INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Jan. 1982, p. 1–14
0020-7713/82/010001-14$02.00/0
Vol. 32, No. 1

Rhodococcus luteus nom. nov. and *Rhodococcus maris* nom. nov.

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Two groups of bacteria isolated from natural substrates were assigned to the genus *Rhodococcus* Zopf 1891, emend. Goodfellow and Alderson 1977. We propose the name *Rhodococcus luteus* nom. nov. for the first group, which corresponds to the description of the organism previously known as "*Mycobacterium luteum*" Söhngen 1913. The type strain of *R. luteus* is IMV 385 (= AUCNM A-594). The name proposed for the second group of strains, which corresponds to the description of the organism previously known as "*Flavobacterium maris*" Harrison 1929, is *Rhodococcus maris* nom. nov. The type strain of *R. maris* is IMV 195 (= AUCNM A-593). The properties of the two species are described, and the characters useful for the identification of the species are given.

From soils of the Soviet Union and from skins and the intestinal tracts of carp (*Cyprinus carpio*), two groups of bacteria, distinguishable by their morphological and colonial characters, were isolated on mineral salt agar enriched with *n*-alkanes. These isolates were assigned to the genus *Rhodococcus* Zopf 1891, as emended by Goodfellow and Alderson (11) to include strains previously identified either as members of the genus "*Gordona*" (42) or of the "rhodochrous" complex (7). (Names in quotation marks were not included on the Approved Lists of Bacterial Names [37] and have not been validly published since 1 January 1980; therefore, they have no standing in bacterial nomenclature.)

Among the various characters used in describing these organisms, chemical markers are of special value in distinguishing *Rhodococcus* from related genera (13, 28). Nocardiae, mycobacteria, rhodococci, and true corynebacteria contain *meso*-diaminopimelic acid (DAP), arabinose, and galactose in their whole-cell hydrolysates (cell wall chemotype IV) (27), whereas brevibacteria have only *meso*-DAP, and arthrobacters contain no *meso*-DAP or arabinose (13, 23, 32, 35, 44). The above-mentioned organisms can be distinguished from one another by thin-layer chromatography of ethanol-ether extracts of their cells. The true corynebacteria, nocardiae, and rhodococci contain a characteristic lipid component (LCN-A), composed of free mycolic acid (14, 23, 30), whereas mycobacteria, brevibacteria, and arthrobacters do not (23, 30, 32). The LCN-A of *Nocardia* has a higher R_f value than that of many rhodococci; the lowest mobility of LCN-A was observed with the *Corynebacterium* strains (23, 30, 32). Differences in mobility of LCN-A on thin-layer chromatography depend on differences in the molecular-weight ranges of the free mycolic acids: *Nocardia*, 48 to 58 carbons; *Rhodococcus*, 34 to 50 carbons; and *Corynebacterium*, 22 to 38 carbons (2, 28, 29). Thus, the R_f value of LCN-A may be useful in distinguishing among nocardiae, rhodococci, and true corynebacteria.

Analyses of other types of lipids may also be valuable in differentiating rhodococci from related bacteria. Menaquinone analyses, for example, indicate that representatives of some animal corynebacteria, rhodococci (including the type species, *Rhodococcus rhodochrous*; see 11), and *Brevibacterium linens* all have menaquinones with eight isoprene units and one hydrogenated double bond [MK-8(H_2)] as the prevalent type; arthrobacters, glutamic acid-producing saprophytic corynebacteria, and mycobacteria have MK-9(H_2) as the main menaquinone component; in contrast, representatives of *Nocardia* have MK-8(H_4) as the main menaquinone component (28). The non-hydroxylated fatty acids of nocardiae, rhodococci, and mycobacteria contain high proportions of straight-chain and unsaturated acids and of 10-methyloctadecanoic (tuberculostearic) acid, whereas most true corynebacteria do not contain tuberculostearic acid; the simple fatty acids of arthrobacters and *B. linens* are composed mainly of iso- and anteiso-acids (12, 28).

Analyses of deoxyribonucleic acid base compositions revealed that the guanine-plus-cytosine contents of the deoxyribonucleic acids of *Mycobacterium, Nocardia, Rhodococcus, Arthrobacter*, and *B. linens* are in the range of 60 to 70 mol%, whereas those of true corynebacteria range from 48 to 59 mol% (13, 28).

In the study reported here, the characters and

the taxonomic positions of the isolated strains were determined.

MATERIALS AND METHODS

Bacterial strains. A list of the strains studied and their sources are given in Tables 1 and 2. The strains were isolated on mineral salt agar (KNO_3, 1 g; $MgSO_4$, 0.1 g; Na_2HPO_4, 0.6 g; KH_2PO_4, 0.14 g; NaCl, 1 g; mixture of *n*-alkanes (C_{12} to C_{22}), 20 g; tap water, 500 ml; distilled water, 500 ml) by the method of Yamada et al. (45). The temperature of incubation was 28°C.

Morphological and cultural tests. The cell form and size and the reaction to the Gram stain were determined on smears from 16- to 24-h-old, 72-h-old, and 12-day-old cultures, on nutrient agar (NA) (4), glycerol agar (GA) (17), and wort agar (WA) (4) slants. Motility and acid fastness were tested in cultures grown on NA for 18 h. The mode of cell division was studied by time-lapse microscopy. Microcultures of the bacteria were made on NA by the method of Komagata et al. (24). The macroscopic appearance of the growth was examined on NA plates and on NA, GA, and WA slants 2 weeks after inoculation.

Physiological tests. Hugh and Leifson's (21) test was used to determine the fermentation or oxidation of glucose. The production of acid from different carbohydrates, the utilization of organic acids, the production of urease, the reduction of nitrate to nitrite, and the decomposition of casein, guanine, hypoxanthine, starch, tyrosine, and xanthine were determined by the methods of Gordon and Smith (18, 19) and Gordon and Mihm (16, 17). Phosphatase production was determined by the method of Giane-Williams and Skerman (10). The hydrolysis of Tweens was investigated by the method of Sierra (36), and the utilization of acetamide and benzamide as sole carbon and nitrogen sources was studied by the method of Tsukamura (42). The production of *p*-nitrophenoloxidase was determined by the method of Bönicke and Juhasz (6). The ability to utilize *n*-alkanes was studied on the mineral salt agar mentioned above and by the method described by Kvasnikov et al. (26). Descriptions of the other tests utilized have been previously published (4, 38).

