Recognition of *Oerskovia* Species in the Clinical Laboratory: Characterization of 35 Isolates

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Fifty-seven clinical isolates of previously unidentified gram-positive, fermentative, nonsporeforming rods were studied and compared to the type strains of *Oerskovia turbata* and *O. xanthineolytica*. Thirty-five of the isolates were identified as *Oerskovia* species: 9 were identified as *O. turbata*, and 26 were identified as *O. xanthineolytica*. The *Oerskovia* cultures could be differentiated from the other isolates on the basis of the development of filamentous colonies.

The genus *Oerskovia*, described by Prauser et al. (8), Sukapure et al. (11), and emended by Lechevalier (6), consists of yellow-pigmented organisms with branched hyphae. These hyphae break up into motile, rodlike elements that "appear bacterial" in smears. These rods are gram positive and non-acid fast. Previously described sources of the organisms are soil, aluminum hydroxide gel antacid, and dry grass cuttings (6). During the last 20 years, the Special Bacteriology Section (SBS), Bacteriology Division, Center for Disease Control (CDC), has received for identification a number of motile, gram-positive, nonsporeforming, yellow pigment-producing organisms isolated from clinical sources. These organisms were not recognized as belonging to any established species and were arbitrarily designated as group A *Corynebacterium* sp. Later, this group was divided into five subgroups, primarily on the basis of carbohydrate reactions. When these isolates were observed to have characteristics similar to those reported for the genus *Oerskovia*, a collaborative study between the Actinomycete Laboratory of CDC's Mycology Division and the SBS was initiated to further characterize and classify them. *Listeria denitrificans* was included in this study because two of our colleagues, D. Hollis and G. Wiggins, had noted during earlier work on the genus *Listeria* that many biochemical characteristics of this species were similar to those of the group A strains.

MATERIALS AND METHODS

Isolates. The isolates studied are listed in Table 1. The type strains *Oerskovia turbata* ATCC 25835 and *O. xanthineolytica* ATCC 27402 were supplied by M. P. Lechevalier. An isolate of *L. denitrificans*, ATCC 14870, was sent to us by H. J. Welshimer.

Morphology. All cultures were incubated at 35°C unless otherwise noted. Gram stains were made from 24-h heart infusion agar slant cultures. A mod-

ified Ziehl-Neelsen method (4) was used for acid-fast stains. Flagella were stained by the Leifson technique (7). Each isolate was cultured on heart infusion agar (HIA; Difco) and Trypticase soy agar (TSA; BBL) plates. Colonial morphology was observed by a light microscope under low power ($\times 100$ magnification) after 24 h, 48 h, and 7 days of incubation.

Biochemical tests. Tests and media were those used routinely in the SBS and the Actinomycete Laboratory, and have been previously described (1, 2, 5).

Whole cell analyses. The methods used for diamino pimelic acid analysis and monosaccharide analysis are those described by Berd (1).

RESULTS

Gram stains from 24-h HIA slant cultures of the group A-1 and A-2 isolates examined showed variation from gram positive to gram negative. Most appeared to be gram variable. Cellular morphology varied a great deal. Some isolates were similar to the type strain of *O. turbata* (Fig. 1a) and were filamentous, with rudimentary branched and round forms (Fig. 1b). Other isolates resembled the type strain of *O. xanthineolytica* (Fig. 1c) and were predominantly coccoid, with a few medium-long rods (Fig. 1d). Most isolates showed variations between these two extremes. *L. denitrificans* stained gram positive and had short, straight, to long, curved rods (Fig. 2). Groups A-3, A-4, and A-5 stained gram variable or gram positive and, although most isolates were short to medium, rather thin rods, two isolates were decidedly diphtheroidal, and two others were filamentous, with rudimentary branching.

Flagella stains were done on all of the cultures studied. One to five polar or lateral flagella, or both, were seen on smears of all motile isolates (Fig. 3). Flagella were not detected on the three nonmotile group A-1 and A-2 isolates (C9205-6, B6934, D4746) or on the other nonmotile group A isolates.

