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Lysobacter, a New Genus of Nonfruiting, Gliding Bacteria with a High Base Ratio

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Highly mucoid, cream, pink and yellow-brown gliding organisms having deoxyribonucleic acid guanine-plus-cytosine contents of 62 to 70.1 mol% have been isolated by several workers, but since these organisms have never been observed to produce typical myxobacterial fruiting bodies, their taxonomy has been problematical. Forty-six isolates were studied in detail, among them Ensign and Wolfe's organism AL-1 and Cook's isolate 495, both of which produce important proteases, as well as Cook's culture 3C, which elaborates the potent, wide-spectrum antibiotic myxin. A new genus, *Lysobacter*, has been established for these organisms, and four new species and one new subspecies have been named and described: *L. antibioticus* (type strain, ATCC 29479), *L. brunescens* (type strain, ATCC 29482), *L. enzymogenes* (type strain, ATCC 29487), *L. enzymogenes* subspecies *cookii* Christensen (type strain, ATCC 29488), and *L. gummosus* (type strain, ATCC 29489). The dimensions of the thin, gliding, flexing cells of *Lysobacter* are 0.3 to 0.5 by 1.0 to 15.0 (sometimes up to 70) μm. These soil and water organisms all degrade chitin, two degrade alginate, three degrade pectate, three degrade carboxymethylcellulose, and one degrades starch, but none decomposes filter paper or agar. They are strongly proteolytic and characteristically lyse a variety of microorganisms such as gram-negative, gram-positive (including actinomycetes), and blue-green bacteria, fungi, and green algae, as well as nematodes. The genus has been placed in a new family, *Lysobacteraceae*, within a new order, *Lysobacterales*.

The existence of albuminous, nonfruiting, gliding bacteria, many of which lyse other microorganisms and have high guanine-plus-cytosine (G+C) contents in their deoxyribonucleic acids (DNAs) has been noted by various authors (9, 12, 14, 18, 23, 27, 28, 30, 34-36; E. J. Napier, British Patent 1,048,887, 1966). Some of these organisms are known to produce important proteases (1, 10, 12-17; L. Y. Huang and J. C. Ensign, Bacteriol. Proc., P22, p. 127, 1971) and polysaccharases (15), one elaborates a potent, wide-spectrum antibiotic (4, 19-21, 27; F. D. Cook, O. E. Edward, D. C. Gillespie, and E. R. Peterson, U. S. Patent 3,609,153, 1971), and another produces an extremely viscid gum. Several of them were tentatively assigned to the formerly recognized genus *Sorangium* (14, 27, 34, 38-42), to *Cytophaga* (12, 25, 26, 35; Napier, British Patent 1,048,887, 1966), or to *Flexibacter* (34), but the majority have been referred to as "unidentified myxobacters."

In a paper presented at the 23rd Annual Meeting of the Canadian Society of Microbiologists in Edmonton, Alberta, in 1973, Christensen and Cook introduced the new genus *Lysobacter*. Three species and one subspecies were described, and the genus was at that time placed within the order *Myxobacterales*. However, presentation of new names at meetings does not constitute effective publication, and thus the purpose of the present report is to effect the valid publication of the names of these and other taxa and to discuss the taxonomic relationships of these taxa. The names proposed in this paper are *Lysobacter* gen. nov., *L. antibioticus* sp. nov., *L. brunescens* sp. nov., *L. enzymogenes* sp. nov., *L. enzymogenes* subsp. *cookii* sp. nov., *L. gummosus* sp. nov., *Lysobacteraceae* fam. nov., and *Lysobacterales* ord. nov.

