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Arylsulfatase Activity for Differentiating *Mycobacterium avium* and *Mycobacterium intracellulare*

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Arylsulfatase activities (96-h reaction) of various strains of *Mycobacterium avium* and *M. intracellulare*, as identified by a DNA probe test, were measured. The enzyme activities of *M. avium* strains were significantly higher than those of *M. intracellulare* strains ($P < 0.005$ to $P < 0.025$). The enzyme activities did not vary with serovar; that is, the activities of serovars 1, 2, 8, and 9 (belonging to *M. avium*) were similar to each other, as were the activities of serovars 7, 12, 13, 14, and 16 (belonging to *M. intracellulare*). The results indicate the usefulness of the arylsulfatase test in distinguishing *M. avium* from *M. intracellulare* in an accurate manner.

Since conventional culturing and biochemical tests do not provide a clear-cut differentiation between *Mycobacterium avium* and *Mycobacterium intracellulare*, these species are often referred to as *M. avium* complex (MAC). The known biochemical differences between the two species lie in the activities of arylsulfatase and nitrite reduction (15–17), although the features are not always consistent (6, 13, 15). One major reason for this confusion is that *M. intracellulare*, classified by old criteria based on conventional culture and biochemical properties and serovars (serovars 1 to 3 were regarded as *M. avium* and serovars 4 to 28 were thought to be *M. intracellulare*) (3), includes some organisms now known to belong to *M. avium*. Recently, the Gen-Probe rapid diagnostic system for MAC was developed; this system uses *M. avium*- and *M. intracellulare*-specific DNA probes. A number of investigations, including ours (1, 2, 5, 11, 12), have demonstrated the usefulness of this system in identifying MAC. Moreover, this DNA probe system enables one to distinguish *M. avium* from *M. intracellulare* in a precise manner. Using various serovar reference strains (3, 7), we found that MAC strains belonging to serovars 1 to 6, 8 to 11, and 21 reacted with the *M. avium* probe, while those belonging to serovars 7, 12 to 20, and 25 reacted with the *M. intracellulare* probe (10, 11). However, the MAC strains belonging to serovars 22 to 24 and 26 to 28 included some strains lacking in reactivity to the MAC DNA probes and some strains reactive to the *M. intracellulare* probe. When the growth of various MAC strains, identified by the DNA probe test as either *M. avium* or *M. intracellulare*, at 45°C was examined (this property is fairly useful for the discrimination of authentic *M. avium* from *M. intracellulare*) (15, 17), a majority of the *M. avium* strains grew at this temperature, but the *M. intracellulare* strains did not (11). Therefore, MAC strains which grow at 45°C can be presumptively identified as *M. avium*. This is an example of how some conventional culture and biochemical characteristics may be applicable for the discrimination of *M. avium* from *M. intracellulare*. Here, we studied the arylsulfatase activities of various MAC strains identified by the DNA probe test to determine the practical usefulness of the arylsulfatase test in distinguishing *M. avium* from *M. intracellulare*.

The 43 MAC strains used were as follows: (i) 6 authentic *M. avium* (reference cultures kept at this laboratory) strains; (ii) *M. avium* serovar 1 (6 strains), serovar 2 (2 strains), serovar 8 (5 strains), and serovar 9 (2 strains) (these strains were isolated from humans, except for 1 strain [N-418] of serovar 2, which was isolated from an environmental source); and (iii) *M. intracellulare* serovar 7 (1 strain), serovar 12 (4 strains), serovar 13 (1 strain), serovar 14 (5 strains), and serovar 16 (11 strains) (these strains were isolated from humans, except for 2 strains [N-381 and N-382] of serovar 16, which were isolated from environmental sources). Isolates were serotyped as previously reported (9). Briefly, to determine the agglutinin titers, 0.1-ml volumes of progressive dilutions of antisera in phosphate-buffered saline and equal volumes of the respective bacterial suspensions were mixed in scratch-free tubes (7 by 50 mm), incubated at 35°C, and scored for agglutination.

All the test MAC strains were identified by the DNA probe test (Gen-Probe Inc., San Diego, Calif.) as previously described (11). Arylsulfatase activities were measured by the method of Tarshis (14), with slight modifications. Briefly, 20 mg of bacterial cells harvested from a 3-week-old culture of a test strain on 1% Ogawa egg medium (8) was suspended in 4 ml of 1 mM phenolphthalein potassium disulfate in a tube (13 by 125 mm) with a screw cap and incubated at 37°C for 24 or 96 h. The reaction mixture was centrifuged at 20,000 × *g* for 30 min, and the supernatant (3 ml) was mixed with 0.2 ml of 1 M Na_2CO_3. The mixture was allowed to stand for a few minutes at room temperature, and its optical density (OD) at 553 nm (OD_{553}) was measured with a spectrophotometer (model 100-60; Hitachi Co., Tokyo, Japan) within 10 min. Enzyme activity was represented by color development in the reaction mixture (the OD_{553} value). An OD_{553} of 1.0 corresponds to an enzyme activity of 30.2 pmol/mg of cells per h. (In preliminary experiments, these exprimental conditions were found to be most useful for the differentiation of *M. avium* from *M. intracellulare*.)

