Further Evidence for the Exclusiveness of the *Mycobacterium leprae*-specific DNA Probe

TO THE EDITOR:

Sequence analysis of long reverse transcriptase generated stretches of the primary structure of 16S ribosomal ribonucleic acid (16S rRNA) from mycobacteria supported the phylogenetic position of *Mycobacterium leprae* within the subgroup of slow-growing pathogenic mycobacteria ([7, 8]). Based on sequence information, a 22-mer synthetic DNA oligonucleotide probe directed against a stretch of positions 206 to 227 (according to the IUB numbering system for *Escherichia coli*) was developed. The specificity of the DNA probe was tested in dot-blot hybridization using *M. leprae, M. tuberculosis, M. avium, M. scrofulaceum*, and *M. phlei* as reference organisms ([10]). Although the probe was exclusive for *M. leprae*, fur-

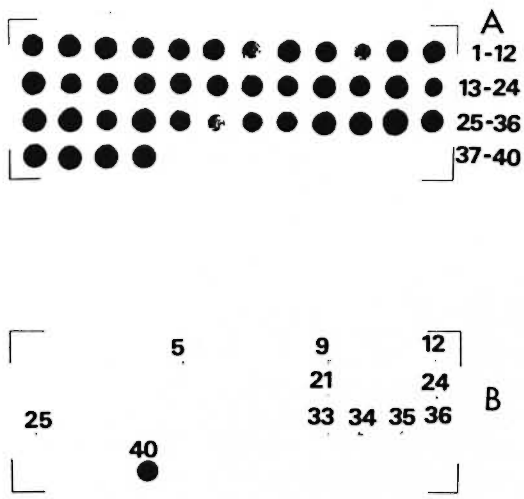
THE TABLE. *Mycobacterial strains used for the isolation of RNA.*

1. *M. ulcerans* ATCC[a] 19423	21. *M. neoaurum* ATCC 25795
2. *M. terrae* TMC[b] 1450	22. *M. lactae* ATCC 25854
3. *M. malmoense* ATCC 29571	23. *M. vaccae* ATCC 15483
4. *M. flavescens* TMC 1541	24. *M. komossense* ATCC 33013
5. *M. szulgai* NCTC[c] 10831	25. *M. chitae* ATCC 19627
6. *M. kansasii* TMC 1204	26. *M. gilvum* NCTC 10742
7. *M. bovis* (Memsen)[d]	27. *M. chelonei* TMC 1544
8. *M. tuberculosis* H37 Rv	28. *M. gadium* ATCC 27726
9. *M. intracellulare* ATCC 23434	29. *M. borstelense* TMC 1524
10. *M. cookii* sp. n. ATCC 49103	30. *M. thermoresistible* ATCC 19527
11. *M. simiae* ATCC 25275	31. *M. fortuitum* TMC 1545
12. *M. gordonae* TMC 1324	32. *M. aurum* ATCC 23366
13. *M. avium* TMC 724	33. *M. sphagni* ATCC 33027
14. *M. scrofulaceum* TMC 1323	34. *M. aichiense* T 49002
15. *M. gastri* ATCC 15754	35. *M. phlei* SN 109 (Bönicke)[f]
16. *M. asiaticum* ATCC 25276	36. *M. duvalli* NCTC 358
17. *M. triviale* TMC 1453	37. *M. phlei* TMC 1516
18. *M. marinum* ATCC 927	38. *M. sphagni* ATCC 33026
19. *M. obuense* ATCC 27023	39. *M. bovis* (Vallée)
20. *M. chubuense* T[e] 48012	40. *M. leprae* LTB[g]

[a] ATCC = American Type Culture Collection, Rockville, Maryland, U.S.A.
[b] TMC = National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A.
[c] NCTC = National Collection of Type Culture, London, U.K.
[d] Memsen = *M. bovis* strain used for tuberculin production.
[e] T = Collection of Dr. M. Tsukamura, Obu, Japan.
[f] Bönicke = Collection of mycobacteria, Borstel, Federal Republic of Germany.
[g] LTB = Leprosy Tissue Bank, Research Institute, Borstel, Federal Republic of Germany.

ther experiments were needed to demonstrate the exclusive specificity of this probe for *M. leprae* by using a larger variety of *Mycobacterium* strains.