MATERIALS AND METHODS

Bacterial strains. Dilutions were prepared from soils enriched with chitin, ground mushrooms, or *Arthrobacter* cells for at least 1 month and then spread on yeast cell agar (32), which consists of 0.5% bakers' yeast in a 1.5% agar medium. The number of colonies capable of lysing autoclaved yeast cells (Fig. 1) increased dramatically with these enrichment procedures (Fig. 2). The "predators" belonged mainly to one of two groups—those that produced cream-colored colonies ("495 types," now *L. enzymogenes*) or those that produced pink-colored colonies ("3C types," now *L. antibioticus*). Thirty of the cream organisms and 24 of the pink ones were isolated and purified. Two other cream strains were acquired. ATCC 27796 was kindly donated by R. S. Wolfe, and the American Type Culture Collection supplied ATCC 21123. In all,

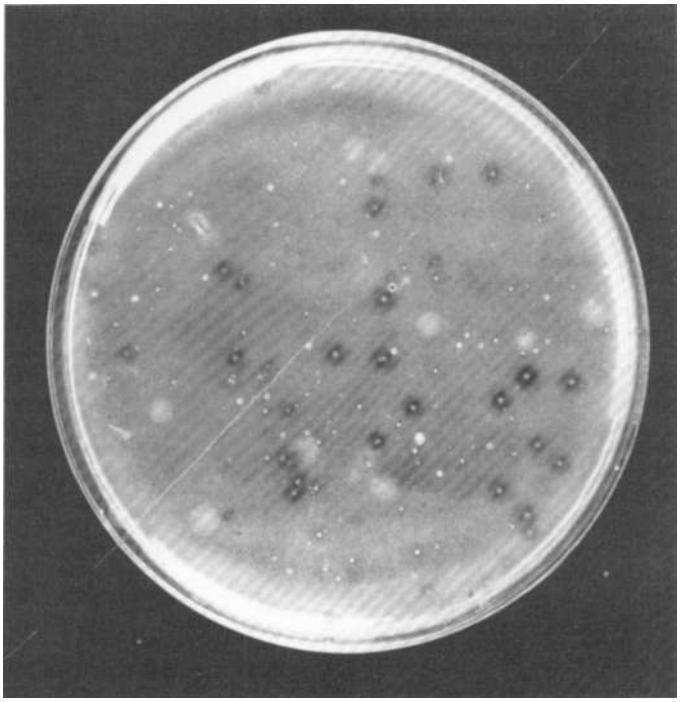

FIG. 1. *One-tenth milliliter of a soil dilution, spread on a yeast cell agar plate, after 3 days of incubation at 26°C. Note the lytic zones around some of the colonies.*

19 strains of the cream type and 16 of the pink type, together with one unusual, gummy, off-white isolate, were used in the present study. These and the other strains used here are listed, together with their sources, in Table 1. Most of the cream organisms had at least two cultural variants—a dirty-white mucoid colony and a yellowish nonmucoid one—which were tested separately in the physiological and biochemical tests.

Water samples were also plated on yeast cell agar, and these yielded several lytic colonies. Ten yellow-brown isolates were purified, and all of these were used in the present study (see Table 1).

Media. The media used for growing the organisms were plate count agar (PCA; Difco), Cook's cytophaga agar (CCA; 8), and skim acetate agar (SAA) or broth (8); incubation was at 25°C unless otherwise noted.

Methods. The methods used in this study were the same as those published in a previous study (7) with the following additions.

Induction of fruiting. Fruiting was induced by inoculating onto sterile rabbit pellets in water agar (32) and by the glycerol technique (11).

Lipolysis. Separately autoclaved solutions of Tween 20, 40, 60, and 80 were added to four different batches of basal agar (1.0% peptone–0.02% $CaCl_2$–1.5% agar [pH 7.2 to 7.4]), to achieve a final concentration of 1% (vol/vol), just prior to pouring the plates (31). Two-day-old plates were inoculated with the test organisms, and, after 5 days, observation with the naked eye was made to see whether crystals of the calcium soaps had been formed as a result of lipolytic action.

Indole, methyl red, Voges-Proskauer, citrate. The method used for detecting indole production was reported previously (7). The medium used for the methyl red (MR) and Voges-Proskauer (VP) tests contained 0.5% peptone, 0.5% glucose, and 0.5% K_2HPO_4. Simmons citrate agar (3) was used to determine the utilization of citrate as a sole carbon source. The VP test was read after 2 days, and the MR and citrate tests were read after 4 days (3).