Chemotaxonomic tests. The monosaccharides and the form of DAP in whole-cell hydrolysates were investigated as previously reported (31). Lipid LCN-A was detected by the method of Mordarska et al. (30). For preparation of the chromatographic plates, we used the 1- to 2-h fraction (or 10 to 30 μm) of silica gel "L" 5/40 μm (Lachema, n.p. Brno, Czechoslovakia). The chromatograms were developed in the system *n*-hexane–diethyl ether–glacial acetic acid (50:50:2, vol/vol) (23). The spots of LCN-A detected in the bacteria under investigation were always compared with those of three reference strains: *Rhodococcus* sp. ("*'M.' rhodochrous*") strain NCTC 576, *R. erythropolis* (*N. calcarea*) NCIB 8863, and "*C. divaricatum*" (*B. divaricatum*) NCIB 9379. Free mycolic acids of *Rhodococcus* sp. strain NCTC 576 contains from 38 to 47 carbon atoms (22), and those from *R. erythropolis* NCIB 8863 contain 34 to 46 carbons (29). "*C. divaricatum*" NCIB 9379 contains the mycolic acid analogs characteristic of *C. diphtheriae*: 26 to 38 carbons (23, 28). The lipid LCN-A of *Rhodococcus* sp. strain NCTC 576 has a high R_f value (0.59), and that of "*C. divaricatum*" NCIB 9379 has a low value (0.54); the LCN-A of *R. erythropolis* NCIB 8863 occupies an intermediate position (0.56).

TABLE 1. Isolated strains used in this study

Serial no.	Laboratory no.[a]	Site of isolation
Rhodococcus luteus (group I bacteria)		
1	IMV 8	Soil[b]
2	IMV 21	Soil
3	IMV 24	Soil
4	IMV 27	Soil
5	IMV 68	Soil
6	IMV 103	Soil
7	IMV 111	Soil
8	IMV 115	Soil
9	IMV 120	Soil
10	IMV 158	Soil
11	IMV 163	Soil
12	IMV 177	Soil
13	IMV 202	Soil
14	IMV 206	Soil
15	IMV 242	Soil
16	IMV 269	Soil
17	IMV 270	Skin of carp
18	IMV 323	Soil
19	IMV 333	Soil
20	IMV 372	Soil
21	IMV 374	Intestinal tract of carp
22	IMV 385	Soil
23	IMV 401	Soil
24	IMV 406	Soil
25	IMV 416	Intestinal tract of carp
26	IMV 417	Soil
27	IMV 419	Soil
28	IMV 427	Soil
29	IMV 445	Soil
30	IMV 455	Soil
31	IMV 462	Soil
32	IMV 604	Soil
Rhodococcus maris (group II bacteria)		
33	IMV 195	Soil
34	IMV 217	Soil
35	IMV 277	Soil
36	IMV 283	Intestinal tract of carp
37	IMV 324	Soil
38	IMV 330	Skin of carp

[a] IMV, Institute of Microbiology and Virology, Kiev, USSR.

[b] Soils, as a rule, were impregnated with oil.

The deoxyribonucleic acid base composition was determined by the method described by Sukapure et al. (41).

The simple fatty acids were detected by the method described by Andreev and Galchenko (3). Gas-liquid chromatography was performed with a Hewlett-Packard 5380A apparatus.

Menaquinones were analyzed by the method of Batrakov et al. (5).

Identification of strains. The 66 isolates were divided into two groups on the basis of their morphological, cultural, and physiological characteristics. The properties of the groups were compared with those of the type strains (Table 2) (11, 34) of most of the currently recognized *Rhodococcus* species.

TABLE 2. Culture collection strains used in this study

Serial no.	Laboratory no.	Names	Strain[a]	Source
1	IVM 733	*B. divaricatum*	C94 (= NCIB 9379)	D. Jones, School of Biological Sciences, The University, Leicester, England
2	IMV 734	*"B. maris"*	AUCNM B-464	AUCNM
3	IMV 735	*"M. luteum"*	AUCNM B-868	AUCNM
4	IMV 736	*"M. luteum"*	587	R. E. Gordon, Waksman Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J.
5	IMV 737	*R. bronchialis*	N654 (= ATCC 25592 = NCTC 10667)[b]	M. Goodfellow, Department of Microbiology, The Medical School, The University, Newcastle upon Tyne, England
6	IMV 738	*R. coprophilus*	CUB 687 (= ATCC 29080)[b]	T. J. Rowbotham, Postgraduate School of Studies in Biological Sciences, University of Bradford, England
7	IMV 739	*R. corallinus*	N657 (= ATCC 25593 = NCTC 10668[b]	M. Goodfellow
8	IMV 740	*R. equi*	R71 (= ATCC 25729 = NCTC 1621)[b]	M. Goodfellow
9	IMV 741	*R. erythropolis*	AJ 9126 (= ATCC 4277 = NCIB 9158)[b]	I. Komura, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan
10	IMV 742	*R. erythropolis (N. calcarea)*	N41 (= NCIB 8863)	M. Goodfellow
11	IMV 743	*R. rhodnii*	N445 (= NCIB 11279)	M. Goodfellow
12	IMV 744	*R. rhodochrous*	ATCC 13808[b]	M. Tsukamura, Chuba Chest Hospital, Obu, Aichi, Japan
13	IMV 745	*R. ruber*	M-1	M. Tsukamura
14	IMV 746	*R. rubropertinctus*	N4 (= ATCC 14352 = NCIB 9664)[b]	M. Goodfellow
15	IMV 747	*Rhodococcus* sp.	NCTC 576	NCTC
16	IMV 748	*R. terrae*	N659 (= ATCC 25594 = NCTC 10669)[b]	M. Goodfellow

[a] AUCNM, All-Union Collection of Nonpathogenic Microorganisms, Moscow, USSR; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, England; CUB, Actinomycete Culture Collection, University of Bradford, Bradford, England; AJ, Culture Collection of Central Research Laboratories of Ajinomoto Co., Inc., Kawasaki, Japan; C, N, and R, Laboratory numbers of strains from D. Jones and M. Goodfellow.

