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Assay of mycobactins from different mycobacterial species by its effect on growth of *Mycobacterium avium* subsp. *paratuberculosis*

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Genus *Mycobacterium* consists of pathogenic and saprophytic organism which meets the demand of iron by synthesizing mycobactins and exochelins. *Mycobacterium avium* subsp *paratuberculosis* (MAP) is unable to synthesize required amount of mycobactin and require exogenous mycobactin. Fast growers such as *Mycobacterium fortuitum* subsp *fortuitum* MTCC929, *M. phlei* MTCC 1724, *M. smegmatis* MTCC 940, *M. vaccae* and slow growers such as *M. bovis* AN5, *M. avium* D4, *M. avium* 1723, *M. microti* MTCC 1727, *M. tuberculosis* H37Rv and *M. avium* subsp. *paratuberculosis* ATCC 19698 obtained from Biological products division, IVRI were used for study. For studying mycobactin production Dorset and Henley's media was prepared with iron at a concentration of 200µg/liter for mycobactin production. Fast growers were incubated for 3 weeks and slow growers were incubated for 8 weeks at 37°C. After incubation mycobactin were extracted from cells by ethanol and chloroform extraction and purified by aluminium oxide chromatography. Among all the mycobacterial species studied, highest mycobactin production was noticed in *M. fortuitum* followed by *M. tuberculosis*, *M. smegmatis* and *M. phlei*. When production of mycobactin is analyzed per gram of cells, efficient mycobactin producer was *M. smegmatis*. MAP produced least amount of mycobactin in high iron media. MAP was unable to grow in medium and low iron medium. Assay of mycobactins was done by analyzing growth rate of MAP in iron deficient media for 2 months with extracted mycobactins. Out of all extracted mycobactins used for the growth assay, mycobactins extracted from *M. tuberculosis*, *M. phlei* and MAP had produced best growth of MAP & mycobactin extracted from *M. microti* was least efficient growth promoter. Mycobactin of *M. tuberculosis*, *M. phlei* and MAP is to be used for large scale production and for primary culturing of MAP.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*, Mycobactins, growth analysis.

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