Nocardia amarae sp. nov., an Actinomycete Common in Foaming Activated Sludge

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One hundred and seven strains of nocardiae have been isolated from samples of activated sludge or from foam forming on activated sludge coming from 10 sewage-treatment plants in five states. Thirty-five of these strains belong to a previously undescribed species containing a novel type of nocardomycolic acid whose α branch is mono-unsaturated. The name proposed for this new species is *Nocardia amarae*. The type strain is IMRU W3960 (= ATCC 27808).

Numerous filamentous organisms have been reported to be present in activated sludge (2, 3), but actinomycetes are only rarely mentioned (20, 21). At times, activated-sludge sewage-treatment plants are plagued with the production of a viscous scum or foam (24). Microscope examination of such foams revealed that they may be composed of a mass of actinomycetic hyphae (R. F. Lewis, personal communication). We have examined a number of foam samples from plants in New Jersey, New York, Florida, Virginia, and Texas and have found that the most common actinomycete present was a nocardia which does not correspond to any described species known to us. The purpose of this paper is to describe and name this new organism.

MATERIALS AND METHODS

Bacterial strains. The strains included in this study are listed in Table 1.

Isolation of cultures. Samples of sewage foam, either shipped to us in dry ice by air express or collected locally, were diluted (wt/vol) in sterile distilled water, and appropriate dilutions were plated out by pipetting 0.1 ml onto the surface of previously poured and solidified agar plates, and by spreading with a sterile glass spreader. The media most useful in isolating the actinomycetes were yeast extract-Czapeks (YCZ) (12), Glycerol Agar (GA) (11), or potato-dextrose agar (PDA)—300 g of peeled old potatoes were cooked in tap water until soft and filtered. The extract was made up to 1 liter, and 15 g of agar and 20 g of dextrose were added. The pH was not adjusted. The medium was autoclaved at 15 lbs for 20 min. Incubation of plates was carried out for 10 days at 28 C. Many other common bacteriological media which were examined did not support good growth of *N. amarae* on isolation plates.

Maintenance of cultures. Cultures were maintained on yeast extract dextrose (YD) (23) or YCZ slants.

Physiological tests. The tests employed were those of Gordon and Mihm (9) and Gordon (5, 7). Phosphatase production was estimated according to the method of Barber and Kuper (1), and growth on 5% NaCl in YD agar according to the method of Goodfellow (4).

Cell-wall and whole-cell analyses. Cell-wall analyses were made according to the method described by Šuput et al. (22), and whole-cell analyses according to the procedure described by Lechevalier (15).

Mycolic-acid analyses. Mycolic acids were isolated according to the method of Lechevalier et al. (16, 19). The unsaturation in the α branch of the nocardomycolates from the strains studied was verified by the retention times of the pyrolysis fragments not only on OV-1 columns as above but also on 15% DEGS columns (60/80 chromosorb W, AW, DMCS) and by the reaction of the mycolates by using a modified solvomercuration-demercuration technique (18). In this reaction, the elements of methanol are added across a double bond, and the resulting monomethoxy derivative elutes later than the starting compound. The modified procedure is as follows. To 5 mg of methyl nocardomycolate solubilized in 1 to 2 drops of methylene chloride are added 10 mg of $\text{Hg}(\text{OAc})_2$ in 1 ml of methanol. After 18 h at 23 C, 1.5 ml of 15-mg NaBH_4 in 1% NaHCO_3 are added. After standing 1 h to coagulate the precipitated mercury, the supernatant is extracted with one half its volume of methylene chloride. The extract is examined by gas chromatography as previously described, the derivatized nocardomycolates giving rise to mono-methoxy pyrolysis fragments whose retention times are compared to authentic mono-methoxy fatty esters prepared in the same way.