[b] Type strain (37).

RESULTS AND DISCUSSION

Forty-nine of the isolates were placed in group I, and the following description is based on 32 of these strains.

Cell morphology of group I bacteria. Smears of cultures grown on GA and NA for 16 to 24 h showed straight or slightly curved rods (0.6 to 1.0 μm by 3.0 to 6.0 μm) arranged in an angular or parallel fashion. Rods and branching filaments (15 to 16 μm) rarely occurred. On WA, the 16- to 24-h-old cultures consisted, as a rule, of rods that were shorter than the cells grown on NA and GA (1.5 to 2.5 μm); many coccoid forms were also found. The morphological transformation of cells during growth on agar slants depended on the composition of the medium. Thus, the rod-shaped cells cultivated on WA generally transformed rapidly to the coccoid form. However, on NA and GA the rods became shorter but ordinarily did not become coccoid.

As shown by time-lapse microscopy, the rod-shaped bacteria elongated and bent within 16 h of cultivation (Fig. 1); sometimes branches were formed. During elongation, septa were formed, and 16 h later, filaments fragmented into rod-shaped elements. The latter then divided, as a rule, by snapping or bending and formed characteristic V forms. In 48 h, the microcolony consisted of rods (2 to 4 μm in length) characteristically arranged in Vs.

Group I bacteria were gram-positive, were not (or rarely only partly) acid-fast, and were nonmotile; endospores were not formed.

Cultural characteristics of group I bacteria. Growth on GA and WA slants was usually abundant, butyrous or mucoid, and intensely yellow; growth on NA slants was moderate or poor, butyrous, and deep yellow to pale orange;

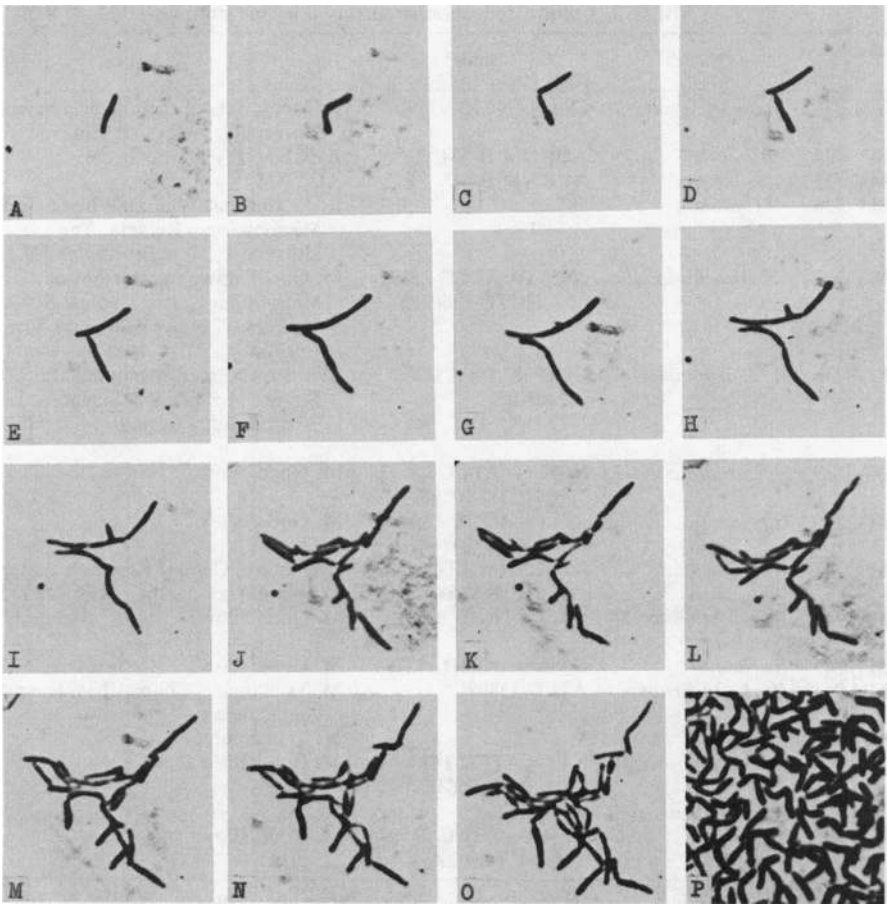

FIG. 1. Growth of *R. luteus* strain 177 on NA at 28°C. Phase contrast, ×2,600. A, 5 h; B, 7 h; C, 9 h; D, 11 h; E, 12 h; F, 14 h; G, 15 h; H, 16 h; I, 16 h 45 min; J, 18 h 50 min; K, 19 h; L, 20 h; M, 21 h; N, 21 h 40 min; O, 23 h; P, 48 h.

on Löwenstein-Jensen medium growth was abundant and yellow-orange. Colonies on the surfaces of NA plates were flat, opaque, raised, glistening, and smooth; colonies that had raised centers and that were opaque and smooth or gently folded also occurred. In nutrient broth, a membrane formed on the surface and the broth remained clear.

Physiological and chemotaxonomic characteristics of group I bacteria. The results of the physiological tests are listed in Tables 3–5. All of the strains actively oxidized glucose. Catalase was produced. Casein (91% of the strains were negative), cellulose, guanine, hypoxanthine (88%), starch, tyrosine, and xanthine were not attacked, but urea was. Tweens 20, 40, 60, and 80 were hydrolyzed. Acetylmethylcarbinol, indole, phosphatase (94% of the strains were negative), and *p*-nitrophenoloxidase were not produced; NH_3 was produced from peptone. No growth occurred with acetamide or benzamide as a sole carbon and nitrogen source. Benzoate, oxalate, and tartrate were not utilized. Acetate, γ-aminobutyrate, butyrate (94% of the strains were positive), *cis*-aconitate, citrate, fumarate, α-ketoglutarate, lactate, malate, pyruvate, and succinate were utilized. Acid was produced from arabinose, fructose, galactose, glucose, glycerol, mannitol, mannose, sorbitol, sucrose, and xylose (87% of the strains were positive). No acid was produced from adonitol, cellobiose, dulcitol, inositol, lactose, maltose, α-methyl-D-glucoside, raffinose (97% of the strains were negative), rhamnose, or salicin. Growth occurred in the presence of 5 and 7% NaCl and of C_9 to C_{17}, C_{19}, and C_{23} *n*-alkanes. No growth occurred with methane, ethane, or C_8 *n*-alkane. No growth occurred at 45°C. Nitrate was reduced to nitrite (85% of the strains were negative). Litmus milk became alkaline (91% of the strains were positive). Properties such as the liquefaction of gelatin, the production of H_2S,

