Adaptation of *Mycobacterium psychrophilum* (L) to Mesophilic Growth on Water-soluble Palmitic Acid Complex Agar Media

TO THE EDITOR:

Cold-loving strains of mycobacteria were regularly grown optimally on water-soluble palmitic acid complex media at +10°C from *Mycobacterium leprae*-infected human, armadillo, and nude mice tissues ([5-7]). Preparation of the culture media and some characteristics of the cultures were described, but the strains were not fully identified ([6, 7, 9]).

Investigations were carried out to improve growth conditions, increase yield in the cultures, and estimate growth kinetics for optimal timing of transfer into subcultures. Results thus obtained are described below.

1. Counting of cells in the liquid media was difficult and impossible to quantitate, due to the detergent properties of several ingredients in the media (ammonium thioglycolate, Na palmitate, methylated cyclodextrin).

2. Since growth kinetics were not established, timing of transfer from liquid-to-liquid subcultures was only guesswork. Consequently, subcultures were difficult and rare to obtain. However, a few subcultures were obtained if transfers were made from cultures younger than 30 to 45 days old.

3. Growth in liquid media occurred as a sediment and subcultures from the centrifuged, packed sediment into the homolog semisolid agar media were not obtained.

4. In the liquid media optimal growth in the primary and in the rarely obtained subcultures occurred at +10°C, with low yield at 22°C and no growth at 32°C. Entirely different results were obtained when host-grown *M. leprae* cell masses were directly inoculated to the surface of the palmitic acid-cyclodextrin complex agar media.

5. On the surface of the agar slants, the inoculum increased in size 5 to 10 days following inoculation with *M. leprae* cells. The growth developed into visible, opaque, ivory-colored colonies of 0.5- to 1-mm diameter within 20 to 30 days and reached up to 2 mm in diameter. It was estimated that cultures fully developed in about 50 to 60 days. At this time cultures were transferred into fresh agar media.

6. On the agar media, subcultures were obtained by serial transmission at 50 to 75 days of incubation.

7. Without DMSO in the media the latency period was considerably longer: 15 to 25 days; colony formation was slower and the size of colonies seldom reached 1–2 mm.

8. On the surface of the agar media hardly any growth (colony formation) was observed in the early, young primary cultures at 20°C and 32°C incubation.

9. Since 20-to-60-day-old primary cultures grown at +10°C were transferred to homolog agar media, growth also occurred in mesophilic conditions at 22°C and 32°C. As estimated visually, the mesophilic growth at 22°C or 32°C was comparable to the psychrophilic growth at +10°C.

10. Subcultures from the liquid media lost infectivity in the foot pad of nude mice. The results from solid media are not yet available.

The above observations raise several unanswered questions.

A. Are the obtained cultures psychrophilic or facultative psychrophilic strains? In the host they grow at body temperature; in the primary cultures at +10°C. Why is it then that within a few weeks of incubation at +10°C the same culture will grow at 22°C–32°C in the same culture media, containing the water-soluble palmitic acid complex, a SH group compound, and DMSO. However, the possible adaptation to mesophilic growth occurred only on the surface of the agar media under highly aerobic conditions.

B. Are the obtained cultures aerobic or semiaerobic with growth at low partial O_2 tension? Results clearly show that with low O_2 tension at the bottom of 8-cm high liquid media the growth is limited to the primary cultures, with loss of infectivity and death of the cells since no subcultures were obtained when transferred to the surface of agar media. The fact that clear colony formation was registered on the agar surface in primary cultures and subcultures is definite evidence that the cultures are highly aerobic. Since palmitic acid is biologically easily available, the water-soluble form is

offered to the cells in the media as an oxidizable source of energy, and since palmitic acid with 16 carbons in its structure is a high yield source of energy ([2, 3]), the energy-generating process certainly requires an adequate supply of readily available O_2, thus strictly aerobic conditions.

This does not contradict the claims of Ishaque ([4]) or the observations of Dhople and Lamoureux ([1]) since these authors did not use palmitic acid as oxidizable substrates in their systems, when advocating semiaerobic requirements for multiplication of leprosy-derived mycobacteria—perhaps *M. leprae*—and *M. lepraemurium*.

C. The role of DMSO as a growth-promoting ingredient in the media did not become evident. DMSO is known for its wide range of biological actions ([8]), as a membrane penetrant, a solute carrier across membranes. It is an electron donor as a source of protons and as an acceptor of H-ligaments. It forms complexes with metals, acts as a nucleophilic reactor and as a reducing agent. DMSO has an oxidation-reduction potential. DMSO was originally incorporated into our media because of its documented solute carrier properties, to promote transport of substrates and nutrients across cell walls and membranes. It did not become clear in our experiments which of these remarkable properties of this versatile compound promoted growth of mycobacteria in the media containing water-soluble palmitic acid-methylated cyclodextrin complex.

For further cultivation trials of *M. leprae* with soluble palmitic acid complex as the major source of carbon and energy, psychrophilic and aerobic conditions in primary cultures, followed by adaptation to mesophilic but strictly aerobic growth in the subcultures, are promising conditions to achieve axenic growth of host-grown mycobacteria from *M. leprae*-infected tissues.

In the above physical conditions and on semisolid agar media containing both known energy and carbon sources for *M. leprae*, the water-soluble form of palmitic acid and ammonium thioglycolate, 12 facultative psychrophilic strains of mycobacteria have been isolated so far from 12 *M. leprae*-infected foot pads of nude mice.

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