Lactose plates. Organisms were streaked on MacConkey agar (Difco) and on eosin-methylene blue agar (Difco), and the color of any colonies that grew was noted at 2 days.

G+C content of the DNA. For determining the G+C content of DNA, the methods of Marmur and Doty were followed (22).

RESULTS

(Note: The organisms forming the two colony types of *L. enzymogenes* [see Tables 3 to 5] behaved identically in all physiological and biochemical tests; therefore they are not recorded separately in the other tables.)

Cell morphology. The 46 isolates were gram-negative rods or filaments (Table 2) which were nonmotile by means of flagella but which glided along solid/liquid interfaces. All except UASM 66, UASM Q9, and UASM 402 showed flexing movements in liquid media. Flexing and gliding were more readily observed with longer cells.

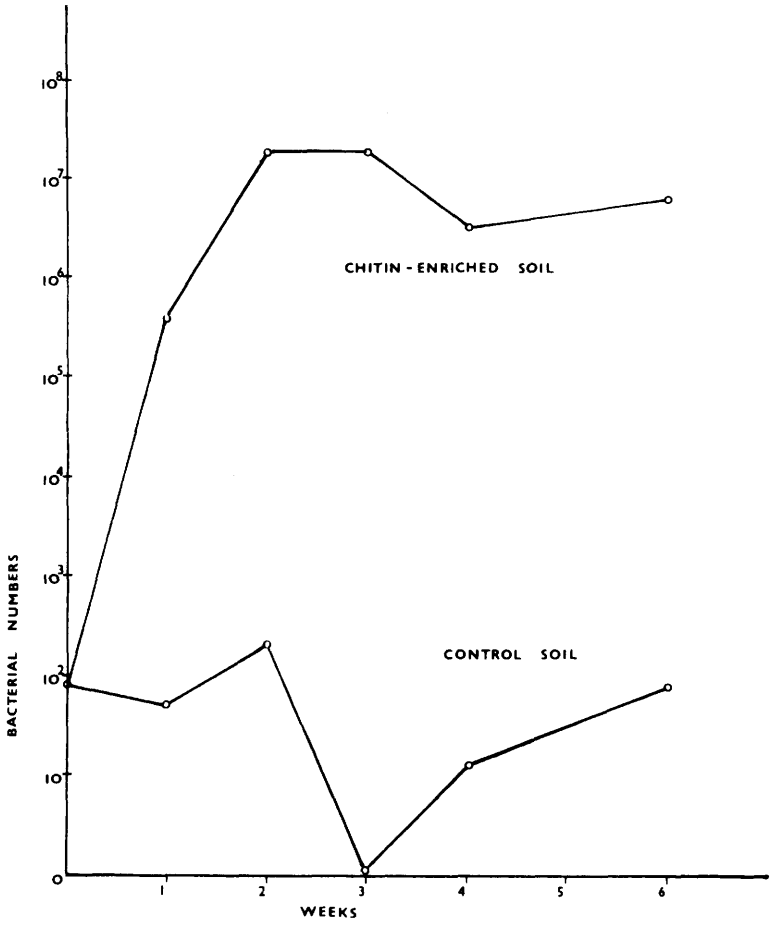

FIG. 2. *Numbers of bacteria lysing autoclaved yeast cells. One set of numbers is from untreated (control) soil at 25% moisture, the other set is from the same soil which had been enriched with chitin.*

Cultural characteristics. Liquid cultures of all the strains except UASM Q9 showed a characteristic silkiness when gently tapped. This silkiness also appeared to be associated with the length of the cells, for it was less pronounced in strains with shorter cells. Liquid cultures of all four species were somewhat viscous, and *L. gummosus* was so much so that a 2-day-old tube culture could be inverted with no spillage.