TABLE 3. Tests positive with *R. luteus* (group I bacteria), "*M. luteum*" AUCNM B-868, and "*M. luteum*" 587

Acetate utilization
Acid from the following carbohydrates
D-(−)-Arabinose
Fructose
Galactose[a]
Glucose
Glycerol
Mannitol
Mannose
Sorbitol
Sucrose
cis-Aconitate utilization
γ-Aminobutyrate utilization[a]
Catalase production
Cell wall chemotype IV[b]
Citrate utilization
Decomposition of Tweens 20, 40, 60, and 80
Fumurate utilization
Gram positive
Growth intensely yellow on NA, GA, and WA without aerial mycelium
Growth with C_9 to C_{17}, C_{19}, C_{23} *n*-alkanes
Growth with 7% NaCl
α-Ketoglutarate utilization
Lactate utilization
Malate utilization
Oxidation of glucose in Hugh and Leifson O-F medium
Presence of lipid LCN-A
Production of NH_3 from peptone
Pyruvate utilization
Rod-like cells (3 to 16 μm) after 16 to 18 h of incubation on NA and GA
Succinate utilization
Urease production

[a] "*Mycobacterium luteum*" 587 is negative.
[b] Cell wall chemotype IV: arabinose, galactose and *meso*-DAP are in whole cell hydrolysates.

and growth in the presence of C_3, C_6, and C_7 *n*-alkanes were quite variable within the group (Table 5).

All strains contained *meso*-DAP, arabinose, and galactose in their cells as well as lipid LCN-A that on plates with silica gel moved similarly to that from *Rhodococcus* sp. strain NCTC 576. The guanine-plus-cytosine content of the deoxyribonucleic acid of strain 385 was 64.1 mol% by thermal denaturation. Fatty acid analysis of stationary-phase cells of strain 385 revealed that the main acids were (percentage of total detected): $C_{14:0}$ (12%), $C_{16:1}$ (12%), $C_{16:0}$ (20%), $C_{18:1}$ (22%), and $10CH_3C_{18}$ (13%). Strain 385 contained, as the major component, menaquinone having eight isoprene units with one hydrogenated double-bond, MK-8(H_2); small amounts of MK-7(H_2) and traces of MK-8 were detected as well.

On the basis of the following characteristics, we classify these organisms as members of the genus *Rhodococcus* in the family *Nocardiaceae* (9, 11, 28): gram-positive, nonmotile, do not form endospores, aerobic, form a primary mycelium that soon fragments into rod-shaped elements, produce soft colonies without aerial hyphae, have a cell wall of chemotype IV, have a lipid LCN-A of the *Rhodococcus* type, have a guanine-plus-cytosine content of the DNA of 64.1 mol%, have fatty acids containing a high proportion of tuberculostearic acid ($10CH_3C_{18}$), straight-chain, and unsaturated acids, and have menaquinone MK-8(H_2) as the major isoprenolog.

We compared the properties of group I bacteria with those of eight type strains and two reference strains of 10 established *Rhodococcus* species (Table 6) and concluded that the members of group I should be classified as a distinct species of *Rhodococcus*. As to selecting a name for these organisms, we should like to point out the following. The characters of the bacteria of

TABLE 4. Tests negative with *R. luteus* (group I bacteria), "*M. luteum*" AUCNM B-868, and "*M. luteum*" 587

Acetylmethylcarbinol production
Acid from the following carbohydrates:
Adonitol
Cellobiose
Dulcitol
Inositol
Lactose
Maltose[a]
α-Methyl-D-glucoside
Rhamnose
Salicin
Sorbose
Benzoate utilization[a]
Decomposition of the following substrates:
Cellulose
Guanine
Starch
Tyrosine[b]
Xanthine
Endospore formation
Growth with:
Acetamide
Benzamide
Ethane
Methane
C_8 *n*-alkane
Growth at 45°C
Indole production
Motility
p-Nitrophenoloxidase production
Oxalate utilization[a]
Tartrate utilization
Methyl red test

[a] "*M. luteum*" 587 is positive.
[b] "*M. luteum*" AUCNM B-868 is positive.

TABLE 5. Tests giving different results with strains of *R. luteus* (group I bacteria)

Characteristic	No. of strains positive	Results with: Type strain (IMV 385)	"*M. luteum*" AUCNM B-868	"*M. luteum*" 587	Serial no. of strains that gave the less common result
Acid from raffinose	1/32	−	−	−	28
Acid from xylose	28/32	+	+	−	15, 18, 24, 31
Alkaline reaction in litmus milk	29/32	+	+	+	11, 19, 23
Decomposition of casein	3/32	−	−	−	25, 26, 30
Decomposition of hypoxanthine	4/32	+	+	−	10, 22, 29, 30
Gelatin liquefaction	12/32	−	−	−	5, 6, 10, 17, 18, 19, 20, 21, 24, 28, 29, 30
Nitrate reduction	5/32	−	−	+	1, 5, 9, 13, 16
Production of H_2S	12/32	+	+	+	2, 3, 7, 12, 13, 19, 20, 22, 23, 25, 26, 32
Production of phosphatase	2/32	−	−	−	1, 29
Utilization of butyrate	30/32	+	+	+	14, 32
Growth with C_3 *n*-alkane	19/32	+	Not tested	Not tested	4, 6, 9, 13, 14, 16, 18, 23, 24, 26, 28, 30
Growth with C_6 *n*-alkane	25/32	+	Not tested	Not tested	2, 3, 5, 9, 12, 26, 30
Growth with C_7 *n*-alkane	11/32	−	Not tested	Not tested	1, 2, 6, 7, 8, 11, 14, 24, 25, 26, 30

group I correspond to the description of "*M. luteum*" given by Söhngen (39) and later supplemented by Krasil'nikov (25). In Söhngen's report (39) we found nothing about the strains on which the author based his original description of "*M. luteum*," and a type or neotype strain of "*M. luteum*" has not been officially established (39, 40). According to Gordon and Mihm (16) and Gordon (15), Söhngen's strain 587 of "*M. luteum*" from Kluyver agrees with the original description of "*M. luteum*" (39), and it is accepted by Gordon and Mihm as authentic for the species. These authors (16) assigned this strain to " '*M.*' *rhodochrous*" ("*rhodochrous*" complex).