Although enzyme activity was detectable even after 24 h of incubation, many values for *M. intracellulare* strains overlapped those for some *M. avium* strains (Fig. 1). However, we could clearly distinguish them after 96 h of incubation. Therefore, we set the incubation time for the arylsulfatase reaction as 96 h, unless otherwise specified.

Table 1 summarizes the results of three separate experiments performed on the MAC strains identified as *M. avium* or *M. intracellulare*. In the case of the *M. avium* strains,

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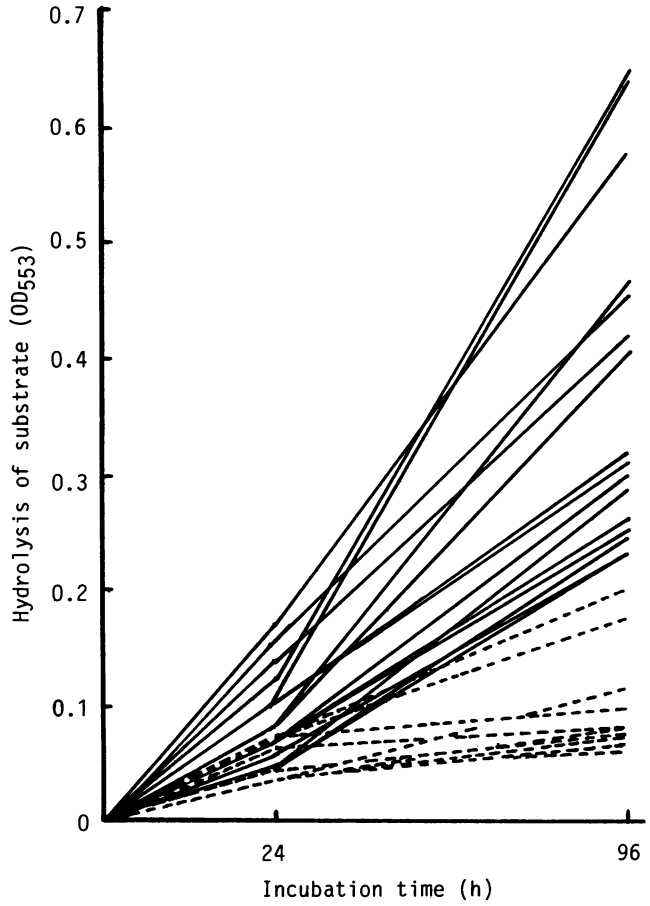

FIG. 1. Time course of arylsulfatase activities of various strains of *M. avium* (strains N-288, N-307, N-339, N-361, N-444, N-445, N-458, N-463, and N-464) (broken lines) and *M. intracellulare* (strains N-240, N-242, N-245, N-252, N-256, N-283, N-284, N-285, N-291, N-292, N-299, N-305, N-338, N-453, and N-477) (solid lines).

arylsulfatase activities were generally low and did not exceed an OD of 0.20 for the reaction mixture, except for 2 of 63 assays (ODs for strains N-418 and N-302 in experiment 2 were 0.202 and 0.245, respectively) (data for individual experiments are not shown). On the contrary, in the case of the *M. intracellulare* strains, much higher activities were recorded: ODs were always greater than 0.20, except for 1 of 66 assays (the OD for strain N-382 in experiment 1 was 0.178) (data for individual experiments are not shown). Therefore, if the cutoff value is set at an OD of 0.20 under the present enzyme assay conditions, the identification of *M. avium* and *M. intracellulare* on the basis of arylsulfatase activities can be done with 97 and 98% specificities, respectively.

Table 2 summarizes the relationship between serovar and arylsulfatase activity. There was no significant difference among the individual enzyme activities of serovars 1, 2, 8, and 9 (*M. avium*), but there was a statistically significant difference ($P < 0.05$; Student's t test) between those of authentic *M. avium* and serovar 8. Similarly, there was no significant difference among the individual enzyme activities of serovars 7, 12, 13, and 16 (*M. intracellulare*). Statistically significant differences were observed when the activities of any of the *M. avium* serovars were compared with the activities of any of the *M. intracellulare* serovars ($P < 0.005$ to $P < 0.025$). There was also a highly significant difference between the average arylsulfatase activity of all the strains identified as *M. avium* and those of all strains identified as *M. intracellulare* ($P < 0.005$). Therefore, it seems that the arylsulfatase activities of MAC strains vary depending on species but not on serovars.