Likewise, all four species were mucilaginous on agar media. The rubbery colonies of *L. gummosus* were particularly difficult to handle. All strains except those of *L. gummosus* produced brown, water-soluble pigments in broth and on agar media, and this pigment production was more marked the older the culture became. The colors of the colonies were distinctive: *L. gummosus* had off-white-colored colonies, *L. enzymogenes* had cream-colored colonies which became brown, the yellow colonies of *L. brunescens* quickly became deep chocolate brown, and the pink colonies of *L. antibioticus* became reddish brown. Deep reddish-brown crystals developed within the slime of several strains of *L. antibioticus,* and these were identified as crystals of the antibiotic myxin in strain UASM 3C (27; Cook et al., U. S. Patent 3,609,153, 28 September, 1971). The identity of the remaining pigments is under investigation in H. Reichenbach's laboratory, Braunschweig-Stöckheim, Federal Republic of Germany.

Colonies of *L. brunescens* spread in the typical "cytophaga" fashion, but those of *L. antibioticus* and *L. enzymogenes* were more mucoid, and we believe that this concealed the typical spreading. However, all strains of the latter two species were observed to produce a thin advancing fringe of cells at the edge of the colony. *L. gummosus* was never observed to spread, and this seemed to be associated with both the

TABLE 1. *Origins and designations of strains used in this study*[a]

Organism	Strain designation	Source
Actinomycete	UASM 4432	F. D. Cook (soil)
Actinomycete	UASM 4441	F. D. Cook (soil)
Arthrobacter sp.	UASM 4165	F. D. Cook ← Prairie Regional Lab; Saskatchewan
Bacillus subtilis	UASM 4611	F. D. Cook ← Prairie Regional Lab; Saskatchewan
Chlorella sp.		Dept. of Botany, U. of Alberta
Cytophaga hutchinsonii	NCIB 10782	NCIB ← N. Palleroni
C. hutchinsonii	Stanier 6	R. Y. Stanier
Escherichia coli	UASM PC20	P. Christensen (creek water, Edmonton, Alberta)
Lysobacter antibioticus[b]	UASM 3C[c,d] (= ATCC 29479)	Soil, CEF, Ottawa, Ontario
L. antibioticus	UASM 66	Variant from UASM 3C culture
L. antibioticus	UASM L17 (= ATCC 29480)	Soil, CEF, Ottawa, Ontario
L. antibioticus	UASM Q9	Soil, Edmonton, Alberta
L. antibioticus	UASM Q15	Soil, Edmonton, Alberta
L. antibioticus	UASM 4045 (= ATCC 29481)	Soil, CEF, Ottawa, Ontario
L. antibioticus	UASM 4169	Soil, Looma, Alberta
L. antibioticus	UASM 4551	Soil, Carmangay, Alberta
L. antibioticus	UASM 4572	Soil, Sylvan Lake, Alberta
L. antibioticus	UASM 4574	Soil, near Sylvan Lake, Alberta
L. antibioticus	UASM 4578	Soil, near Sylvan Lake, Alberta
L. antibioticus	UASM 4593	Soil, Edmonton, Alberta
L. antibioticus	UASM 4598	Soil, Ellerslie, Alberta
L. antibioticus	UASM 8I	Soil, CEF, Ottawa, Ontario
L. antibioticus	UASM 10I	Soil, CEF, Ottawa, Ontario
L. antibioticus	UASM 12I	Soil, CEF, Ottawa, Ontario
L. brunescens	UASM D[d] (= ATCC 29482)	Lake A water, Martin House, NWT
L. brunescens	UASM 2 (= ATCC 29483)	Lake B water, Martin House, NWT
L. brunescens	UASM 6 (= ATCC 29484)	Lake C water, Martin House, NWT
L. brunescens	UASM 4541	Slough water, Alberta
L. brunescens	UASM CB1	N. Saskatchewan River, Edmonton, Alberta
L. brunescens	UASM CB2	N. Saskatchewan River, Edmonton, Alberta
L. brunescens	UASM CB4	Fallis Creek, Alberta
L. brunescens	UASM CB5	Fallis Creek, Alberta
L. brunescens	UASM CB6	Burma Creek, Saskatchewan
L. brunescens	UASM CB7	Burma Creek, Saskatchewan
L. enzymogenes[f]	UASM 495[c,d] (= ATCC 29487)	Soil, CEF, Ottawa, Ontario
L. enzymogenes	UASM AL-1 (= ATCC 27796)	Soil, R. S. Wolfe, Illinois
L. enzymogenes	ATCC 21123[e]	ATCC ← Kyowa Fermentation Industries Co., Japan
L. enzymogenes	UASM 18L[c] (= ATCC 29485/6)	Soil, CEF, Ottawa, Ontario
L. enzymogenes	UASM 4553	Soil, Carmangay, Alberta
L. enzymogenes	UASM 4554	Soil, Angus Ridge, Alberta
L. enzymogenes	UASM 4555	Soil, Malmo, Alberta
L. enzymogenes	UASM 4556	Soil, Vegreville, Alberta
L. enzymogenes	UASM 4557	Soil, Breton, Alberta
L. enzymogenes	UASM 4558	Soil, Peace River, Alberta
L. enzymogenes	UASM 4559	Soil, Lac la Biche, Alberta
L. enzymogenes	UASM 4560	Soil, Lloydminster, Alberta
L. enzymogenes	UASM 4561	Soil, Olds, Alberta
L. enzymogenes	UASM 4562	Soil, Vermilion, Alberta
L. enzymogenes	UASM 4563	Soil, Alberta
L. enzymogenes	UASM 4564	Soil, Alberta
L. enzymogenes	UASM 4565	Soil, Alberta
L. enzymogenes	UASM Q1	Soil, Edmonton, Alberta
L. enzymogenes subsp. *cookii*	UASM 13B[d] (= ATCC 29488)	Soil, CEF, Ottawa, Ontario

