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原　著

Mycobacterium gadium Casal & Rey Calero 1974 に関する知見補遺

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A SUPPLEMENTARY STUDY ON *MYCOBACTERIUM GADIUM* CASAL & REY CALERO 1974

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Mycobacterium gadium Casal & Rey Calero 1974 (Tubercle, 55: 299–308, 1974) is a rapidly growing, scotochromogenic *Mycobacterium*, which exists in the "Approved Lists of Bacterial Names" (Int. J. Syst. Bacteriol. 30: 225–420, 1980). It was documented that the type strain ATCC 27726 (M. Casal 1066) was differentiated from all other rapidly growing, scotochromogenic mycobacteria and was considered to belong to a distinct species (Tsukamura, M. et al.: Int. J. Syst. Bacteriol. 31: 263–275, 1981). However, since it was described on a single strain by Casal and Rey Calero, it is desired to characterize the species using more strains. We received three additional strains from Prof. M. Casal and studied on four strains, including the type strain ATCC 27726.

The characters of the type strain are shown in Table 1, and characters useful for differentiating among rapidly growing, scotochromogenic mycobacteria are shown in Table 2.

The species *M. gadium* together with other three, *Mycobacterium aurum, M. thermoresistibile* and *M. duvalii,* are characteristic in their negative arylsulfatase activity after 14 days. Among rapidly growing, scotochromogenic mycobacteria, only these four species show a negative arylsulfatase activity after 14 days. Differentiation of *M. gadium* from *M. aurum, M. thermoresistibile* and *M. duvalii* is made as shown in Table 3.

Mycobacterium gadium は Casal & Rey Calero[1] によつて1974年に記載された迅速発育性，暗発色性抗酸菌で，分離源は患者喀痰である。Casal[2] はその後1977年に追加研究を行なつて既知抗酸菌との区別点を明らかにしている。我々は前に Prof. M. Casal から *M. gadium* の type strain を受けとつて研究し，この菌が他のいずれの迅速発育性，暗発色性抗酸菌とも異なることを確かめた[3]。しかし，*M. gadium* の記載は唯1株についてのみ行なわれているので[1]，更に菌株を追加して，菌の特徴を明らかにすることが望ましいと考えた[3]。この我々の意見に応えて Prof. Casal から *M. gadium* と同定された3株が我々の許に送られてきた。そこで，先の株と併せて計4株の *M. gadium* の特徴を研究しえたので報告する。

研究方法

被検株。被検株は次の4株である。Casal 1066＝ATCC 27726 (type strain) (03001), Casal 122B (E 10211), Casal 2315 (E10212), Casal 3021 (E10213)。第1の株は1973年に，以下の3株は1981年に Prof. M. Casal, Department of Microbiology, Medical Faculty, University of Córdoba, Córdoba, Spain から受領した。( )内は，これらの菌株に対して与えた我々の研究室番号である。

性状。検査した性状は表1に示してある。検査方法は既報によつた[3][4]。

計数分類学的解析。検査した成績から，Liston, Wiebe

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Table 1. Characters of *Mycobacterium gadium* Strain ATCC 27726 (type strain)