The present study indicates the usefulness of the arylsulfatase test described here in differentiating *M. avium* from *M. intracellulare*. It is possible to accurately differentiate the two species on the basis of arylsulfatase activity as follows: for *M. avium*, the OD_{553} (96-h reaction) is <0.20; for *M.*

TABLE 1. Serovars and arylsulfatase activities of MAC strains identified as *M. avium* or *M. intracellulare* by the DNA probe test

Species	Strain	Serovar	% Hybridization[a] in DNA probe test with probe for:		Arylsulfatase activity (OD_{553}) (mean ± SEM)[b]
			M. avium	*M. intracellulare*	
M. avium	N-288	1	52.6	6.8	0.079 ± 0.013
	N-445	1	51.5	3.9	0.077 ± 0.005
	N-458	1	57.5	1.7	0.059 ± 0.003
	N-461	1	47.9	1.2	0.059 ± 0.002
	N-464	1	54.9	1.6	0.081 ± 0.005
	N-472	1	60.2	1.5	0.122 ± 0.003
	N-418	2	25.5	7.2	0.175 ± 0.015
	N-544	2	60.2	1.8	0.064 ± 0.014
	N-307	8	59.4	2.0	0.091 ± 0.014
	N-339	8	52.3	1.1	0.131 ± 0.015
	N-361	8	48.6	1.4	0.108 ± 0.008
	N-444	8	60.0	3.3	0.198 ± 0.002
	N-463	8	52.7	1.9	0.070 ± 0.006
	N-254	9	50.1	1.8	0.087 ± 0.013
	N-302	9	56.7	3.6	0.172 ± 0.032
	E38686	NT[c]	46.4	1.8	0.065 ± 0.005
	Nagoya 59	NT	43.2	1.9	0.061 ± 0.010
	Flamingo	NT	43.8	1.7	0.068 ± 0.008
	ATCC 15769	NT	42.3	5.4	0.067 ± 0.007
	4110	NT	47.8	3.2	0.081 ± 0.002
	4121	NT	42.1	2.2	0.084 ± 0.003
M. intracellulare	N-242	7	4.5	52.2	0.526 ± 0.054
	N-252	12	2.2	47.4	0.380 ± 0.076
	N-338	12	5.3	38.5	0.332 ± 0.044
	N-385	12	4.7	40.0	0.331 ± 0.014
	N-453	12	4.5	45.5	0.356 ± 0.033
	N-249	13	6.3	51.8	0.511 ± 0.081
	N-244	14	4.3	43.0	0.356 ± 0.069
	N-245	14	1.2	44.8	0.290 ± 0.024
	N-256	14	4.4	37.4	0.349 ± 0.092
	N-291	14	8.0	48.2	0.398 ± 0.087
	N-299	14	3.3	41.1	0.292 ± 0.033
	N-240	16	1.2	36.7	0.411 ± 0.065
	N-241	16	2.6	47.9	0.609 ± 0.108
	N-260	16	3.9	45.6	0.349 ± 0.045
	N-283	16	3.7	40.5	0.412 ± 0.023
	N-284	16	6.2	45.8	0.386 ± 0.082
	N-285	16	5.3	47.4	0.302 ± 0.029
	N-292	16	9.8	52.3	0.567 ± 0.095
	N-305	16	3.2	45.6	0.261 ± 0.039
	N-381	16	2.7	42.4	0.644 ± 0.142
	N-382	16	3.3	47.4	0.223 ± 0.020
	N-477	16	2.9	45.8	0.433 ± 0.070

[a] Reactivity of each strain to DNA probes specific for *M. avium* and *M. intracellulare*. Values higher than 15% were taken as positive (12).
[b] Data were from three experiments.
[c] NT, Serovars of reference *M. avium* strains kept in our laboratory were not determined.

TABLE 2. Relationship between serovar and arylsulfatase activity within species of the MAC[a]

Species	Serovar	No. of strains	Arylsulfatase activity (OD_{553}) (mean ± SEM)[b]
M. avium	1	6	0.080 ± 0.009
	2	2	0.120 ± 0.039
	8	5	0.119 ± 0.019
	9	2	0.130 ± 0.030
	Authentic	6	0.071 ± 0.003
M. intracellulare	7	1	0.526
	12	4	0.350 ± 0.010
	13	1	0.511
	14	5	0.337 ± 0.018
	16	11	0.418 ± 0.040

[a] Summary of the data presented in Table 1.
[b] Totals for *M. avium* and *M. intracellulare*, 0.095 ± 0.009 and 0.396 ± 0.023, respectively.

intracellulare, the OD_{553} (96-h reaction) is >0.20. With our strains, even in a single test, the identification would have been correct in over 97% of cases. When the test was performed in triplicate, there was no overlap between the mean enzyme activities of *M. avium* strains and those of *M. intracellulare* strains (Table 1). The color development at this cutoff value corresponded to the intensity midway between the ± and 1+ color standards for the standard arylsulfatase test (4). In this study, we tested MAC strains representing the serovars isolated from humans and environmental sources in Japan. We are currently investigating other serovars of MAC strains to confirm that the property of arylsulfatase activity is as useful as this study suggests.

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