TABLE 1—*Continued*

Organism	Strain designation	Source
L. gummosus	UASM 402[d] (= ATCC 29489)	Soil, CEF, Ottawa, Ontario
Myxococcus sp.	UASM K54	F. D. Cook (soil)
Penicillium notatum		N. Colotelo, Edmonton
Pseudomonas aeruginosa	ATCC 9027	ATCC ← C. P. Hegarty (ear infection)
Rhizopus sp.		N. Colotelo, Edmonton
Sclerotinia sclerotiorum		N. Colotelo, Edmonton
Serratia marcescens		Provincial Laboratory of Public Health, Edmonton
Yeast (probably *Saccharomyces cerevisiae*)		Fleischmann's fast-rising active dry yeast, Standard Brands, Ltd. Montreal

[a] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md. 20852; CEF, Central Experimental Farm; NWT, Northwest Territories; UASM, University of Alberta Soil Microbiology Lab., Edmonton, Alberta, Canada.

[b] All *Lysobacter* strains were isolated by F. D. Cook, and within each species all strains except one are from different soil or water samples; none are known to be siblings.

[c] Formerly placed in the genus *Sorangium* (14, 27, 38–42).

[d] Type strain.

[e] Listed as *Cytophaga johnsonae* in ATCC catalogue.

[f] Type species of the genus.

shorter cells and the large amount of gum produced on every medium on which it was grown (Tables 3 to 5).

Fruiting bodies characteristic of members of the order *Myxobacterales* were not observed when these strains were inoculated on dung pellets, and microcyst production by these strains was not induced by the glycerol method.

Physiology. The physiological data on the strains are presented in Tables 6 to 8. The temperatures for optimum growth of these organisms were relatively high (25 to 40°C) for soil and water organisms.

All strains except UASM AL-1 utilized ammonia, all except UASM CB1 and UASM CB2 utilized asparaginate, and all utilized glutamate and nitrate as N sources. The use of urea was restricted to the single strain of *L. gummosus* and to UASM 3C, ATCC 21123, UASM 18L, and UASM 13B. In general, the addition of yeast extract to the salts-glucose-nitrate agar, or to the chitin medium, stimulated growth.

