Description of a New Species, *Oerskovia xanthineolytica*, and Emendation of *Oerskovia* Prauser et al.

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A new species belonging to the genus *Oerskovia* Prauser et al. of the order *Actinomycetales* is described under the name *O. xanthineolytica*. It differs from *O. turbata* mainly in its ability to hydrolyze xanthine and hypoxanthine, in certain carbohydrate reactions, in the production of a phosphatase, and in its ability to grow at 42 C on Trypticase-soy agar. The type strain of this species is IMRU 3959 (LL G62) (=ATCC 27402). The description of the genus *Oerskovia* is emended to take into account the fact that members of this genus can grow anaerobically on Trypticase-soy medium with the production of catalase-negative biomasses.

Actinomycetes belonging to the genus *Oerskovia* Prauser et al. (10, 13) are characterized by the formation of branched substrate hyphae which break up into flagellated motile elements, by a lack of aerial mycelium, and by a cell wall composition of type VI (15) plus galactose. Other actinomycetes with a type VI cell wall include members of the genera *Actinomyces*, *Rothia*, *Arthrobacter*, and *Promicromonospora*. Of these, the only taxon presenting any similarity to *Oerskovia* is *Promicromonospora*, first described by Krasil'nikov et al. in 1961 (9). *Promicromonosporae* produce highly branched substrate hyphae which break down into short fragments, but these are never motile. *Promicromonosporae* also produce aerial hyphae on many media, and their cell walls lack galactose.

Up to now, the genus *Oerskovia* included only *O. turbata*, previously placed in the genus *Nocardia* (3, 12). In addition, there are numerous *Oerskovia*-like actinomycetes which may be isolated from soil and other sources which are nonmotile but which do not have a clearcut similarity to *promicromonosporae*. These will be referred to as "NMOs" (nonmotile *Oerskovia*-like strains).

Recently, strains of *Oerskovia*, first identified as *Arthrobacter* spp., have been reported by J. W. Mann and J. D. Macmillan (Bacteriol. Proc., p. 62, 1971) to break down the walls of various yeasts. Among the enzymes that were elaborated by these organisms, the authors found a β -1-6 glucanase, a β -1-3 glucanase, and an

α -mannanase. Jones and Ballou (8) also reported an α -mannanase from an "*Arthrobacter*" sp. which I have found to be an NMO. *Oerskoviae* have been reported to deacetylate steroid acetates (17).

Lately, I have isolated strains of *oerskoviae*, *promicromonosporae*, and NMOs from various sources including soil, decaying organic matter, saliva, and aluminum hydroxide compressed gel antacid. These recent isolates have been characterized physiologically, and a certain number of tests have been found useful in distinguishing *oerskoviae* and NMOs from *promicromonosporae*. The *oerskoviae* studied form two natural subgroups; hence, I am describing a new species, *O. xanthineolytica*, and redefining *O. turbata*.

MATERIALS AND METHODS

Strains. The strains studied are listed in Table 1.

Physiological tests. To determine whether glucose was oxidized or fermented, OF (Difco) medium, containing 1% glucose tubed 7 cm deep and covered with 5 cc of sterile mineral oil, was used and read after 5 days of incubation at 28 C. The cytochrome oxidase test was performed on 18 to 24-hr growth according to Ewing and Johnson (4), β -D-galactosidase was determined on 18- to 72-hr growth and read after 24 hr using Differentiation Discs ONPG (Difco), and deoxyribonuclease was detected by using growth on DNase agar (Difco) incubated for 14 days at 28 C. Phosphatase activity was determined according to Barber and Kuper (1) after 1 day of incubation at 28 C. To determine whether nitrate could serve as an oxygen acceptor under anaerobic conditions, the

TABLE 1. *Strains included in this study*

Name	Culture collection no. ^a	Source
<i>Oerskovia turbata</i>	SSIC 891 = Ørskov 27 = ATCC 25835	Soil
<i>O. turbata</i>	IMRU 689	Soil
<i>O. turbata</i>	IMRU 761 = ATCC 27403	Soil
<i>O. turbata</i>	IMRU 762	Soil
<i>O. turbata</i>	IMRU 763	Soil
<i>O. turbata</i>	IMET 7130	Soil
<i>O. turbata</i>	LL 11-49 = ATCC 27400	Soil
<i>Oerskovia xanthineolytica</i>	LL 17-11 = ATCC 27404	Soil
<i>O. xanthineolytica</i>	LL Y13-3	Soil
<i>O. xanthineolytica</i>	LL Y13-4 = ATCC 27401	Soil
<i>O. xanthineolytica</i>	LL 20-2	Soil
<i>O. xanthineolytica</i>	LL Mann 1	Soil
<i>O. xanthineolytica</i>	LL Mann 3	Soil
<i>O. xanthineolytica</i>	IMRU 3959 = ATCC 27402	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G63	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G79	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G86	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G89	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G104	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G109	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL G975	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	LL ST3	Dry grass cuttings
<i>O. xanthineolytica</i>	LL ST4	Dry grass cuttings
NMO ^b	LL Ballou	Soil
NMO	LL 17-13	Soil
NMO	LL 18-4	Soil
NMO	LL 19-1	Soil
NMO	LL 20-1	Soil
<i>Promicromonospora citrea</i>	I 8	L. V. Kalakoutskii, Academy of Sciences, Moscow
<i>P. citrea</i>	I 25	L. V. Kalakoutskii
<i>Promicromonospora</i> sp.	LL G165	Aluminum hydroxide gel antacid
<i>Promicromonospora</i> sp.	LL G173	Aluminum hydroxide gel antacid
<i>Promicromonospora</i> sp.	LL G201	Aluminum hydroxide gel antacid