Electron microscopy. The surface of a 1-week-old culture of strain Se 149B on YD agar was covered with a 1-mm layer of 2% water agar. After solidification, cubes with a small colony in the center were cut and placed in double aldehyde fixative (1% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M phosphate buffer) for 30 min at room temperature. After washing in phosphate buffer, then in Veronal acetate buffer, secondary fixation was accomplished by placing the cubes overnight in the dark in 1% osmium

TABLE 1. Sources of strains used in this study

Strain designation ^a	Identity	Source
SN 5101	<i>Nocardia rhodochrous</i> ^b	R. Bonicke
SN 5104	<i>Nocardia rhodochrous</i> ^b	R. Bonicke
A 12974	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 3639	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 1240	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
NC 8139	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 1082S	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 624	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 369	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 462	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 515	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 570	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
I 625	<i>Nocardia rhodochrous</i> ^c	R. E. Gordon
N 10	<i>Nocardia rhodochrous</i>	M. Goodfellow
N 31	<i>Nocardia rhodochrous</i>	M. Goodfellow
N 60	<i>Nocardia rhodochrous</i>	M. Goodfellow
N 239	<i>Nocardia rhodochrous</i>	M. Goodfellow
NC 1621	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
A 7005	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
A 11890	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
I 384	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
I 549	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
I 564	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
I 1256	Strain related to <i>N. rhodochrous</i> ^d	R. E. Gordon
Mil 14	<i>N. rhodochrous</i>	Abnormal foam, Milwaukee, Wis., R. F. Lewis
Mil 15	<i>N. rhodochrous</i>	Abnormal foam, Milwaukee, Wis., R. F. Lewis
SJ 2	<i>N. rhodochrous</i>	Abnormal foam, San José, Calif., R. F. Lewis
Se 113	<i>N. rhodochrous</i>	Abnormal foam, Delran, N. J.
Se 135	<i>N. rhodochrous</i>	Abnormal foam, Middletown, N. J.
Se 141	<i>N. rhodochrous</i>	Abnormal foam, Ocean Township, N. J.
Se 167	<i>N. rhodochrous</i>	Abnormal foam, Hartford, Conn.
Se 187	<i>N. rhodochrous</i>	Abnormal foam, Hartford, Conn.
Se 188	<i>N. rhodochrous</i>	Abnormal foam, Hartford, Conn.
Se 189	<i>N. rhodochrous</i>	Abnormal foam, Bordentown, N. J.
Se 192	<i>N. rhodochrous</i>	Abnormal foam, Bordentown, N. J.
Se 194	<i>N. rhodochrous</i>	Abnormal foam, Bordentown, N. J.
Se 3	<i>Nocardia amarae</i>	Abnormal foam, Andover (Miami), Fla.
Se 6	<i>Nocardia amarae</i>	Abnormal foam, Andover (Miami), Fla.
Se 45	<i>Nocardia amarae</i>	Abnormal foam, Andover (Miami), Fla.
Se 51	<i>Nocardia amarae</i>	Abnormal foam, Riverdale (Miami), Fla.
Se 53	<i>Nocardia amarae</i>	Abnormal foam, Riverdale (Miami), Fla.
Se 61	<i>Nocardia amarae</i>	Abnormal foam, Ocean Township, N. J.
Se 64	<i>Nocardia amarae</i>	Abnormal foam, Ocean Township, N. J.
Se 65	<i>Nocardia amarae</i>	Abnormal foam, Ocean Township, N. J.
Se 85	<i>Nocardia amarae</i>	Abnormal foam, Austin, Tex.
Se 87	<i>Nocardia amarae</i>	"Normal" froth, Austin, Tex.
Se 90	<i>Nocardia amarae</i>	Abnormal foam, Austin, Tex.
Se 91	<i>Nocardia amarae</i>	Abnormal foam, Austin, Tex.
Se 96	<i>Nocardia amarae</i>	Sludge return, Austin, Tex.
Se 97	<i>Nocardia amarae</i>	Abnormal foam, Bordentown Township, N. J.
Se 102	<i>Nocardia amarae</i>	Abnormal foam, Bordentown Township, N. J.
Se 106	<i>Nocardia amarae</i>	Abnormal foam, Eversham, N. J.
Se 107	<i>Nocardia amarae</i>	Abnormal foam, Eversham, N. J.
Se 110	<i>Nocardia amarae</i>	Abnormal foam, Delran, N. J.
Se 111	<i>Nocardia amarae</i>	Abnormal foam, Delran, N. J.
Se 117	<i>Nocardia amarae</i>	Abnormal foam, Matawan, N. J.