Members of the genus *Lysobacter* lysed both gram-negative and gram-positive bacteria (including actinomycetes), filamentous fungi, a yeast, and an alga (Table 8). Lysobacters did not attack gram-negative as well as they did gram-positive bacteria. It should be noted that lysobacters are themselves gram negative, and this may be a self-protective feature.

Biochemical reactions. The biochemical reactions of the strains are presented in Tables 9 and 10. All strains degraded chitin and all except the strains of *L. brunescens* degraded carboxymethyl-cellulose (CMC). None of the strains degraded filter paper or agar in tubes. Lysobacters were highly proteolytic; all strains liquefied gelatin, grew and produced NH_3 in both casein and salts-Casitone broths, and grew well on tryptone agar. All strains produced catalase, oxidase (*L. gummosus* UASM 402 untestable), and phosphatase and gave negative results for the indole, MR, and VP tests. Citrate was used as a sole carbon source by all strains of *Lysobacter* except those of *L. brunescens*. (Strains UASM 4553–4565 inclusive were not tested for MR, VP, or citrate.)

According to our determinations, the G+C content of the DNA of the lysobacters ranged from 65.4 to 70.1 mol% (Table 11).

DISCUSSION

As non-photosynthetic, gliding bacteria, the organisms under study fell within the confines of part 2 of the 8th edition of *Bergey's Manual* (5), which contains two orders—*Myxobacterales* and *Cytophagales*. *Lysobacter* shows certain similarities to the *Myxobacterales*, notably in its ability to lyse procaryotic and eucaryotic microbes and its high DNA base ratio (65 to 70 mol% G+C for *Lysobacter*, 67 to 71 mol% G+C for the *Myxobacterales*). However, the myxobacteria are strict aerobes whereas lysobacters are not; some myxobacteria but none of the lysobacters degrade filter-paper cellulose; and, most important of all, lysobacters do not form fruiting bodies or microcysts of any kind. The possibility has been raised that these organisms are myxobacters that have lost the ability to fruit. We reject this possibility because we have observed 67 freshly isolated strains, and none has ever been seen to fruit during our use of appropriate techniques. Clearly *Lysobacter* does not belong in the order *Myxobacterales*.

TABLE 2. *Cell dimensions of Lysobacter strains*

Strain	Length (μm)	Width (μm)
L. antibioticus		
UASM 3C (ATCC 29479)	6.5	0.4
UASM 66	40	0.4
UASM L17 (ATCC 29480)	9	0.4
UASM Q9	6.5	0.4
UASM Q15	22	0.4
UASM 4045 (ATCC 29481)	23	0.4
UASM 4169	16	0.4
UASM 4551	21	0.4
UASM 4572	4	0.4
UASM 4574	35	0.4
UASM 4578	40	0.4
UASM 4593	19	0.4
UASM 4598	22	0.4
UASM 8I	6	0.4
UASM 10I	38	0.4
UASM 12I	37	0.4
L. brunescens		
UASM D (ATCC 29482)	11	0.3–0.5
UASM 2 (ATCC 29483)	35	0.3–0.4
UASM 6 (ATCC 29484)	15	0.2–0.5
UASM 4541	70	0.3–0.5
UASM CB1	24	0.3–0.5
UASM CB2	7	0.4–0.5
UASM CB4	12	0.3–0.5
UASM CB5	13	0.4–0.5
UASM CB6	25	0.4–0.5
UASM CB7	15	0.4–0.5
L. enzymogenes		
UASM 495 (ATCC 29487)	38	0.5
UASM AL-1 (ATCC 27796)	27	0.3–0.4
ATCC 21123	37	0.4
ATCC 18L (ATCC 29485/6)	50	0.4
UASM 4553	4	0.4–0.5
UASM 4554	5	0.5
UASM 4555	6	0.4–0.5
UASM 4556	5	0.4
UASM 4557	5	0.3–0.5
UASM 4558	6	0.4
UASM 4559	6	0.4
UASM 4560	7	0.4
UASM 4561	8	0.3–0.4
UASM 4562	7	0.4
UASM 4563	5	0.4–0.5
UASM 4564	6	0.3
UASM 4565	11	0.4
UASM Q1	41	0.4
L. enzymogenes subsp. *cookii*, UASM 13B (ATCC 29488)	37	0.5
L. gummosus UASM 402 (ATCC 29489)	2	0.4