TABLE 1. *Isolates used in this study*

Name	SBS no.	Source
<i>Oerskovia turbata</i> ATCC 25835	KC1355	Soil
<i>O. turbata</i>	C4239	Unknown (human)
<i>O. turbata</i>	C5519	Heart valve
<i>O. turbata</i>	C5520	Heart valve
<i>O. turbata</i>	C5614	Heart valve
<i>O. turbata</i>	C5918	Tissue
<i>O. turbata</i>	C5919	Tissue
<i>O. turbata</i>	C6926	Heart tissue
<i>O. turbata</i>	C6927	Heart tissue
<i>O. turbata</i>	C8821	Blood
<i>Oerskovia xanthineolytica</i> ATCC 27402	KC1356	Aluminum hydroxide gel antacid
<i>O. xanthineolytica</i>	KC1332	Sputum
<i>O. xanthineolytica</i>	2425	Urine
<i>O. xanthineolytica</i>	2681	Blood
<i>O. xanthineolytica</i>	5488	Blood
<i>O. xanthineolytica</i>	9359	Right ethmoid
<i>O. xanthineolytica</i>	A3824	Heart
<i>O. xanthineolytica</i>	A4212	Blood
<i>O. xanthineolytica</i>	A7595	Cerebrospinal fluid
<i>O. xanthineolytica</i>	A8249	Blood
<i>O. xanthineolytica</i>	A8307	Tear duct
<i>O. xanthineolytica</i>	B559(1)	Sputum
<i>O. xanthineolytica</i>	B4827	Urine
<i>O. xanthineolytica</i>	B6165	Urine
<i>O. xanthineolytica</i>	B6934(A)	Leg wound
<i>O. xanthineolytica</i>	C2886	Left tibia
<i>O. xanthineolytica</i>	C4861	Urine
<i>O. xanthineolytica</i>	C5541	Blood
<i>O. xanthineolytica</i>	C7119	Blood
<i>O. xanthineolytica</i>	C7315	Left eye drainage
<i>O. xanthineolytica</i>	C9205-6	Liver biopsy
<i>O. xanthineolytica</i>	D334	Lung
<i>O. xanthineolytica</i>	D1217	Granuloma, hand
<i>O. xanthineolytica</i>	D3494	Leg wound
<i>O. xanthineolytica</i>	D3580	Blood
<i>O. xanthineolytica</i>	D4746	Blood
<i>O. xanthineolytica</i>	D4865	Wound, knee
SBS Group A-3	A3936	Blood
SBS Group A-3	C2508	Blood
SBS Group A-3	C8262	Cerebrospinal fluid
SBS Group A-3	D2991	Cerebrospinal fluid
SBS Group A-3	D3060	Pilonidal cyst
SBS Group A-3	D3505	Blood
SBS Group A-4	C4603	Ear
SBS Group A-4	C5206	Urine
SBS Group A-4	C6391	Pleural fluid
SBS Group A-4	C8412	Enfamil
SBS Group A-4	C8759	Unknown
SBS Group A-4	D225	Blood
SBS Group A-4	D640	Incision drainage
SBS Group A-4	D3562	Sputum
SBS Group A-5	6905	Blood
SBS Group A-5	9846	Cerebrospinal fluid
SBS Group A-5	A5085	Cerebrospinal fluid
SBS Group A-5	B3576	Wound
SBS Group A-5	B4407	Cerebrospinal fluid
SBS Group A-5	C2375	Blood
SBS Group A-5	C6166	Back lesion
SBS Group A-5	C6210	Blood
<i>Listeria denitrificans</i> ATCC 14870	KC1082	Ox blood

FIG. 1. Gram stain from 24-h HIA slants showing variation in cellular morphology within *Oerskovia* species. Magnification, $\times 1,375$. (a) *Oerskovia turbata* (KC1355); (b) *O. xanthineolytica* (A8307); (c) *O. xanthineolytica* (KC1356); (d) *O. turbata* (C5614).

FIG. 2. *Listeria denitrificans* (KC1082). Gram stain from 24-h HIA slant. Magnification, $\times 1,375$.

The colonial morphology of all group A-1 and A-2 isolates on 24- and 48-h HIA and TSA plates was similar to that of both of the type strains of *Oerskovia* sp. Colonies varied from 1 to 2 mm in diameter, and all had vegetative hyphae in 24 h. Young colonies were filamentous with distinct centers. Mature colonies developed dense centers with a filamentous fringe that was so short on some colonies that it was barely visible (Fig. 4). No aerial mycelia were seen after 7 days of incubation. All except two isolates (C6927 and D3494) produced a yellow, nonsoluble pigment. *L. denitrificans* was nonpigmented and produced smooth, convex colonies with entire edges in 24 h. After 48 to 72 h, filaments were formed which radiated from the center of the colony and grew into and on the surface of the agar (Fig. 5). Hyphae were not seen on any of the group A-3, A-4, or A-5 cultures during 7 days of observation.

