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## Identification of genes in biofilm of slow and fast growing mycobacteria by using microarray and other molecular tools including electron microscopy

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Electron microscopy used to investigate the detailed structure and configuration of the mycobacteria which may lead to an understanding the role of infections and in transmitting the diseases. Many Mycobacteria can develop biofilm, a multicellular structure largely combining bacteria and their extracellular polymeric substances (EPS). The formation of biofilm results in an alternative existence in which microbes ensure their survival in adverse environments. Bacterial biofilms are often associated with infections especially with medical implants such as catheters and other medical devices. In the natural world more than 99% of bacteria exist as biofilms and according to NIH report more than 75% of all human infections are associated with biofilms formation. Biofilms are slimy, glue-like substance that excreted by bacteria and aggregate on living surface. Biofilms are formed to protect the bacteria from host defenses, antibiotics and from harsh environmental conditions. Biofilms are found almost everywhere in nature, including soil, water pipes, and even inside the human body. Attachment of mycobacteria involved in biofilm formation in the liquid air interface is a complex process, with many variables such as pH, nutrient levels, iron, oxygen, ionic strength and temperature, affecting the outcome. We had taken four mycobacterial species for study of mycobacterial biofilm. The isolates were sub-cultured and characterized biochemically and molecularly. The large quantity of biofilm was produced by *M. smegmatis* at temperature 37°C and 42°C as compared to 30°C. *M. fortuitum* developed more amount of biofilm at 30°C as compared to 37°C and 42°C. *M. avium* developed strong amount of biofilm at 30°C and 42°C as compared to 37°C. *M. tuberculosis* (H37Rv) developed strong biofilm at 37°C and no biofilm at 30°C and 42°C in MB 7H9 media and Sauton's media. The selected non tuberculosis mycobacteria and H37Rv developed strong biofilm in the presence of OADC enrichment in MB7H9 as well as Sauton's medium. Antibiotic susceptibility of biofilms at ultra-structural level was also studied in fast growing clinical isolates *M. smegmatis* in presence of streptomycin, isoniazid rifampicin, ethambutol and pyrazinamide. Electron microscopy revealed that control (no drug) biofilms consisted primarily of bacterial clusters a mid fibrillar elements. Isoniazid showed strong inhibited biofilm in fast grower and sensitive isolates. However, pyrazinamide and isoniazid inhibited biofilm of *M. tuberculosis* (H37Rv) and in MDR isolates ethionamide and moxifloxacin inhibited biofilm in slow grower and fast grower mycobacteria. However, many mycobacterial species are known to form biofilms, little is known about either the genetic requirements, patterns of gene expression. In micro array hybridization, we have found that six genes were expressed in *M. avium*. In *M. tuberculosis*, MDR isolates seven genes were expressed and two genes Rv0359 and Rv3526 were homologous as earlier reported in *P. aeruginosa* and *M. avium* which might be responsible for biofilm formation.

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