1. Strong acid-fastness	+	53. Salicylamidase	−
2. Weak or partial acid-fastness	+	54. Allantoinase	−
3. Permanent mycelium	−	55. Succinamidase	−
4. Fragmenting mycelium	−	56. Glutamate as simultaneous N and C sources	+
5. Long rods (more than 7 μm in length)	−	57. Serine as simultaneous N and C sources	−
6. Intermediate rods (3∼6 μm in length)	+	58. Glucosamine as simultaneous N and C sources	−
7. Short rods (less than 2 μm in length)	+	59. Acetamide as simultaneous N and C sources	−
8. Cross-barred	−	60. Benzamide simultaneous N and C sources	−
9. Rough colonies	−	61. Monoethanolamine as simultaneous N and C sources	−
10. Pigmentation of colonies in dark	+	62. Trimethylene diamine as simultaneous N and C sources	−
11. Photochromogenicity	−	63. Acetate as C source	+
12. Growth after 3 days	+	64. Citrate as C source	+
13. Growth at 28°C	+	65. Succinate as C source	+
14. Growth at 37°C	+	66. Malate as C source	+
15. Growth at 42°C	−	67. Pyruvate as C source	+
16. Growth at 45°C	−	68. Benzoate as C source	−
17. Growth at 52°C	−	69. Malonate as C source	−
18. Resistance to 0.2% p-aminosalicylate	+	70. Fumarate as C source	+
19. Degradation of p-aminosalicylate	−	71. Glucose as C source	+
20. Resistance to $NH_2OH.HCl$ (125 μg/ml)	+	72. Fructose as C source	+
21. Resistance to $NH_2OH.HCl$ (250 μg/ml)	−	73. Sucrose as C source	−
22. Resistance to $NH_2OH.HCl$ (500 μg/ml)	−	74. Ethanol as C source	+
23. Growth on Sauton agar medium	+	75. n-Propanol as C source	+
24. Tolerance to 0.1% salicylate in Sauton agar	+	76. Propylene glycol as C source	−
25. Degradation of salicylate	−	77. 1,3-Butylene glycol as C source	+
26. Tolerance to 0.1% pciric acid in Sauton agar	+	78. 1,4-Butylene glycol as C source	+
27. Tolerance to 0.2% picric acid in Sauton agar	+	79. 2,3-Butylene glycol as C source	−
28. Arylsulfatase (3 days)	−	80. n-Butanol as C source	−
29. Arylsulfatase (14 days)	−	81. iso-Butanol as C source	−
30. Resistance to thiophene-2-carboxylic acid hydrazide (1 μg/ml)	+	82. Mannose as C source	+
31. Resistance to 0.05% sodium salicylate	+	83. Galactose as C source	−
32. Resistance to ethambutol (5 μg/ml)	−	84. Arabinose as C source	−
33. Resistance to p-nitrobenzoic acid (0.5 mg/ml)	+	85. Xylose as C source	−
34. Resistance to rifampicin (25 μg/ml)	−	86. Rhamnose as C source	−
35. Tolerance to 0.1% $NaNO_2$ in Sauton agar	+	87. Trehalose as C source	−
36. Tolerance to 0.2% $NaNO_2$ in Sauton agar	+	88. Inositol as C source	+
37. Niacin production	−	89. Mannitol as C source	+
38. Tween 80 hydrolysis (7 days)	+	90. Sorbitol as C source	+
39. Tween 80 hydrolysis (14 days)	+	91. Serine as N source	+
40. Catalase (foam height more than 45 mm)	−	92. Methionine as N source	+
41. α-Esterase	+	93. Acetamide as N source	+
42. β-Esterase	+	94. Benzamide as N source	−
43. β-Galactosidase	+	95. Urea as N source	+
44. Acid phosphatase	−	96. Pyrazinamide as N source	+
45. Nitrate reduction (6 hours)	−	97. Nicotinamide as N source	+
46. Nitrate reduction (24 hours)	+	98. Isonicotinamide as N source	+
47. Acetamidase	−	99. Succinamide as N source	+
48. Benzamidase	−	100. Nitrate as N source	−
49. Urease	+	101. Nitrite as N source	−
50. Isonicotinamidase	−	102. Resistance to isoniazid (10 μg/ml)	+
51. Nicotinamidase	−	103. Resistance to 5% NaCl	+
52. Pyrazinamidase	−		

All tests on resistance were carried out in Ogawa egg medium, unless specially noted. All tests on the utilization of carbon compounds as sole carbon source were carried out in the presence of ammoniacal nitrogen, and all tests on the utilization of nitrogen compounds as sole nitrogen source in the presence of glycerol carbon. The methods used were described previously (Tsukamura, M. et al.: Int. J. Syst. Bacteriol. 31: 263∼275, 1981).

Table 2. Differentiation among Rapidly Growing, Scotochromogenic Mycobacteria

Character	Percentage of strains showing positive reaction																	
	M. thermoresistibile (14)	*M. tokaiense* (3)	*M. obuense* (4)	*M. rhodesiae* (6)	*M. aichiense* (5)	*M. sphagnii* (3)	*M. vaccae* (6)	*M. parafortuitum* (5)	*M. duvalii* (4)	*M. flavescens* I (21)	*M. flavescens* II (9)	*M. phlei* (10)	*M. komossense* (3)	*M. chubuense* (5)	*M. neoaurum* (19)	*M. aurum* (35)	*M. gilvum* (3)	*M. gadium* (4)
Photochromogenicity	0	0	0	0	0	0	83	100	0	0	0	0	0	0	0	0	0	0
Growth at 52°C	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
Degradation of p-aminosalicylate	0	0	100	0	0	33	0	0	0	0	0	0	67	0	0	0	0	0
Arylsulfatase (14 days)	0	100	100	100	100	100	100	100	25	100	100	100	100	100	100	29	67	0
Resistance to ethambutol (5 μg/ml)	0	0	100	17	0	0	0	0	0	0	0	0	67	0	100	3	67	0
α-Esterase	100	67	25	0	0	0	0	0	0	81	100	100	100	0	11	14	0	100
Nitrate reduction (24 hours)	100	0	0	0	0	0	0	20	100	100	100	100	0	100	74	17	100	100
Nicotinamidase	100	100	100	0	0	0	100	100	100	86	11	100	0	100	100	74	100	0
Pyrazinamidase	100	100	100	67	20	0	100	100	100	86	22	100	0	100	100	91	100	0
Glucosamine as N and C sources	0	100	100	100	100	33	100	100	0	0	0	30	100	100	100	97	67	0
Acetamide as N and C sources	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	74	0	0
Monoethanolamine as N and C sources	0	0	25	0	0	0	0	0	0	0	0	0	0	0	100	77	0	0
Trimethylene diamine as N and C sources	0	100	75	100	0	0	100	100	0	0	0	100	0	100	100	100	0	0
Citrate as C source	0	0	0	0	0	0	33	80	0	0	0	0	100	0	89	9	0	100
Succinate as C source	71	100	100	100	100	0	0	100	100	0	0	100	100	100	100	100	100	100
Ethanol as C source	100	100	100	100	100	0	0	100	0	0	0	90	100	100	100	91	100	100
1,3-Butylene glycol as C source	43	0	0	83	20	0	0	0	0	95	89	90	0	0	68	43	100	100
Arabinose as C source	0	67	100	0	0	0	50	80	0	0	0	100	0	20	68	89	0	0
Xylose as C source	0	100	0	100	0	0	67	80	0	0	0	60	0	100	68	97	0	0
Inositol as C source	0	33	50	100	100	100	67	0	0	0	0	0	0	20	100	80	67	100
Sorbitol as C source	0	100	25	0	0	0	0	0	0	52	89	80	100	20	0	11	0	100
Nitrate as N source	100	0	25	0	0	0	0	100	0	5	78	50	0	60	100	49	100	0