The morphological, cultural, chemotaxonomic, and most of the physiological properties of group I bacteria are similar to those of the culture of "*M. luteum*" 587 studied in this work (Tables 3, 4, and 6). However, in contrast to strain 587, our isolates did not utilize benzoate or oxalate but did utilize γ-aminobutyrate and did form acid from galactose and xylose (87% of the strains were positive) but did not form acid from maltose. In contrast to the description of "*M. luteum*" by Krasil'nikov (25), group I bacteria did not peptonize milk. Group I bacteria are also very closely related to "*M. luteum*" AUCNM B-868 (Tables 3–6).

Thus, "*M. luteum*," corresponding to the isolates of group I, must be considered as a species of *Rhodococcus* (16; our data). However, "*M. luteum*" was not included on the Approved Lists (37), and hence it has no standing in nomenclature. Furthermore, since we here regard "*M. luteum*" as a member of the genus *Rhodococcus*, it would serve no useful purpose to revive the name "*M. luteum*." Consequently, we propose the name *Rhodococcus luteus* for the group I bacteria. This name cannot be regarded as a new combination, and the organism cannot be regarded as a new species since it was already recognized as such by Söhngen in 1913. We therefore regard *R. luteus* as a new name, realizing that this use of "nom. nov." will require an amendment of rule 34a of the Bacteriological Code to accommodate this and similar situations.

Strain IMV 385 (= AUCNM A-594) is the type strain of *R. luteus*. The characters of this strain are given in Table 6. In general, the description of IMV 385 agrees with the original description of "*M. luteum*" (39). However, in contrast to this description, but similar to strains AUCNM B-868 and 587 of "*M. luteum*," strain IMV 385 produces urease. In contrast to "*M. luteum*" 587, strain IMV 385 decomposes hypoxanthine, does not reduce nitrate to nitrite, does not utilize benzoate or oxalate but utilizes γ-aminobutyrate, and produces acid from galactose and xylose but does not form acid from maltose. The properties of strain IMV 385 are similar to those of *M. luteum* AUCNM B-868 except for its ability to decompose tyrosine (Table 6).

The members of *R. luteus* may be differentiated from the type or reference strains of 10 species of *Rhodococcus* by a number of tests (see Table 10).

The following description of group II bacteria is based on 6 of the 17 isolates placed in this group.

Cell morphology of group II bacteria. After 16

to 18 h of incubation on NA, GA, and WA slants, the cells were short ovoids (0.6 to 1.0 μm by 1.0 to 2.0 μm) arranged in an angular fashion.

Time-lapse microscopy revealed (Fig. 2) that the coccoid cells (0.8 μm in length) elongated to 2.0 μm within 6 h of cultivation. The rods then divided by snapping, and V forms were produced. The angles between the attached daughter cells became smaller, and the cells were then arranged in parallel. After several divisions of the rods, microcolonies were produced. Growth and division of cells occurred at the edges of the colonies, but at the centers of the microcolonies, the cells did not elongate; with each new division, the cells became shorter and eventually transformed into coccoid forms. After 36 to 48 h, the microcolonies consisted of short, straight rods and coccoid forms.

Group II organisms were gram-positive, were not acid-fast, were nonmotile, and did not form endospores.

Cultural characteristics of group II organisms. Growth on NA, GA, and WA slants was moderate or poor, butyrous, and orange. Colonies on the surface of NA were raised, butyrous, glistening, and circular with an entire edge. Nutrient broth became turbid.

Physiological and chemotaxonomic characteristics. The results of the physiological tests are listed in Tables 7–9. Growth occurred in the aerobic and anaerobic tubes of Hugh and Leifson medium, but as a rule, the indicator was not changed to the acid color. Catalase was produced. Casein, cellulose, guanine, hypoxanthine, starch, tyrosine, and xanthine were not attacked. Acetylmethylcarbinol, indole, *p*-nitrophenoloxidase, phosphatase, and H_2S were not produced. Tweens 20, 40, 60, and 80 were hydrolyzed. No growth occurred with either acetamide or benzamide (84% of the strains were negative) as the sole carbon and nitrogen source. γ-Aminobutyrate, benzoate, *cis*-aconitate, α-ketoglutarate, lactate, oxalate, and tartrate were not utilized. Acetate, butyrate, fumarate, malate, pyruvate, and succinate were utilized. Acid was produced from fructose, glucose, and glycerol. No acid was produced from adonitol, arabinose, cellobiose, dulcitol, galactose, inositol, lactose, maltose, mannitol, α-methyl-D-glucoside, raffinose, rhamnose, salicin, sorbitol, sorbose, sucrose (84% of the strains were negative), or xylose. Growth occurred in the presence of 5 and 7% NaCl and of C_6, C_7, C_8 (83% of the strains were positive), C_9 to C_{17}, C_{19}, and C_{23} *n*-alkanes. No growth occurred with methane or ethane. No growth occurred at 45°C. Nitrate was reduced to nitrite. There was no change in litmus milk. The liquefaction of gelatin, the production of NH_3 from peptone, the decomposition of urea, the utilization of citrate, and the production of acid from mannose were quite variable within the group (Table 9).

All strains contained *meso*-DAP, arabinose, and galactose in their whole-cell hydrolysates and had a lipid LCN-A chromatographically similar to that of *R. erythropolis* NCIB 8863.