TABLE 1.—(Cont'd.)

Strain designation	Identity	Source
Se 118	<i>Nocardia amarae</i>	Abnormal foam, Matawan, N. J.
Se 119	<i>Nocardia amarae</i>	Abnormal foam, Matawan, N. J.
Se 120	<i>Nocardia amarae</i>	Abnormal foam, Matawan, N. J.
Se 122	<i>Nocardia amarae</i>	Artificial foam lab isolate, Bordentown, N. J.
Se 139	<i>Nocardia amarae</i>	Abnormal foam, Ocean Township, N. J.
Se 144	<i>Nocardia amarae</i>	Abnormal foam, Ocean Township, N. J.
Se 149	<i>Nocardia amarae</i>	Abnormal foam, Jamaica Bay, N. Y.
Se 149B	<i>Nocardia amarae</i>	Abnormal foam, Jamaica Bay, N. Y.
Se 151	<i>Nocardia amarae</i>	Abnormal foam, Jamaica Bay, N. Y.
Se 154	<i>Nocardia amarae</i>	Abnormal foam, Jamaica Bay, N. Y.
Se 156	<i>Nocardia amarae</i>	Sample no. 1, abnormal foam, Arlington, Va.
Se 157	<i>Nocardia amarae</i>	Sample no. 1, abnormal foam, Arlington, Va.
Se 160	<i>Nocardia amarae</i>	Sample no. 1, abnormal foam, Arlington, Va.
Se 162	<i>Nocardia amarae</i>	Sample no. 2, abnormal foam, Arlington, Va.
Se 164	<i>Nocardia amarae</i>	Sample no. 2, abnormal foam, Arlington, Va.

^a A, American Type Culture Collection, Rockville, Md.; I, Institute of Microbiology, Rutgers University; NC, National Collection of Type Cultures, London, England; N, Collection of the University of Newcastle Upon Tyne (England).

^b Received as *N. pellegrini*.

^c Received as "*Mycobacterium*" *rhodochrous*.

^d Received as related to "*Mycobacterium*" *rhodochrous*.

tetroxide in Veronal-acetate buffer containing 0.1% tryptone and 0.05% sodium chloride. After washing in buffer and dehydrating in ethanol, the cubes were transferred to propylene oxide and were then placed in a 50% propylene oxide-50% DER332-732 mixture for 1 h at 45 C. After 1 h at 37 C in pure resin, the cubes were placed in capsules containing the same resin. Curing of the resin was carried out for 14 h at 37 C, then 24 h at 45 C followed by 24 h at 60 C. Sections 5,000 to 7,000 nm thick were cut on a Sorvall MT-1 microtome with a diamond knife and stained by immersion for 30 min in Reynold's lead citrate. The electron microscope used was a JEM 120 operated at 80 kV.

RESULTS AND DISCUSSION

Samples of foam and sludge from activated-sludge sewage-treatment plants were received from two localities in the Miami, Fla., area (Riverdale and Andover), Arlington, Va., and Austin, Tex. Similar samples were collected in Ocean, Bordentown, Evesham, Delran, and Matawan Townships in N.J., and Jamaica Bay, N.Y. All of these samples were found to be rich in actinomycetic hyphae when examined by phase-contrast microscopy.

Foaming problems appeared at first to be limited to seasons or areas where the average temperature of the water was >55 C. Recently, however, some northern plants have been experiencing this problem at lower temperatures.

All of the strains of the new organism had a

cell wall of type IV (17) and a whole-cell sugar pattern of type A (15), typical of members of the genus *Nocardia*. Other genera having this type of cell wall include *Pseudonocardia*, *Mycobacterium*, *Corynebacterium* (diphtheriae type), *Micropolyspora*, and *Saccharomonospora*. They contained nocardomycolic acids (16) of a novel type whose main α branch is mono-unsaturated. Thus, the mycolates of these strains give rise, on pyrolysis, to major amounts of mono-unsaturated fatty esters having 16 and 18 carbons, accompanied for some strains by minor amounts of analogous saturated fragments. This is in contrast to most *Nocardia* strains we have examined to date which contain nocardomycolates whose pyrolysis fragments are saturated fatty esters having 12 to 18 carbons. Nevertheless, minor amounts of unsaturated fragments have been noted in the pyrolysates of the mycolic acids of numerous nocardiae, particularly members of the *N. rhodochrous* group (M. P. Lechevalier, unpublished results). We have also found this novel type of nocardomycolate in two other nocardial strains: *N. vaccinii* IMRU 3500 and *N. carnea* 3419. It has been reported (13) that corynomycolates isolated from the free lipids and cord factor of a strain of *Nocardia asteroides* contained mono-unsaturated α branches. Conventional nocardomycolic acids having a saturated α branch were found in the wall-bound lipids of this strain. We have