However, does it belong in the order *Cytophagales*? The G+C content of *Lysobacter* DNA (65 to 70 mol%) is quite different from that of the *Cytophagales* (31 to 53 mol%). The wide range in G+C content as well as the morphological and biochemical characteristics of members of this order indicate that it is too heterogeneous and that it will undoubtedly be divided into more discrete units after urgently needed studies are undertaken.

We shall, therefore, not add to the heterogeneity within the order *Cytophagales* but instead propose that the genus *Lysobacter* be placed within a new family, *Lysobacteraceae,* within a new order, *Lysobacterales,* to be placed in part 2 of *Bergey's Manual.*

Lysobacterales ord. nov. (Lys.o.bac.te.ra'les. M.L. masc.n. *Lysobacter* name of the type genus of the order; *-ales* ending to denote an order; M.L. fem.pl.n. *Lysobacterales* the *Lysobacter* order.)

Gram-negative, rod-shaped cells which may become quite long (up to 70 μm) and which are motile by means of gliding. Fruiting bodies are not produced. The DNA base ratio ranges from 65 to 70 mol% G+C.

Lysobacteraceae fam. nov. (Lys.o.bac.-te.ra'ce.ae. M.L. masc.n. *Lysobacter* type genus of the family; *-aceae* ending to denote a family; M.L. fem.pl.n. *Lysobacteraceae* the *Lysobacter* family.)

Description as for the order.

Lysobacter gen. nov. (Lys.o.bac'ter. Gr. adj. *lysis* loosing; M.L. n. *bacter* masc. equivalent of Gr. neut.n. *bactrum* a rod; M.L. masc.n. *Lysobacter* the loosing rod; intended to mean the lysing rod.)

Flexible, gliding, gram-negative rods, 0.2 to 0.5 by 2 to 70 μm. Colonies are slimy or mucoid and are white, cream, yellow, pink or brown; many strains also produce a brown water-soluble pigment; the nature of the pigments is unknown. Growth in broth culture is silky.

Aerobic; mesophilic; pH range for growth, 5 to 10; growth of most strains is reduced by 1% NaCl.

Chemoorganotrophic. Metabolism is usually respiratory; molecular oxygen is used as the terminal electron acceptor.

NO_3^-, NH_4^+, glutamate, and asparaginate are used as N sources.

Chitin, but not filter-paper cellulose or agar, is hydrolyzed.

Strongly proteolytic.

Catalase, oxidase, and phosphatase are produced. The indole, MR, and VP tests are negative.

Lyses gram-negative, gram-positive (including actinomycetes), and blue-green bacteria, filamentous fungi, yeasts, and algae; also lyses nematodes.

Habitat: Soil and fresh water.

The G+C content of the DNA ranges from 65.4 to 70.1 mol%.

TABLE 3. *Colonial characteristics of Lysobacter strains on CCA after 5 days of incubation*