The methods used were described previously (Tsukamura, M. et al.: Int. J. Syst. Bacteriol. 31: 263~275, 1981).
The number in parentheses shows the number of strains tested.

& Colwell[5] の方法により4株の "hypothetical median pattern" (HMO) を作つた。これに対する各株の類似度 (*M.* value) を次の式によつて計算した。

$M(\%) = \{n_s/(n_s+n_d)\} \times 100\%$

ここに n_s は2株の間で同じ code symbol (++ または −−) を示した性状数, n_d は異なる code symbol (+−) を示した性状数である。

なお, 比較のための他の迅速発育性抗酸菌の性状は当研究室で蓄積した data を利用した[3]。*M. parafortuitum*, *M. aurum* および *M. neoaurum* については文献4の方法で検査した未発表成績を使用した。

研究成績および考察

1. *M. gadium* type strain Casal 1066 (ATCC27726) の性状

M. gadium type strain の性状を表1に示す。Liston et al.[5] の方法で作つた HMO に対する M. value は 03001 (type strain) が 97%, E10211 が 98%, E10212 が 99%, E10213 が 98% で, 平均値および標準偏差は 98.0±0.8% ($n=4$) であつた。すなわち4株の性状は極めて高い類似性を示した。他の迅速発育性, 暗発色性抗酸菌についてのこの数値は93.3±2.6%~99.3±0.5%であるから菌種内の均一度は充分高いと考えられた[6]。また, 先の我々の研究で, *M. gadium* の type strain は他のすべての迅速発育性, 暗発色性抗酸菌から独立していると認められた故[3], この菌種は菌種として独立させてよいと思われる。

2. *M. gadium* と他の迅速発育性, 暗発色性抗酸菌との区別点および *M. gadium* 同定のための Key Characters

Tsukamura et al.[3] の研究では, *M. gadium* は独立の

Table 3. Differentiation of *Mycobacterium gadium* from *Mycobacterium aurum*, *Mycobacterium thermoresistibile* and *Mycobacterium duvalii*

Character	Percentage of strains showing positive reaction			
	M. aurum 35 strains	*M. thermoresistibile* 14 strains	*M. duvalii* 4 strains	*M. gadium* 4 strains
Arylsulfatase (14 days)	29	0	0	0
Growth at 52°C	100	0	0	0
Nicotinamidase	74	100	100	0
Pyrazinamidase	91	100	100	0
Glucosamine as simultaneous N and C sources	97	0	0	0
Trimethylene diamine as simultaneous N and C sources	100	0	0	0
Citrate as sole C source	9	0	0	100
Ethanol as sole C source	91	100	0	100
Xylose as sole C source	97	0	0	0
Sorbitol as sole C source	0	0	0	100

Remark. The above four species, *M. aurum*, *M. thermoresistibile*, *M. duvalii* and *M. gadium*, are differentiated from other rapidly growing, scotochromogenic mycobacteria by their negative arylsulfatase activity after 14 days.

菌種であるとは思われたが，*M. gadium* が唯1株であつたので，菌種の性状記載も他の抗酸菌との区別点の呈示も行なわなかつた。唯1株では菌種の性状偏差がどの程度かわからないからである。今回，4株を入手しえたので他の迅速発育性，暗発色性抗酸菌との性状比較を表2に示す。

表2の性状から注目されるのは，*M. gadium* が arylsulfatase 14日反応陰性の点である。この反応はほとんど全部の抗酸菌で陽性で，陰性はむしろ例外的存在である[4]。*M. gadium* 以外で陰性を示すのは，*M. thermoresistibile*, *M. aurum*, *M. duvalii* の3者にすぎない。したがつて，arylsulfatare 14日反応陰性を指標にすれば，区別を要する菌種は3菌種に限定され，これらと *M. gadium* の区別は表3に示すごとく比較的容易である。*M. gadium* はすべて喀痰から分離されており，我が国の患者喀痰からも分離される可能性があるので，その特徴および同定のポイントを本報に示した。

総　括

Mycobacterium gadium の性状を明らかにし，他の迅速発育性，暗発色性抗酸菌との区別点を明らかにした。この菌は喀痰から分離されているので，我が国でも分離される可能性がある。

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