TABLE 2. A comparison of the physiological characteristics of *N. amarae* with other species of *Nocardia*

Determination	<i>N. amarae</i> (35 strains)	<i>N. asteroides</i> (137 strains ^a)	<i>N. brasiliensis</i> (62 strains ^a)	<i>N. caviae</i> (24 strains ^a)
Hydrolysis of:				
Casein	0 ^b	0	98	0
Hypoxanthine	0	4	94	100
Tyrosine	0	1	100	0
Xanthine	0	0	0	100
Adenine	0	0	3	4
Starch	86(w)	67	55	54
Gelatin	0	34 ^c		
Esculin	100	100	100	100
Urea	100	96	100	92
Production of:				
Nitrate reductase	100	86	90	100
Growth at/on/in:				
10 C(YD)	6	15	37	13
45 C(YD)	0	41	2	50
Lysozyme broth	9	100	100	100
Acid from:				
Adonitol	0	0	0	0
Arabinose	0	0	0	4
Dulcitol	0	0	0	0
Erythritol	0	7	2	0
Galactose	0	27	94	0
Glucose	100	98	97	100
Glycerol	100	99 ^d	98 ^d	100 ^d
Inositol	92	3	100	100
Lactose	0	0	0	0
Maltose	100	6	4	18
Mannitol	100	1	94	90
Mannose	100	17	68	36
Melibiose	0			0
α -Methyl-D-glucoside	0	0	0	0
Raffinose	0	0	0	0
Rhamnose	92	32	0	5
Salicin	100	25 ^c		
Sorbitol	0	0	0	0
Xylose	0	0	0	5
Utilization of:				
Acetate	100	100 ^d	100 ^d	100 ^d
Benzoate	0	0 ^d	2 ^d	0 ^d
Citrate	0	38	98	29
Lactate	100	31 ^e		
Malate	100	97	100	100
Oxalate	0	0 ^e		
Propionate	100	100 ^d	100 ^d	100 ^d
Pyruvate	100	99 ^d	100 ^d	100 ^d
Succinate	100	92	100	100
Survival:				
50 C/8 h	0	94	0	88

^a Gordon and Horan (8).^b Percent positive; w, weak.^c Gordon and Mihm (9) (79 strains of *N. asteroides*).^d Gordon and Mihm (10) (142 strains of *N. asteroides*; 15 strains of *N. caviae*; 62 strains of *N. brasiliensis*).^e R. E. Gordon, personal communication to M.P.L.

obtained this organism through the kindness of J. Rozanis but have found only normal nocardomycolates in its free and bound lipids. Under our conditions of growth, it is possible

that the percentage of the corynomycolates in the mycolate fractions is too low to be detected.

F. Mariat, of the Institut Pasteur, in Paris,

TABLE 3. A comparison of the physiological characteristics of *N. amarae* with other species of *Nocardia*

