Psychrophilic Mycobacteria in *M. leprae*-infected Tissues

TO THE EDITOR:

A multifactorial medium (MFM) was proposed for the *in vitro* cultivation of *Mycobacterium leprae* ([2, 3]). In the MFM, Na-thioglycolate served as a source of energy and mycobactin with exochelin for iron acquisition ([1–4]). Slow growth of leprosy-derived mycobacteria (LDM) occurred on the semisolid medium at pH 5.8 and incubation temperature of 32°C.

I am now able to report that a considerably higher yield and more rapid growth can be achieved in a liquid medium at an incubation temperature of 16°C to 18°C if Na-thioglycolate is replaced by ammonium thioglycolate and β-cyclodextrin replaces mycobactin-exochelin.

In a closed Erlenmeyer flask, 0.05 g of thioctic acid (Fluka Chemical Corporation, Hauppauge, New York, U.S.A.) and 5 g of β-cyclodextrin (Chinoin, Budapest, Hungary) were dissolved in 10 ml of hot ammonium thioglycolate (Fluka) (60% v/w in water).

A poor nutrient, multifactorial liquid medium was used. This contained in 1 liter of distilled water: KH_2PO_4, 2.5 g; Na_2HPO_4, 4.0 g; $(NH_4)_2SO_4$, 2 g; $MgSO_4$, 0.2 g; ferric ammonium citrate, 0.05 g; and 10 ml of the above thioctic acid-β-cyclodextrin-ammonium thioglycolate solution. The pH was adjusted to 7.0, using the PO_4 buffers. The solution, distributed 10 ml/25 ml screw-cap tubes, was autoclaved for 25 min. Optimal growth of the primary cultures and subcultures was registered at 16°C to 18°C. These results indicate that the physicochemical properties of the β-cyclodextrin might replace the iron acquisition growth factors.

No visible growth was observed at 4°C and very slow growth was seen at 32°C. At 16–18°C the inoculum increased in size into a visible growth within 2 to 8 weeks, depending on the size and quality of the inoculum. This growth consisted of strongly acid-fast cells with characteristics as previously described ([3]).

Twenty-four such cultures are now maintained, being transferred into subcultures at 6- to 10-week intervals and grown at 16°C incubation temperature.

These leprosy-derived cultures, ranging from the 2nd to the 17th subcultures respectively, are tentatively designated as "*M. psychrophilum* L.," indicating that further characterization and identification are nec-

essary. However, the designation "*M. psychrophilum* L." has been selected because the mycobacterium grows under psychrophilic conditions and cultures are obtained from *M. leprae*-infected tissues. Results indicate that LDM might have a role in the pathology of leprosy, as advocated earlier by this author.

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