^a SSIC, Statens Seruminstitut, Copenhagen, Denmark; ATCC, American Type Culture Collection, Rockville, Md., USA; IMRU, Institute of Microbiology, Rutgers University, New Brunswick, N.J.; LL, author's collection; IMET, Institut für Mikrobiologie und Experimentelle Therapie, Jena, Germany.

^b NMO, Nonmotile oerskovia-like strains.

nitrate agar of Sutter (16) was used. Cellulase production was determined in the cellulose strip-peptone medium described by Skerman (14). Ability to grow anaerobically (Gaspak, BBL) and at 42°C was determined on Trypticase-soy agar (BBL). Absence of nitrate in Trypticase-soy agar was confirmed by reduction with zinc dust and lack of positive reaction with the sulfanilic acid- α -naphthylamine reagent for nitrite. The inability of strains to utilize fumarate or sulfate as an electron acceptor was determined by adding 0.05% magnesium sulfate or 0.14% sodium fumarate to soil extract agar (7) and incubating anaerobically as above. Other tests and maintenance conditions were described previously (2, 5, 6, 11).

RESULTS

Promicromonosporae were originally reported to bear monospores on the substrate hyphae. I have never been able to observe their formation either in solid or liquid culture. On solid media, the hyphae fragment in situ and give rise to small branchlets, but no true spores seem to be formed.

Physiologically, we have been able to differentiate *promicromonosporae* from *oerskoviae* and NMOs by the following (Table 2): *promicromonosporae* cannot attack glucose fer-

TABLE 2. *Properties of strains of Oerskovia, nonmotile oerskovia-like strains (NMOs), and promicromonosporae^a*

Properties ^b	<i>O. turbata</i> (7 strains)	<i>O. xanthineolytica</i> (15 strains)	<i>O. xanthineolytica</i> IMRU 3959	NMOs		<i>P. citrea</i> (2 strains)	<i>Promicromono-</i> <i>spora</i> spp. (3 strains)
				A (3 strains)	B (2 strains)		
OF glucose	F ^c	F	F	F	F	O	O
Anaerobic growth (TS)	+	+	+	+	+	—	—
Nitrate/EA	+	+	+	+	+	—	—
Cellulase production	—	—	—	+	+	—	—
Motility	+	+	+	—	—	—	—
Cell wall	VI + Gal	VI + Gal	VI + Gal	VI	VI	VI	VI
Hydrolysis of:							
Casein	+	+	+	—(66)	—	+	+
Tyrosine	—	—(76)	—	—	—	+	+
Xanthine	—	+	+	+	—	V	—
Hypoxanthine	—	+	+	+	—	—	—
Adenine	NG	NG	NG	NG	NG	NG	NG
Gelatin	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+
DNA	+	+	+	+	+	+	+
Urea	—(86)	—(88)	—	—(66)	+	—	—(66)
Production of:							
Phosphatase	—	+	+	+	—	+	+
Galactosidase (ONPG)	+	+	+	+	+	+	+
Cytochrome oxidase	—	—	—	—	—	—	—
Catalase	+	+	+	+	+	+	+
Nitrate reductase	+	+	+	+	+	V	+
Growth at 42 C (TS)	—	+	+	—(66)	—	+	—
53 C	—	—	—	—	—	—	—
Survival 50 C/8 hr.	+	+	+	+	+	+	—(66)
Survival 60 C/4 hr.	+	+	+	+	+	+	—(66)
Survival in lysozyme broth	—	—	—	—	—	—	—
Acid from: ^d							
Inositol	—	—	—	—	—	—	—(66)
Mannitol	—	—(82)	—	—	—	+	+
Melibiose	—(86)	+	+	—(66)	—	+	+

Raffinose	—	+	+	+	+	+	+
Rhamnose	—	—	—	—	—	—	—
Sorbitol	+(86)	+	+	+	+	+	+
Utilization of:							
Acetate	+	+	+	+	+	+	+
Citrate	—	—	—	—	—	—	—
Lactate	+	+	+	+	+	+	+
Malate	—	—	—	—	—	—	—
Propionate	—(86)	—	—	—	—	—	—
Pyruvate	+	+	+	+	+	+	+
Succinate	—	—	—	—	—	—	—
Tartrate	—	—	—	—	—	—	—

^a Numbers in parentheses indicate percentages of positive or negative strains in variable tests.

^b TS, Trypticase-soy; EA, electron acceptor.

c F, Fermentative; O, oxidative; VI, cell wall type VI: major constituents are alanine, glutamic acid, glucosamine, muramic acid, lysine and aspartic acid; Gal, galactose; NG, no growth; V, variable.

lactose; NG, no growth; V, variable.