Determination	<i>N. amarae</i>	<i>N. rhodochrous</i>		<i>Nocardia</i> sp. related to <i>N. rhodochrous</i>		<i>N. rhodo- chrous</i> (sewage) (12 strains) ^b	<i>N. vac- cinii</i> IMRU 3500 ^b	<i>N. carnea</i> IMRU 3419 ^b
	(35 strains)	(97 strains) ^a	(17 strains) ^b	(36 strains) ^a	(7 strains) ^b			
Hydrolysis of:								
Casein	0 ^c	0		0		0	—	—
Hypoxanthine	0		0		0	0	—	—
Tyrosine	0	74		5		58	—	—
Xanthine	0	0		0		0	—	—
Adenine	0		35		71	75	—	—
Starch	86(w) ^d	97	35	97	43	25	—	—
Gelatin	0		0		0	0	—	—
Esculin	100		76		86	75	+	+
Urea	100	73 ^e			100	83	+	—
Production of:								
Phosphatase	83 ^d		100		100	100	—	+
Nitrate reductase	100	82 ^e			100	58	+	+
Growth at/in/on:								
10 C (YD)	6 ^d	100	100	97	100	94	—	+
45 C (YD)	0	46	53	11	14	66	—	—
Lysozyme broth	9 ^d		59		28	94	+	+
5% NaCl (YD)	6 ^d		100		100	83	—	+
Adenine agar	6 ^d		100		100	100	+	+
Acid from:								
Adonitol	0		0		0	0	—	—
Arabinose	0	8		0		0	—	—
Cellobiose	0		6		0	0	—	—
Dulcitol	0	0		0		0	—	—
Erythritol	0		12		0	0	—	—
Fructose	100		100		43	100	+	+
Galactose	0		6		0	17	+	+
Glucose	100	99		100		100	+	+
Glycerol	100		94		57	100	+	+
Inositol	92 ^d	21		0		75	+	—
Lactose	0	0		0		0	—	—
Maltose	100	27 ^e	35		14	8	—	—
Mannitol	100	99	100	0	0	100	+	+
Mannose	100	99		61		100	+	—
Melibiose	0		0		0	0	—	—
α-Methyl-D-glucoside	0	0		0		0	—	—
Raffinose	0	0		0		0	—	—
Rhamnose	92 ^d	9		0		0	+	—
Salicin	100		29		0	25	—	—
Sorbitol	0	100	100	0	0	100	—	+
Sucrose	100		100		29	100	—	—
Trehalose	100	97		24		92	+	+
Xylose	0		41		0	42	+	—
β-Methyl-D-xyloside	0		0		0	0	—	—
Utilization of:								
Acetate	100		100		100	100	+	+
Benzoate	0	70		19		83	—	—
Citrate	0	90		8		100	+	—
Lactate	100	100		100		100	+	—
Malate	100	99		94		100	+	+
Oxalate	0	0		0		0	—	—
Propionate	100		100		100	100	+	+
Pyruvate	100		100		100	100	+	+
Succinate	100	100		97		100	+	+
Tartrate	0		12		0	0	—	—
Survival:								
50 C/8 h	0		82		86	92	ND ^f	ND

^a R. E. Gordon (6).^b Run by authors.^c Percent positive; w, weak.^d Reactions of type strain (ATCC 27808): Starch hydrolysis —; phosphatase production +; growth at 10 C —; growth in/on: lysozyme broth —, 5% NaCl (YD) —, adenine agar —; acid from: inositol +, rhamnose +.^e Based on tests of 60 strains (Gordon and Mihm, 1957).^f ND, Not determined.

FIG. 1. Phase contrast, oil immersion objective, $\times 8,000$. Strain Se 85 grown 1 week in YD broth in shaken culture at 28 C.

tested eight strains of the organism described here in guinea pigs and mice and found them to be nonpathogenic (personal communication).

When the physiology of these sewage strains was compared with that of strains of *N. asteroides*, *N. brasiliensis*, and *N. caviae*, it was found to differ as indicated in Table 2. These

strains were closest to *N.* ("Mycobacterium") *rhodochrous*, strains of which are also isolated occasionally from foams. A comparison of these two taxa, along with the reactions of strains of *N. rhodochrous* isolated from sewage, *N. vacinii* 3500, and *N. carnea* 3419, are presented in Table 3.

FIG. 2. Electron micrograph. Section through hyphae of strain Se 149B grown 1 week on YD agar. $\times 37,000$.

The principal characteristics differentiating the new organism from *N. rhodochrous* are the following: nocardomycolic acid type, hydrolysis of tyrosine, growth at 10 C, growth on adenine and on 5% NaCl, acid from inositol, maltose, rhamnose, salicin, and sorbitol, utiliza-

tion of benzoate and citrate, and survival at 50 C for 8 h. The principal differences between the new organism and Gordon's (6) *rhodochrous*-related *Nocardia* sp. (which she placed in *M. rhodochrous*) are: nocardomycolic acid type, hydrolysis of adenine, growth at 10 C, growth

FIG. 3. Same as Fig. 2. $\times 52,500$.

on adenine and 5% NaCl, acid from fructose, maltose, mannitol, rhamnose, salicin, sucrose, and trehalose, and survival at 50 C.