Armadillo-derived *M. leprae* were isolated from the liver and spleen tissue of experimentally infected nine-banded armadillos held in the Division of Laboratory Animal Science, Research Institute for Experimental Biology and Medicine, Borstel, Federal Republic of Germany. The animals were kept under conditions at which contamination with other mycobacteria was excluded ([2]). The identity and the purity of *M. leprae* were verified by negative growth on synthetic media ([5]), by positive DOPA oxidase reaction ([6]), and by decolorization with pyridine ([4]). Purification was performed as described earlier ([1]). The isolates were positive in an indirect immunofluorescence technique using *M. leprae*-specific monoclonal antibody against the phenolic glycolipid-I antigen of the organism ([3]). For the isolation of bulk RNA ([7, 8]), 39 strains of 36 species of *Mycobacterium* (The Table) were



THE FIGURE. Autoradiogram of a dot-blot hybridization between a *M. leprae*-specific oligonucleotide probe and bulk RNA from 40 strains of 37 *Mycobacterium* species. (Order of strains is as indicated in The Table.) A = nonstringent washing temperature (46°C); B = stringent washing temperature (65°C). Numbers (excepting No. 40, which is the homologous reaction) refer to those strains whose rRNA gave faint hybridization signals with the probe.

cultivated on Löwenstein-Jensen medium at an optimum growth temperature.

A 22-mer DNA oligonucleotide with the composition 5'ACTCCTGCACCGCAA-AAAGCTT 3' [10] was 5'labeled with $^{32}P\gamma$-ATP and purified electrophoretically. Crude RNA (100 ng) from *M. leprae* and the other 39 mycobacterial strains were applied to Hybond N filters (Amersham) by a dot-blot apparatus (Schleicher & Schüll, Dassel) and fixed by UV 254 nm for 5 min. Prehybridization was in 6 × SSC and 4 × Denhardt's solution for 1 hr at 46°C. Hybridization with the labeled probe (3 × 10^6 cpm, 30 ng) was performed in the same solution for 3 hr at 46°C (20°C below Tm of the probe). Subsequently, the filters were washed at 46°C, 56°C and 65°C, each step for 15 min. Autoradiography was for 12 hr.

The Figure shows the results of dot-blot hybridization. At 46°C (A in The Figure), all RNA dots gave a strong signal because under these washing conditions not only the homologous *M. leprae* rRNA but the heterologous rRNAs as well hybridized with the probe. To eliminate nonspecific binding the filters were washed at increased temperatures—at 56°C and then at 65°C, which corresponds to 1°C below the melting point (Tm) of the homologous hybrid. Hybridization signals of the heterologous mycobacteria rRNA diminished due to the stringent conditions. Only a few strains still showed weak signals which are detectable as very light dots in B in The Figure. However, the homologous *M. leprae* rRNA-probe hybrid retains the distinct signal even at a temperature of 65°C, which in its intensity is comparable to the signal obtained at optimal temperature of 46°C.

These results confirm the high specificity of the *M. leprae* probe. In addition to the information summarized in The Figure, we can also predict the failure of the probe to bind against the rRNA of *M. triviale* and *M. fallax* (Dorsch, Lévy-Frébault and Stackebrandt, unpublished). This is concluded from sufficiently large differences in the sequences of the target site of the two organisms.

In conclusion, the molecular-genetic technique facilitates identification of *M. leprae* using the species-specific probe as already discussed in a previous paper [10], when a very limited number of mycobacterial strains only was tested.

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We were deeply saddened to learn of the death of Dr. Chapman H. Binford at his home on the afternoon of 9 February 1990. Dr. Binford was an Honorary Vice President of the International Leprosy Association, and well known to generations of leprologists.