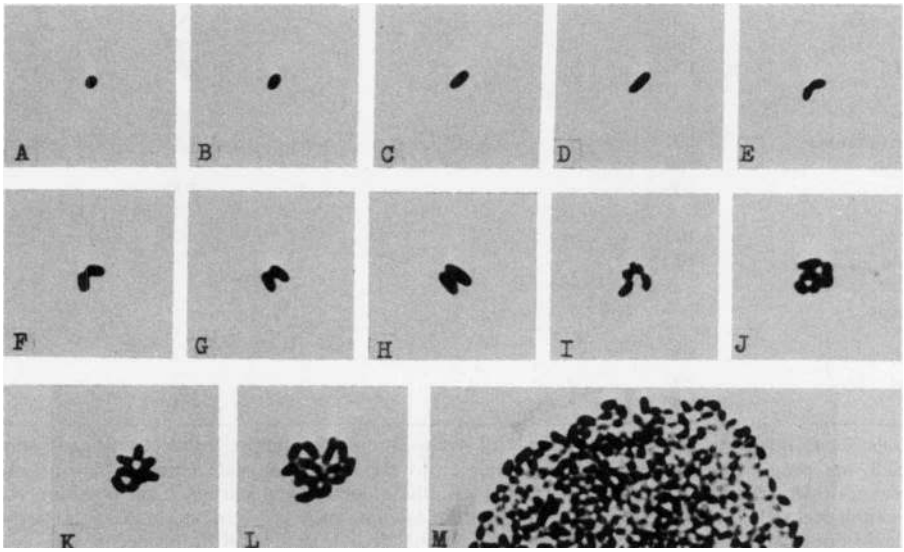

FIG. 2. Growth of *R. maris* strain 283 on NA at 28°C. Phase contrast, ×2,800. A, 0 h; B, 3 h; C, 4 h; D, 6 h; E, 8 h; F, 9 h; G, 11 h; H, 12 h; I, 18 h; J, 23 h; K, 25 h; L, 30 h; M, 48 h.

The guanine-plus-cytosine content of the deoxyribonucleic acid of strain 195 was 73.2 mol% by thermal denaturation. The main fatty acids of stationary-phase cells of this strain were (percentage of total detected): $C_{16:1}$ (12%), $C_{16:0}$ (17%), $C_{17:1}$ (16.5%), $C_{17:0}$ (10%), $C_{18:1}$ (14.5%), and $10CH_3C_{18}$ (9.2%). Strain 195 possessed MK-8(H_2) as the major isoprenolog, with smaller quantities of MK-6 and traces of MK-8.

On the basis of the following characteristics,

TABLE 6. Characteristics of the species of the genus *Rhodococcus*[a]

Character	Group I		"*M. luteum*" AUCNM B-868	"*M. luteum*" 587	Group II		
	R. luteus (32 strains)	*R. luteus* IMV 385			*R. maris* (6 strains)	*R. maris* IMV 195	*R. maris* AUCNM B-464
Nitrate reduction	15[b]	−	−	+	100[b]	+	+
Gelatin liquefaction	36	−	−	−	50	−	−
Litmus milk	91[c]	+	+	+	0	−	−
Decomposition of:							
Casein	9	−	−	−	0	−	−
Hypoxanthine	12	+	+	−	0	−	−
Tyrosine	0	−	+	−	0	−	−
Tween 80	100	+	+	+	100	+	+
Production of:							
H_2S	36	+	+	+	0	−	−
NH_3 from peptone	100	+	±	+	50	−	−
p-Nitrophenoloxidase	0	−	−	−	0	−	−
Phosphatase	6	−	−	−	0	−	−
Urease	100	+	+	+	33	−	+
Utilization of:							
α-Aminobutyrate	100	+	+	−	0	−	−
Butyrate	94	+	+	+	100	+	+
cis-Aconitate	100	+	+	+	0	−	−
Citrate	100	+	+	+	33	−	−
Fumarate	100	+	+	+	100	+	+
α-Ketoglutarate	100	+	+	+	0	−	−
Lactate	100	+	+	+	0	−	+
Malate	100	+	+	+	100	+	+
Succinate	100	+	+	+	100	+	+
Growth with/at							
Acetamide	0	−	−	−	0	−	−
Benzamide	0	−	−	−	16	−	−
n-Alkanes C_8	0	−	−	−	83	+	+
C_{13}	100	+	+	+	100	+	+
7% NaCl	100	+	+	+	100	+	+
45°C	0	−	−	−	0	−	−
Acid from:							
D-(−)-Arabinose	100	+	+	+	0	−	−
Galactose	100	+	+	−	0	−	−
Glycerol	100	+	+	+	100	+	+
Inositol	0	−	−	−	0	−	−
Mannitol	100	+	+	+	0	−	−
Mannose	100	+	+	+	50	−	−
Raffinose	3	−	−	−	0	−	−
Rhamnose	0	−	−	−	0	−	−
Salicin	0	−	−	−	0	−	−
Sorbitol	100	+	+	+	0	−	−
Sucrose	100	+	+	+	16	−	−
Xylose	87	+	+	−	0	−	−

[a] Symbols: +, Positive; −, negative; ±, weak. All strains do not decompose cellulose, guanine, starch, and xanthine; do not produce acetylmethylcarbinol, indole; do not utilize benzoate (except "*M. luteum*" 587), oxalate (except "*M. luteum*" 587), or tartrate, but do utilize acetate and pyruvate; do not form acid from adonitol, cellobiose, dulcitol, lactose, maltose (except "*M. luteum*" 587), α-methyl-D-glucoside, or sorbose, but do form acid from glucose and fructose; all are negative in the methyl red test; all strains decompose Tweens 20, 40, and 60, produce catalase, and grow with 5% NaCl.

[b] Percentage of strains positive.