The new organism also differs from the Russian *N. ucrainica* (14) by eleven characters: acid from galactose, lactose, α -methyl-D-

glycoside, raffinose, rhamnose, and xylose, utilization of citrate, decomposition of tyrosine and casein, and growth on 5% NaCl.

Ruth E. Gordon of our Institute has informed us (personal communication, 1973) that she has no named strains of *Nocardia* in

FIG. 4. Bright-field, high-dry objective. Strain Se 6 growing on Bennett's agar. $\times 2,000$.

the IMRU collection having the same pattern of physiological reactions as our organism.

On the basis of the overall evidence, we regard the organism described here as a new species, for which we propose the name *Nocardia amarae*. (Gr. n. ἀμάρᾱ, sewage duct; M.L. gen. n. *amarae* of a sewage duct.)

Description of *Nocardia amarae* sp. nov.: substrate mycelium. Moderately branching vegetative hyphae are variable in diameter but about $1\ \mu\text{m}$ wide; the hyphae penetrate and grow into the agar medium; they do not fragment in undisturbed cultures; secondary branching is rare to absent; the colony edge is

filamentous, never entire. Many strains on first isolation show banded hyphae (Fig. 1). This characteristic is lost on prolonged cultivation but can be observed in hyphae in sewage. Hyphae are often irregularly swollen and break up into coryneform elements when mounted in water. Sections through banded hyphae show numerous cross walls, cells with irregularly thickened, laminated walls, empty spaces, numerous mesosomes and, within the same hypha, cells containing cytoplasm of varying electron opacities (Fig. 2 and 3).

Aerial mycelium. Aerial mycelium is not visible to the naked eye. Short, branched, and unbranched aerial hyphae may be seen by the use of a microscope. No specialized morphological structures have been noted (Fig. 4).

Appearance on various media. Growth of all strains on various media is always tan, convoluted, and lacking macroscopically visible aerial hyphae. A small amount of brown-black, soluble pigment is produced on YCZ agar by some strains.

Staining characteristics. The filaments are gram-positive and only partially acid-fast, if at all.

Physiological and chemical characteristics. Catalase-positive. Attacks glucose oxidatively. Cell wall of type IV. Contains nocardomycolic acids having an unsaturated α branch. Grows at 23 to 37 C.

Esculin and urea hydrolyzed. No hydrolysis of casein, xanthine, hypoxanthine, tyrosine, adenine, or gelatin. Starch was weakly hydrolyzed (86% of the strains studied were positive). Nitrate reductase produced. Phosphatase produced (83%). No growth at 10 (94%) or 45 C or on 5% NaCl. No growth in lysozyme broth (91%) or on adenine agar (94%). Acetate, lactate, malate, propionate, pyruvate, and succinate utilized. Benzoate, citrate, oxalate, and tartrate not utilized. Acid from fructose, glucose, glycerol, inositol (92%), maltose, mannitol, mannose, rhamnose (92%), salicin, sucrose, and trehalose. No acid from adonitol, arabinose, cellobiose, dulcitol, galactose, lactose, melibiose, α -methyl-D-glucoside, raffinose, sorbitol, xylose, β -methyl D-xyloside. No survival at 50 C for 8 h.

Type strain: Se6 (= ATCC 27808; = IMRU W3960).

Reactions of the type strain are given as a footnote to Table 3.