^d All strains produced acid from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, trehalose, xylose, and β -methyl-D-xyloside. No strain produced acid from adonitol, erythritol, or sorbose.

mentatively, cannot use nitrate as an electron acceptor under anaerobic conditions, and cannot grow anaerobically on Trypticase-soy agar, whereas oerskoviae and NMOs can. All who have studied the oerskoviae up to now, including the present author, have considered these organisms to be strict aerobes. However, they can grow anaerobically through at least five serial transfers on Trypticase-soy agar. The factor in this medium that acts as an electron acceptor does not appear to be nitrate, sulfate, or fumarate. No other medium of 16 tested supports such anaerobic growth. The morphology of anaerobically grown oerskoviae is like that of cultures grown in air. The anaerobic growth is catalase negative. Oerskoviae may be distinguished from the other two groups on the basis of the presence of galactose in the cell wall and the presence of motile forms in cultures 24 to 48 hr old. The NMOs tested to date all produce cellulases, and the members of the other two groups do not.

It is proposed to emend the descriptions of the genus *Oerskovia* and the type species, *O. turbata*, as follows.

Oerskovia Prauser et al. char. emend. Extensively branching vegetative hyphae (ca. 0.5 μm in diameter), growing on the surface of and penetrating into the agar, which break up into motile rodlike elements. Growth appears bacterial in smears. No aerial mycelium is formed. Gram positive. Catalase positive when grown in air. Facultatively anaerobic on Trypticase-soy medium. Attacks glucose both oxidatively and fermentatively. Cell wall is type VI plus major amounts of galactose (15). Aspartic acid may be absent. Percent guanine plus cytosine (%GC) of the DNA = 70.5 to 75.0 (15). The type species is *Oerskovia turbata*. Named after Jeppe Ørskov, who worked at the Statens Seruminstitut of Copenhagen from 1920 to 1959.

Oerskovia turbata (Erikson) Prauser, Lechevalier, and Lechevalier char. emend. Yellow, extensively branching vegetative hyphae breaking into motile, rodlike elements which are usually monotrichous when small and peritrichous when long (ca. 0.4 by 1.1 μ m). Nitrite produced from nitrate. Casein, gelatin, and starch are hydrolyzed. Catalase positive when grown aerobically; catalase negative when grown anaerobically on Trypticase-soy agar. Not acid fast. Mesophilic: incapable of growth at 42 C on Trypticase-soy agar. Isolated from soil. Acetate, lactate, and pyruvate are utilized; acid from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, glycogen, α -methyl-D-glucoside, lactose, maltose, mannose, salicin, sucrose, trehalose, xylose, and

β -methyl-D-xyloside. Adenine, tyrosine, hypoxanthine, xanthine, benzoate, citrate, malate, succinate, and tartrate not utilized; no acid from adonitol, erythritol, mannitol, raffinose, rhamnose, or sorbose. The following tests were variable (percent positive strains): propionate (14%), acid from melibiose (14%), sorbitol (86%), urease (14%). Deoxyribonuclease and β -D-galactosidase positive, phosphatase, cytochrome oxidase, and cellulase negative. Does not grow in lysozyme broth. Survives 50 C for 8 hr and 60 C for 4 hr. *O. turbata* SSIC 891 (Statens Seruminstitut of Copenhagen) (=ATCC 25835) is the type strain.

Oerskovia xanthineolytica sp. nov. (from "xanthine" and "λύω" I dissolve). Yellow, extensively branching vegetative hyphae breaking into motile, rodlike elements which are usually monotrichous when small and peritrichous when long (ca. 0.4 by 1.1 μ m). Nitrite produced from nitrate. Casein, xanthine, hypoxanthine, gelatin, and starch are hydrolyzed. Catalase positive when grown aerobically; catalase negative when grown anaerobically on Trypticase-soy agar. Not acid fast. Thermotolerant: capable of growth at 42 C on Trypticase-soy agar. Isolated from soil, aluminum hydroxide antacid gel, and decaying organic matter. Acetate, lactate, and pyruvate are utilized; acid from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, glycogen, α -methyl-D-glucoside, lactose, maltose, mannose, melibiose, raffinose, salicin, sorbitol, sucrose, trehalose, xylose, and β -methyl-D-xyloside. Adenine, benzoate, citrate, malate, succinate, and tartrate not utilized; no acid from adonitol, erythritol, inositol, rhamnose, or sorbose. Variable reactions (percent positive): tyrosine decomposition (24%), urease production (12%), and mannitol (18%) and propionate (59%) utilization. Phosphatase, deoxyribonuclease, and β -D-galactosidase positive; cytochrome oxidase and cellulase negative. Does not grow in lysozyme broth. Survives 50 C for 8 hr and 60 C for 4 hr. *O. xanthineolytica* IMRU 3959 (LL G62) is designated as the type strain; it has been deposited in the American Type Culture Collection (Rockville, Md.) under the number ATCC 27402. The morphology of the type strain is the same as that given above for the species, and the other properties of this strain are given in Table 2.

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