[c] Litmus milk turns alkaline.

group II bacteria may be classified in the genus *Rhodococcus* in the family *Nocardiaceae* (9, 11, 13): gram-positive, nonmotile, pleomorphic, do not form endospores, produce soft colonies without aerial mycelium, and possess a cell wall of chemotype IV, a lipid LCN-A of the *R. erythropolis* NCIB 8863 type, a guanine-plus-cytosine content of the deoxyribonucleic acid of 73.2 mol%, fatty acids containing a high proportion of tuberculostearic acid, straight-chain, and

TABLE 6—*Continued*

R. rhodochrous ATCC 13803	*R. bronchialis* ATCC 25592	*R. corallinus* ATCC 25593	*R. erythropolis* ATCC 4277	*R. equi* ATCC 25729	*R. rhodnii* NCIB 11279	*R. ruber* M-1	*R. rubropertinctus* ATCC 14352	*R. terrae* ATCC 25594	*R. coprophilus* ATCC 29080
+	+	+	−	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
+	−	−	+	−	+	−	−	−	−
+	+	±	+	+	−	+	+	−	+
−	+	−	−	−	−	−	−	−	−
+	−	+	±	−	±	+	−	+	±
+	+	−	+	−	−	+	+	−	−
−	+	−	−	−	−	−	−	−	−
−	+	+	+	+	+	−	−	+	−
−	−	−	+	±	−	+	−	−	−
+	+	+	+	+	+	+	+	+	+
−	−	+	+	−	−	+	+	±	−
+	+	+	+	−	−	+	+	+	−
+	+	+	+	+	+	+	+	+	−
+	−	+	−	−	−	−	+	+	−
+	+	+	+	+	+	±	+	+	+
+	+	+	+	+	+	+	+	+	−
+	+	+	+	+	+	+	+	+	−
±	−	−	+	+	−	±	+	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
±	±	±	+	+	−	+	+	±	−
+	+	+	+	+	+	+	+	+	−
+	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	−	+	+	+	−
−	+	−	+	−	−	−	−	−	−
+	−	+	+	−	+	+	+	+	−
+	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	+	−
−	−	−	+	+	−	−	−	−	−
+	−	+	+	−	−	+	+	+	−
−	+	+	+	+	−	+	+	+	−
−	−	−	+	−	+	−	−	±	−

TABLE 7. Tests positive with *R. maris* (group II bacteria) and "*B. maris*" AUCNM B-464

Acid from the following carbohydrates:
Fructose
Glucose
Glycerol
Catalase formation
Cell-wall chemotype IV[a]
Coccoid and rodlike cells (0.5 to 2 μm) after 16 to 18 h of incubation on NA and GA
Decomposition of Tweens 20, 40, 60, and 80
Gram positive
Growth orange and poor on NA, GA, and WA without aerial mycelium
Growth with C_6, C_7, C_9 to C_{17}, and C_{23} *n*-alkanes
Growth with 5% NaCl
Growth with 7% NaCl
Nitrate reduction
Presence of lipid LCN-A
Utilization of following substrates:
Acetate
Butyrate
Fumarate
Malate
Pyruvate
Succinate

[a] See footnote *b* to Table 3.

unsaturated acids, and a menaquinone, MK-8(H_2), as the major isoprenolog.

A comparison of group II properties with those of the type or reference strains of 10 established *Rhodococcus* species (Table 6) indicates that the members of group II also belong to a distinct species of *Rhodococcus*. With respect to the name of this species, the following must be taken into consideration. On the basis of morphological, cultural, and physiological properties, the isolates of group II may be identified as members of "*B. maris*" (Harrison 1929) Breed 1953 (synonym: "*Flavobacterium maris*" Harrison 1929) (8, 20). The strains closely resemble "*B. maris*" AUCNM B-464 (Tables 6–9), which has a lipid LCN-A similar to that of *R. erythropolis* NCIB 8863 and a cell wall of chemotype IV; the same wall chemotype was found (44) in another strain of "*B. maris*," viz., AJ 1480 (= IFM S-30).

It is obvious that at present the genus *Brevibacterium* is a heterogeneous taxon. Some of its members have cell walls of chemotype IV and produce glutamic acid; they were thus named *C. glutamicum* and were considered to be members of the genus *Corynebacterium* sensu stricto (1). Many other species with ornithine or lysine in the cell wall were transferred from *Brevibacterium* to either the genus *Curtobacterium* or the genus *Arthrobacter* (44). The chemotaxonomic properties mentioned above indicate that "*B. maris*" cannot be classified as a member of the genus *Arthrobacter* as suggested by Rogosa and Keddie (33). Gordon (15) has pointed out that strains with the properties of "*'M.' rhodochrous*" may previously have been identified as members of the genus *Brevibacterium*.

According to Yamada and Komagata (44), only strains of *B. linens* containing *meso*-DAP in their cell walls can be considered bona fide members of *Brevibacterium*. Subsequently, other authors have stated that *B. linens* contains ribose but not arabinose or mycolic acids in its cell wall (12, 23, 32, 35).

TABLE 8. Tests negative with *R. maris* (group II bacteria) and "*B. maris*" AUCNM B-464

Acid from following carbohydrates:
Adonitol
D-(−)-Arabinose
Cellobiose
Dulcitol
Galactose
Inositol
Lactose
Maltose
Mannitol
α-Methyl-D-glucoside
Raffinose
Rhamnose
Salicin
Sorbitol
Sorbose
Xylose
Acid-fastness
Alkaline reaction in litmus milk
Decomposition of:
Casein
Cellulose
Guanine
Hypoxanthine
Starch
Tyrosine
Xanthine
Endospore formation
Formation of:
Acetylmethylcarbinol
Indole
H_2S
p-Nitrophenoloxidase
Phosphatase
Growth at 45°C
Growth with acetamide
Growth with ethane
Growth with methane
Motility
Methyl red test
Utilization of:
γ-Aminobutyrate
Benzoate
cis-Aconitate
α-Ketoglutarate
Lactate[a]
Oxalate
Tartrate

[a] "*B. maris*" AUCNM B-464 is positive.

