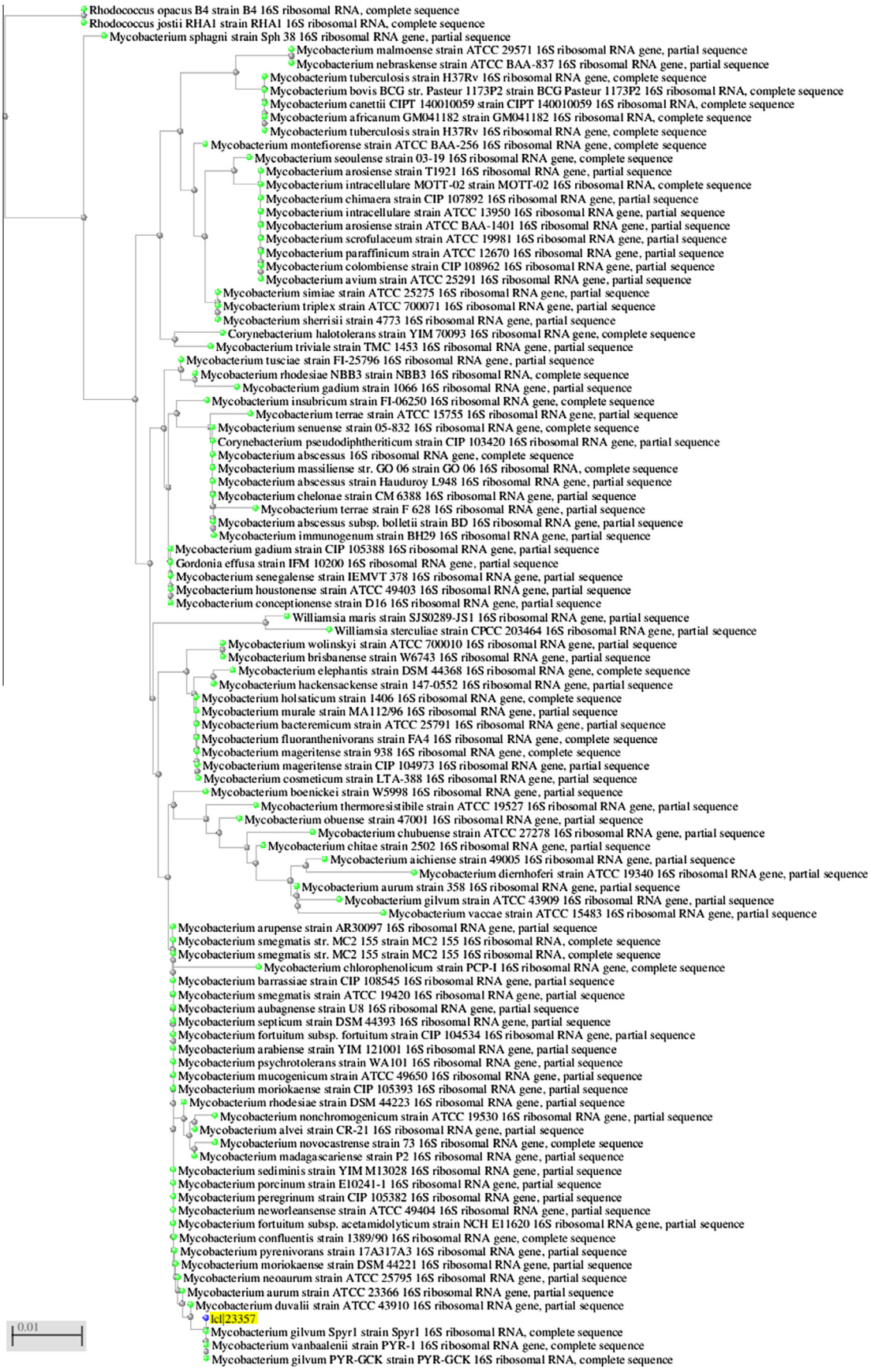

Fig. 2 – Pairwise alignment of blast results among known Mycobacterial species.

the *Mycobacterium vaccae* group (Fig. 1). Blast results showed the similarity of these sequences with *M. gilvum*. This mycobacterial species was first reported by Stanford and Gunthorpe [8] from sputum samples. The species *M. gilvum* is derived from the Latin word *gilvus*, which means pale yellow. This *Mycobacterium* species is a rapid grower (growth within 7 days) and shows resistance to isoniazid, rifampicin, and sodium aminosalicylate. *M. gilvum* is not a pathogenic mycobacterial species and is able to degrade most polycyclic aromatic hydrocarbons (PAH). It has also been isolated from river sediments [9,10]. This mycobacteria recovered from water samples also colonized in free living amoebae (FLA) [11,12]. It was shown that *M. gilvum* after phagocytosis was entering the trophozoites of *Acanthamoeba polyphaga* and survived at this location over a period of 5 days like other NTMs. Many of these NTMs were found to be resistant to the bactericidal activity of amoeba. They can survive easily within the cysts or trophozoites of FLA [13,14]. However, Lamrabet and Drancourt [15] observed that *M. gilvum*, *Mycobacterium rhodesiae* and *Mycobacterium thermoresistibile* mycobacteria did not multiply within amoebae and did not kill the amoebae (Fig. 2).