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LITERATURE CITED

1. Barber, M., and S. W. A. Kuper. 1951. Identification of *Staphylococcus pyogenes* by the phosphatase reaction. *J. Pathol. Bacteriol.* 63:65-68.
2. Cooke, W. B., and W. O. Pipes. 1970. The occurrence of fungi in activated sludge. *Mycopathol. Mycol. Appl.* 40:249-270.
3. Cyrus, Z., and A. Sladká. 1970. Several interesting organisms present in activated sludge. *Hydrobiologia* 35:383-396.
4. Goodfellow, M. 1971. Numerical taxonomy of some nocardioform bacteria. *J. Gen. Microbiol.* 69:33-80.
5. Gordon, R. E. 1966. Some criteria for the recognition of *Nocardia madurae*. *J. Gen. Microbiol.* 45:355-364.
6. Gordon, R. E. 1966. Some strains in search of a genus *Corynebacterium*, *Mycobacterium*, *Nocardia* or what? *J. Gen. Microbiol.* 43:329-343.
7. Gordon, R. E. 1967. The taxonomy of soil bacteria, p. 293-321. In T. Gray, R. G. Parkinson, and B. Parkinson (ed.), *The ecology of soil bacteria*. Liverpool University Press.
8. Gordon, R. E., and A. C. Horan. 1968. *Nocardia dassonvillei*, a macroscopic replica of *Streptomyces griseus*. *J. Gen. Microbiol.* 50:235-240.
9. Gordon, R. E., and J. M. Mihm. 1957. A comparative study of some strains received as nocardiae. *J. Bacteriol.* 73:15-27.
10. Gordon, R. E., and J. M. Mihm. 1962. Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann. N.Y. Acad. Sci.* 98:628-636.
11. Gordon, R. E., and M. M. Smith. 1953. Rapidly growing, acid fast bacteria. I. Species descriptions of *Mycobacterium phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. *J. Bacteriol.* 66:41-48.
12. Higgins, M. L., and M. P. Lechevalier. 1969. Poorly lytic bacteriophage from *Dactylosporangium thailandensis*. *J. Virol.* 3:210-216.
13. Ionedá, T., E. Lederer, and J. Rozanis. 1970. Sur la structure des diesters de tréhalose ("cord factors") produits par *Nocardia asteroides* et *Nocardia rhodochrous*. *Chem. Phys. Lipids* 4:375-392.
14. Krasnikov, E. I., A. Nesterenko, V. A. Romanovskaya, and S. A. Kasumova. 1971. Microorganisms of the genera *Nocardia* Trevisan and *Mycobacterium* Lehmann and Neumann which utilize natural and individual gaseous hydrocarbons. *Microbiology U.S.S.R.* 40:240-246.
15. Lechevalier, M. P. 1968. Identification of aerobic actinomycetes of clinical importance. *J. Lab. Clin. Med.* 71:934-944.
16. Lechevalier, M. P., A. C. Horan, and H. Lechevalier. 1971. Lipid composition in the classifica-

- tion of nocardiae and mycobacteria. *J. Bacteriol.* **105**:313-318.
17. Lechevalier, H. A., and M. P. Lechevalier. 1970. A critical evaluation of the genera of aerobic actinomycetes, p. 393-405. *In* H. Prauser (ed.), *The actinomycetales*. Fischer, Jena.
 18. Lechevalier, M. P., H. A. Lechevalier, and C. E. Heintz. 1973. Morphological and chemical nature of the sclerotia of *Chainia olivacea* Thirumalachar and Sukapure of the Order *Actinomycetales*. *Int. J. Syst. Bacteriol.* **23**:157-170.
 19. Lechevalier, M. P., H. Lechevalier, and A. C. Horan. 1973. Chemical characteristics and classification of nocardiae. *Can. J. Microbiol.* **19**:965-972.
 20. McKinney, R. E., and M. P. Horwood. 1952. Flocc-producing bacteria. *Sewage Ind. Wastes* **24**: 117-123.
 21. Mohanrao, G. J., and R. E. McKinney. 1962. A study of the biochemical characteristics of quaternary carbon compounds. *Int. J. Air Water Pollut.* **6**:153-168.
 22. Šuput, J., M. P. Lechevalier, and H. A. Lechevalier. 1967. Chemical composition of variants of aerobic actinomycetes. *Appl. Microbiol.* **15**:1356-1361.
 23. Waksman, S. A. 1950. *The actinomycetes*. Chronica Botanica. Waltham, Mass.
 24. Wells, W. N., and M. T. Garrett. 1971. Getting the most from an activated sludge plant. *Pub. Works*, May: 63-68.