TABLE 9. Tests giving different results with strains of *R. maris* (group II bacteria)

Characteristic	No. of strains positive	Results with:		Serial no. of strains that gave the less common result
		Type strain IMV 195	"*B. maris*" AUCNM B-464	
Acid from mannose	3/6	−	−	35, 37, 38 are positive; 33, 34, 36 are negative
Acid from sucrose	1/6	−	−	35
Citrate utilization	2/6	−	−	35, 38
Gelatin liquefaction	3/6	−	−	35, 36, 38 are positive; 33, 34, 37 are negative
Growth with benzamide	1/6	−	−	35
Growth with C_8 *n*-alkane	5/6	+	+	34
Oxidation of glucose in Hugh and Leifson O-F medium	1/5	−	−	37
Production of NH_3 from peptone	3/6	−	−	34, 36, 38 are positive; 33, 35, 37 are negative
Urease production	2/6	−	+	35, 37

Our data indicate that "*B. maris*" corresponds to the isolates of our group II and that these organisms should be classified as a species of the genus *Rhodococcus*, for which we propose the name *Rhodococcus maris*. As with "*M. luteum*," the name "*Brevibacterium maris*" was not included on the Approved Lists (37). By reason of the same arguments give above for "*M. luteum*," the name *R. maris* should be regarded as a new name.

Strain IMV 195 (= AUCNM A-593) is the type strain of *R. maris*. A description of this strain is presented in Table 6. (It should be noted that a type or neotype strain was never officially established for "*B. maris*.")

Tests useful for differentiating *R. maris* and *R. luteus* from each other and from the type or reference strains of 10 established *Rhodococcus* species (11, 34) are given in Table 10.

In contrast to the rhodococci studied by Gordon (15), *R. maris* did not oxidize glucose in Hugh and Leifson medium. The morphological cycle of *R. maris* is characterized by a rapid changing of very short rods into coccoid forms without the formation of primary mycelium.

ACKNOWLEDGMENTS

We are much indebted to M. Goodfellow, M. Tsukamura, D. Jones, T. J. Rowbotham, R. E. Gordon, and I. Komura, who kindly provided the collection strains (Table 2); we thank E. F. Lessel for his invaluable help in the preparation of this manuscript.

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TABLE 10. Differentiating characters of *Rhodococcus* species

Character	*R. luteus*	*R. maris*	*R. rhodochrous* ATCC 13808	*R. bronchialis* ATCC 25592	*R. corallinus* ATCC 25593	*R. erythropolis* ATCC 4277	*R. equi* ATCC 25729	*R. rhodenii* NCIB 11279	*R. ruber* M-1	*R. rubropertinctus* ATCC 14352	*R. terrae* ATCC 25594	*R. coprophilus* ATCC 29080
Colonies deep yellow, often mucoid on GA, NA, and WA	100[a]	0	−	−	−	−	−	−	−	−	−	−
Colonies orange on GA and WA	0	100[b]	−	+	−	−	+	−	+	−	−	−
Colonies red-orange on GA and WA	0	0	+	−	+	−	−	−	−	+	+	+
Colonies pink-flesh on GA, WA, and NA	0	0	−	−	−	+	−	+	−	−	−	−
Coccoid and rodlike cells (0.5 to 2 μm) on GA and NA after 16–18 h	0	100	−	−	−	−	−	−	−	−	−	−
Rodlike cells (3 to 16 μm) on GA and NA after 16 to 18 h	100	0	−	+	+	+	+	−	−	+	+	−
Mycelium formed on GA and NA after 16 to 18 h	0	0	+	−	−	−	−	+	+	−	−	+
Partly or slightly acid-fast	0	0	−	+	+	−	−	−	−	+	+	−
Nitrate reduction	15	100	+	+	+	−	+	+	+	+	+	+
Litmus milk turns alkaline	91	0	+	+	+	+	+	+	+	+	+	+
Decomposition of:												
Tyrosine	0	0	+	−	−	+	−	+	−	−	−	−
Tween 80	100	100	+	+	±	+	+	−	+	+	−	+
Production of:												
p-Nitrophenoloxidase	0	0	+	+	−	+	−	−	+	+	−	−
Phosphatase	6	0	−	+	−	−	−	−	−	−	−	−
Urease	100	33	−	+	+	+	+	+	−	−	+	−

TABLE 10—*Continued*

Character	*R. luteus*	*R. maris*	*R. rhodochrous* ATCC 13808	*R. bronchialis* ATCC 25592	*R. corallinus* ATCC 25593	*R. erythropolis* ATCC 4277	*R. equi* ATCC 25729	*R. rhodenii* NCIB 11279	*R. ruber* M-1	*R. rubropertinctus* ATCC 14352	*R. terrae* ATCC 25594	*R. coprophilus* ATCC 29080
Utilization of:												
γ-Aminobutyrate	100	0	−	−	−	+	±	−	+	−	−	−
cis-Aconitate	100	0	−	−	+	+	−	−	+	+	±	−
Citrate	100	33	+	+	+	+	−	−	+	+	+	−
Fumarate	100	100	+	+	+	+	+	+	+	+	+	−
α-Ketoglutarate	100	0	+	−	+	−	−	−	−	+	+	−
Lactate	100	0	+	+	+	+	+	+	±	+	+	+
Malate	100	100	+	+	+	+	+	+	+	+	+	−
Succinate	100	100	+	+	+	+	+	+	+	+	+	−
Growth with/at:												
Acetamide	0	0	±	−	−	+	+	−	±	+	−	−
n-Alkanes C_8	0	83	−	−	−	−	−	−	−	−	−	−
C_{13}	100	100	±	±	±	+	+	−	+	+	±	−
7% NaCl	100	100	+	−	+	+	+	+	+	+	+	−
45°C	0	0	+	−	−	−	−	−	−	−	−	−
Acid from:												
D-(−)-Arabinose	100	0	−	−	−	−	−	−	−	−	−	−
Galactose	100	0	−	−	−	−	−	−	−	−	−	−
Glycerol	100	100	+	+	+	+	+	−	+	+	+	−
Inositol	0	0	−	+	−	+	−	−	−	−	−	−
Mannitol	100	0	+	−	+	+	−	+	+	+	+	−
Rhamnose	0	0	−	−	−	−	−	−	−	−	+	−
Salicin	0	0	−	−	−	+	+	−	−	−	−	−
Sorbitol	100	0	+	−	+	+	−	−	+	+	+	−
Sucrose	100	16	−	+	+	+	+	−	+	+	+	−
Xylose	87	0	−	−	−	+	−	+	−	−	±	−

[a] Percentage of strains positive.
[b] Growth is weaker than that of other *Rhodococcus* strains.

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