In this study this environmental mycobacterial species *M. gilvum* was isolated from a leprosy endemic village (Simonpur of Purulia district of West Bengal). Water samples from the bathing places of the leprosy patients were collected for the study. These environmental samples were investigated for the presence of mycobacterial species. This area is highly endemic with a small leprosy colony of old treated leprosy patients situated nearby The Leprosy Mission Community Hospital, Purulia, West Bengal. The population of the village is 1607 (2011 Census). A total of 66 new leprosy cases were reported from Simonpur during the period 2011–2014.

The explanation for the relation between leprosy and *M. gilvum* is beyond the scope of the present study. Other environmental *Mycobacterium* species like *M. vaccae* have been shown to be immunomodulator which suppress the immune system of the host towards susceptibility to leprosy. *M. gilvum* belongs to the group of *M. vaccae* and might be responsible for such an immunomodulation of the host. Further studies will be needed to explore the interactions between free-living amoebae and the majority of waterborne *Mycobacterium* species and whether these species may influence the susceptibility to infectious diseases like leprosy.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

This work was supported by a grant from the host organization The Leprosy Mission India. We thank Mr. Atul Roy for his support in sample collection.

REFERENCES

[1] J.P. Euzbey, List of bacterial names with standing in nomenclature genus *Mycobacterium*, Available: from http://www.bacterio.net/mycobacterium.html (accessed 23.09.14).
[2] M.R. Ettinger, S.R. Webb, S.A. Harris, S.P. McInnch, C.G. Garman, B.L. Brown, Distribution of free living amoebae in James River, Virginia, USA, Parasitol. Res. 89 (2003) 6–15.
[3] J.O. Falkinham III, C.D. Norton, M.W. LeChevallier, Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare* and other mycobacteria in drinking water distribution systems, Appl. Environ. Microbiol. 67 (2001) 1225–1231.
[4] R. Narang, P. Narang, D.K. Mendiratta, Isolation and identification of nontuberculous mycobacteria from water and soil in central India, Indian J. Med. Microbiol. 27 (2009) 247–250.
[5] E. Tortoli, A. Bartoloni, E.C. Bottger, S. Emler, C. Garzelli, E. Magliano, Burden of unidentifiable mycobacteria in a reference laboratory, J. Clin. Microbiol. 39 (2001) 4058–4065.
[6] D. Parashar, D.S. Chauhan, V.D. Sharma, A. Chauhan, S.V.S. Chauhan, V.M. Katoch, Optimization of procedures for isolation of environmental mycobacteria from soil and water samples of North India, Appl. Environ. Microbiol. 70 (2004) 3751–3753.
[7] R.S. Jadhav, R.R. Kamble, V.S. Shinde, S. Edward, V.K. Edward, Use of reverse transcription polymerase chain reaction for the detection of *Mycobacterium leprae* in the slit-skin smears of leprosy patients, Indian J. Lepr. 77 (2005) 116–127.
[8] J.L. Stanford, W.J. Gunthorpe, A study of some fast growing scotochromogenic mycobacteria including species descriptions of *Mycobacterium gilvum* (new species) and *Mycobacterium duvalii* (new species), Br. J. Exp. Pathol. 52 (1971) 627–637.
[9] B. Brezna, A.A. Khan, C.E. Cerniglia, Molecular characterization of dioxygenases from polycyclic aromatic hydrocarbon-degrading *Mycobacterium* spp., FEMS Microbiol. Lett. 223 (2003) 177–183.
[10] D. Dean-Ross, C.E. Cerniglia, Degradation of pyrene by *Mycobacterium flavescens*, Appl. Microbiol. Biotechnol. 46 (1996) 307–312.
[11] T. Adekambi, S. Ben Salah, M. Khlif, D. Raoult, M. Drancourt, Survival of environmental mycobacteria in *Acanthamoeba polyphaga*, Appl. Environ. Microbiol. 72 (2006) 5974–5981.
[12] I.B. Salah, E. Ghigo, M. Drancourt, Free-living amoeba, a training field for macrophage resistance of mycobacteria, Clin. Microbiol. Infect. 15 (2009) 894–905.
[13] G. Greub, D. Raoult, Microorganisms resistant to free-living amoebae, Clin. Microbiol. Rev. 17 (2004) 413–433.
[14] O. Lamrabet, F. Medie, M. Drancourt, *Acanthamoeba polyphaga*-enhanced growth of *Mycobacterium smegmatis*, PLoS One. 7 (2012) e29833.
[15] O. Lamrabet, M. Drancourt, *Mycobacterium glivum* illustrates size correlated relationships between *Mycobacteria* and *Acanthamoeba polyphaga*, Appl. Environ. Microbiol. 79 (2013) 1606–1611.