Detection of *Mycobacterium Gilvum* First Time from the Bathing Water of Leprosy Patient from Purulia, West Bengal

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**Abstract**

**Introduction:** *Mycobacterium gilvum* is a fast grower with smooth growth having pale yellow colour. This strain was first isolated from the sediment of the Grand Calumet River in North-western Indiana based on its ability to utilize pyrene, a toxic polycyclic hydrocarbon, as a growth substrate.

**Methods:** The identification and characterization of this isolate was done by various conventional and molecular tests including 16S rDNA sequencing. Sequencing of the nearly complete 16S rRNA gene revealed a unique organism *M. gilvum*, distantly related to *M. vaccae* group. Blast results showed similarity of these sequences with *M. gilvum*.

**Conclusion:** Results might shed further light and its association with amoeba in the leprosy endemic area of this rare *Mycobacterium* species.

**Keywords:** Non-tuberculous; Mycobacteria; Pathogens

### Introduction

There are 174 species and 13 subspecies known to be present in the genus *Mycobacterium* [1]. *Mycobacterium* has been further categorized into strict pathogens (*Mycobacterium tuberculosis* complex) and potential pathogens, non-tuberculous mycobacteria (NTM) [1]. Environmental mycobacteria (NTMs) are widely distributed in the environment and are passed on to humans by ingestion, inhalation, and inoculation from such sources. *M. gilvum* is a species of the phylum actinobacteria (Gram-positive bacteria with high guanine and cytosine content, one of the dominant phyla of all bacteria), belong to the genus *Mycobacterium*. However, little is known regarding the clinical impact of this species. *M. gilvum* is considered as non-pathogenic and is resistant to isoniazid, rifampicin, and sodium aminosalicylate. We observed that *M. gilvum* is a fast grower with smooth growth having pale yellow colour. This strain was first isolated from the sediment of the Grand Calumet River in North-western Indiana based on its ability to utilize pyrene, a toxic polycyclic hydrocarbon, as a growth substrate.

**Methods**

The identification and characterization of this isolate was done by various conventional and molecular tests including 16S rDNA sequencing. Sequencing of the nearly complete 16S rRNA gene revealed a unique organism *M. gilvum*, distantly related to *M. vaccae* group. Blast results showed similarity of these sequences with *M. gilvum*.

**Conclusion**

Results might shed further light and its association with amoeba in the leprosy endemic area of this rare *Mycobacterium* species.

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**Report**

In our present study while looking for the presence of *M. leprae* in the environment (soil and water) of leprosy patients residing in an endemic village (Simonpur of Purulia district of West Bengal) effort was directed to search for existence of other environmental mycobacterium species. Several mycobacterial species were isolated which grew on Lowenstein Jensen (LJ) medium. 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.

Water sample was collected from the drain connected to the bathing place of patient of leprosy and was investigated. Decontamination was done by Parashar et al. [3] by treating water samples with 3% SDS, 4% NaOH and 2% cetrimide. 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. The colonies were of pale yellow colour and were smooth and pleomorphic in appearance with rapid growth in 7 days. Sequencing of the 16S rRNA gene revealed a unique organism, *M. gilvum*, distantly related to the *Mycobacterium vaccae* group (Figure 1). Blast results showed similarity of these sequences with *M. gilvum*.

**Discussion**

*M. gilvum* was first reported by Stanford and Gunthorpe [4] from human sputum samples. The species *M. gilvum* has been derived from the Latin word gilvus, which means pale yellow. *M. gilvum* (formerly *M. flavescens*) is an environmental mycobacterium isolated from river sediments and it has the ability to degrade polycyclic aromatic hydrocarbons, such as pyrene, as a sole source of carbon and energy. This mycobacteria recovered from water samples is also able to colonize in free living amoeba (FLA) [5,6]. It was shown that *M. gilvum* after phagocytosis by amoeba was capable in entering the trophozoites of Acanthamoeba polyphaga and can survive at this location over a period of 5 days like other NTMs [6].

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 [7]. However, Lamrabet and Drancourt [8] observed that *M. gilvum*, *M. rhodesiae* and *M. thermoresistibile* did not multiply within amoeba and did not kill the amoeba. Despite the global success of multi-drug therapy, incidences of clinical leprosy have been observed in individuals with no apparent exposure to other cases.

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Figure 1: Pairwise alignment of blast results among known Mycobacterial Species.
suggestive of possible non-human sources of the bacteria. Wheat et al. [9] showed that common FLA can phagocytose M. leprae, and allow the bacillus to remain viable for up to 8 months within amoebic cysts. The explanation for the relation between leprosy and M. gilvum was beyond the scope of the present study. Other environmental Mycobacterium species like M. vaccae have been shown to be an immunomodulator which was found to suppress the immune system of the host towards susceptibility to leprosy. M. gilvum being in the same group of M. vaccae 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 Mycobacte rium species and whether this species influence the susceptibility to infectious diseases like leprosy.

References