Biochemical reactions and other characteristics are listed in Table 2. *O. turbata*, *O. xanthineolytica*, and all of the organisms in groups A-

1 and A-2 liquefied gelatin within the 14-day incubation period. Neither *L. denitrificans* nor the organisms in group A-3 liquefied gelatin, and, since their biochemical reactions were very similar to those of *Oerskovia* sp., the gelatin liquefaction test seems to be useful for differentiation.

Carbohydrate fermentation results listed in Table 2 were obtained in the fermentation broth base (2) routinely used in the SBS laboratory. Numerous fermentation tests were also done in the Actinomycete Laboratory with Gordon's carbohydrate base (3). With the carbohydrates tested, results were the same in both bases. Cellobiose, inulin, dextrin, glycogen, erythritol, melibiose, and melezitose, however, were not tested in Gordon's base. Temperature studies on all group A-1 and A-2 isolates were done on TSA slants, in addition to tryptone-glucose-yeast extract agar slants; the slants were incubated overnight in warm air at 25, 35, and 42°C. Results obtained with the two media varied only slightly. Nine isolates of groups A-1 and A-2 hydrolyzed casein but did not hydrolyze xanthine, hypoxanthine, or tyrosine. They resembled *O. turbata* in this respect. The remaining 26 isolates of groups A-1 and A-2, like *O. xanthineolytica*, hydrolyzed casein, xanthine, and hypoxanthine. *L. denitrificans* did not decompose either casein, xanthine, or hypoxanthine. Although casein and hypoxanthine hydrolysis tests gave reproducible results with all of the isolates, nine cultures of groups A-1 and A-2 were inconsistent in their ability to hydrolyze xanthine. However, when plates were incubated at a constant 28°C rather than at room temperature, this problem was eliminated, and all nine isolates hydrolyzed xanthine in 14 days. None of the isolates in group A-3, A-4, or A-5 hydrolyzed xanthine or hypoxanthine. Casein was hydrolyzed by two of the group A-5 isolates.

Whole cell analyses of the organisms in groups A-1 and A-2 indicated that they contained neither the *meso*- nor the *L*-isomer of diaminopimelic acid. Furthermore, as Lechevalier reported (6), galactose was the only characteristic whole cell sugar found in either the *Oerskovia* sp. or the isolates in groups A-1 and A-2.

DISCUSSION

Fifty-seven clinical isolates received for identification by the SBS during the last 20 years and formerly designated group A *Corynebacterium* sp. were studied. Of these, 35 isolates are now identified as *Oerskovia* sp. All 35 isolates were formerly included in groups A-1 and A-2.

FIG. 3. *O. turbata*. Flagella stain from 24-h rabbit blood agar plate. Magnification, $\times 1,375$.

TABLE 2. Characteristics of isolates of *Oerskovia* sp., group A *Corynebacterium* sp., and *Listeria denitrificans*^a

Characteristics ^b	<i>O. tur- bata</i> ATCC 25835	Groups A-1 and -2 (<i>O. tur- bata</i>), 9 iso- lates	<i>O. xanthi- neolytica</i> ATCC 27402	Groups A-1 and -2 (<i>O. xanthineoly- tica</i>), 26 isolates	Group A-3, 6 iso- lates	Group A-4, 8 iso- lates	Group A-5, 8 iso- lates	<i>L. denitri- ficans</i> ATCC 14870
Vegetative hyphae ^c on TSA and HIA	+	+	+	+	-	-	-	+
Motility	+	+	+	+(3)	+	+(3)	-(3)	+
Yellow growth pigment	+	+(1)	+	+(1)	-	+(2)	+	-
OF glucose	F	F	F	F	F	F	F	F
Gelatinase	+	+	+	+	-	-(1)	-(2)	-
Nitrate reduction	+	-(1)	+	+(1)	+	-(1)	+(1)	+
Acid from: ^d								
Glucose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	-	+
Mannitol	-	-	-	-	-	+	+	-
Lactose	+	+(3)	+	+(7)	+	+	-(3)	+
Sucrose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Glycerol	+	+(2)	+	+(1)	+(1)	-(2)	+(3)	+
Arabinose	+	+	+	+	+	+	-(3)	+
Mannose	+	+	+	+	+	+	+(1)	+
Rhamnose	-	-	-	-	+	+(3)	-(2)	-
Raffinose	-	-	+	-(3)	+(2)	-(2)	-(2)	+
Sorbitol	-	-	-	-	-(2)	-(2)	-(1)	-
Inulin	-	-	-	-(2)	-	-(1)	-	-
Dextrin	+	+	+	+(1)	+	+	+(2)	+
Glycogen	+	+	+	+	-	-(2)	+(1)	+
Melibiose	-	-	+	-(6)	+(1)	+(4)	-(3)	+
Melezitose	-	-	-	-(2)	-(2)	-	-(2)	+
Starch	+	+	+	+	-(2)	+(3)	+	+
Growth on TGY agar at:								
25°C	+	+	+	+	+	+	+(1)	+
35°C	+	+	+	+	+	+	+	+
42°C	-	+(1)	+	+(2)	+(2)	-(1)	+(2)	+
Urea hydrolysis	-	+(2)	+	+(10)	-	-	-(1)	-
Growth in NB with 6% NaCl	-	+(3)	+	+(5)	+(3)	-(2)	-(3)	+
Whole cell analyses:								
DAP ^e	-	-	-	-	-	-(1)	-(1)	-
Galactose	+	+	+	+	+(2)	+	+(2)	-
Hydrolysis of:								
Casein	+	+	+	+	-	-	-(2)	-
Xanthine	-	-	+	+	-	-	-	-
Hypoxanthine	-	-	+	+	-	-	-	-
Tyrosine	-	-	-	-(1)	-	-	-	-

^a Numbers in parentheses indicate the number of isolates that varied from the recorded result. All isolates hydrolyzed esculin and were catalase positive. No isolates utilized citrate, produced indole, or stained acid fast positive. Tests for oxidase, MR-VP, and reactions in litmus milk were variable within all groups.

^b Abbreviations: TSA, Trypticase soy agar; HIA, heart infusion agar; TGY, tryptone-glucose-yeast extract; NB, nutrient broth; F, fermentative; O, oxidative; DAP, diaminopimelic acid.

^c No aerial mycelium produced on plate or slide cultures.

^d Results recorded were obtained in fermentation broth base. All isolates produced acid from galactose, levulose, trehalose, cellobiose, and salicin. No strains produced acid from adonitol, dulcitol, inositol, or erythritol.

^e DAP, when present, was in the *meso* form.

All of the organisms that had been included in groups A-3, A-4, and A-5 apparently do not belong in the genus *Oerskovia*, and their identity remains uncertain at the present time.

We speciated the 35 *Oerskovia* isolates. Nine proved to be *O. turbata*, and 26 were identified as *O. xanthineolytica* solely on the basis of the ability of the latter to hydrolyze xanthine and hypoxanthine. In contrast to Lechevalier's findings, we found that growth on TSA at 42°C was a variable characteristic for both species. Lechevalier (personal communication, 1976) clarified the differences that we noted in the production of acid from raffinose. After examining some of our strains of *O. xanthineolytica*, she now believes that this character should be considered variable for *O. xanthineolytica*.

The nine isolates, now identified as *O. turbata*, were received by the SBS during a 15-month period (1972 to 1974), and all but one (C4239) were sent from the same geographical area (Colorado) (9). Unfortunately, we have been unable to obtain any information about

FIG. 4. *O. xanthineolytica* (KC1356). Colonies producing vegetative hyphae on 48-h TSA plate. Magnification, $\times 100$.

FIG. 5. *L. denitrificans* (KC1082). Colonies producing vegetative hyphae on 72-h TSA plate. Magnification, $\times 100$.

the source of isolate C4239, other than that it was from a human.

As noted in Table 1, the sources of the *O. xanthineolytica* isolates varied widely. The fact that eight cultures were isolated from human blood would seem to indicate clinical significance, but, unfortunately, we have been unable to obtain satisfactory case histories on these patients.

The biochemical characteristics of *L. denitrificans* closely resemble those of the *Oerskovia* species. The only differences that we found were gelatinase production, fermentation of melezitose, and casein hydrolysis. On the basis of whole cell analyses, however, we do not believe that *L. denitrificans* should be included in this genus. *L. denitrificans* produced branched vegetative hyphae on HIA and TSA plates. Stuart and Welshimer have suggested that serious consideration be given to reclassifying *L. denitrificans* (10). Our findings support their suggestion.

ACKNOWLEDGMENTS

We thank Mary P. Lechevalier for her advice and encouragement, Don Howard for photomicrographs, and Libero Ajello for his suggestions in the preparation of this manuscript.

REPRINT REQUESTS

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