Strain	Form			Surface		Edge					Elevation						Optical properties			Brown water-soluble pigment	Color (Munsell notation [6])
	Circular	≅ Circular	Irregular	Smooth	Rough	Entire	Undulate	Lobate	Erose	Filamentous	Effuse	Flat	Raised	Convex	Pulvinate	Umbonate	Transparent	Translucent	Opaque		
L. antibioticus																					
UASM 3C (ATCC 29479)	√			√	√	√					√			√				√	√	+	7.5 YR 2.5/4
UASM 66	√			√						√		√						√		−	1 YR 4/6
UASM L17 (ATCC 29480)	√			√					√				√					√		+	7.5 YR 6/4
UASM Q9		√		√					√				√					√		±	10 YR 7/6
UASM Q15		√		√		√			√				√					√		+	10 YR 6/4
UASM 4045 (ATCC 29481)			√	√			√					√						√		+	7.5 YR 5/4
UASM 4169			√	√					√	√		√						√		+	2.5 YR 5/6
UASM 4551		√		√		√			√			√							√	±	5 YR 5/6
UASM 4572		√		√		√			√			√							√	−	2.5 YR 5/8
UASM 4574		√		√					√				√						√	±	1.5 YR 4/8
UASM 4578	√			√					√				√					√		±	5 YR 4/6
UASM 4593	√			√					√			√							√	−	1.5 YR 4/8
UASM 4598	√			√					√			√						√		+	2.5 YR 4/6
UASM 8I	√			√		√								√					√	+	2.5 YR 5/4
UASM 10I	√			√		√							√						√	+	5 YR 5/4
UASM 12I	√			√		√							√						√	+	2.5 YR 4/4
L. brunescens																					
UASM D (ATCC 29482)			√		√			√			√						√			±	2.5 Y 4/6
UASM 2 (ATCC 29843)			√		√			√			√						√			±	2.5 Y 4/6
UASM 6 (ATCC 29484)			√		√				√		√						√			+	5 Y 3/4
UASM 4541			√		√		√				√			√			√			−	10 YR 5/8
UASM CB1			√		√					√	√					√	√			+	10 YR 5/8
UASM CB2			√		√					√	√					√	√			+	10 YR 4/6
UASM CB4			√		√					√	√					√	√			+	10 YR 4.5/8
UASM CB5			√		√					√	√					√	√			+	10 YR 4/8
UASM CB6			√		√					√	√					√	√			+	2.5 Y 4/6
UASM CB7			√		√					√	√					√	√			+	2.5 Y 4/8
L. enzymogenes																					
UASM 495 (ATCC 29487)			√		√		√				√	√					√			−	2.5 Y 6/6
UASM AL-1 (ATCC 27796)																					
ct" (i)			√	√	√			√					√					√		−	5 YR 8/4
ct (ii)			√	√			√						√					√		−	5 Y 7/6
ct (iii)			√	√					√		√		√				√			−	5 Y 8/5
ATCC 21123	√			√	√				√		√		√				√			−	5 Y 7/6
UASM 18L																					
ct (y) (ATCC 29485)		√		√			√							√			√			−	10 YR 6/8
ct (w) (ATCC 29486)		√		√					√		√			√			√			−	5 Y 7/4
UASM 4553																					
ct (i)		√		√			√						√					√		−	5 Y 7/4
ct (ii)			√		√		√				√		√				√			−	10 YR 6/6

TABLE 3—*Continued*

Strain	Form			Surface		Edge					Elevation						Optical properties			Brown water-soluble pigment	Color (Munsell notation [6])
	Circular	≥ Circular	Irregular	Smooth	Rough	Entire	Undulate	Lobate	Erose	Filamentous	Effuse	Flat	Raised	Convex	Pulvinate	Umbonate	Transparent	Translucent	Opaque		
UASM 4554		√		√			√				√		√					√		–	2.5 Y 7/6
UASM 4555																					
ct (i)	√			√		√					√		√					√		–	5 Y 7/4
ct (ii)		√		√			√				√	√						√		–	5 Y 7.5/6
UASM 4556																					
ct (i)		√		√					√		√		√					√		–	5 Y 7/6
ct (ii)	√			√					√			√					√			–	2.5 Y 7/8
UASM 4557																					
ct (i)		√		√					√		√		√					√		–	5 Y 7/4
ct (ii)	√			√		√						√					√			–	10 YR 6/8
UASM 4558																					
ct (i)		√		√			√				√	√						√		–	4 Y 6.5/4
ct (ii)	√			√					√		√	√						√		–	10 YR 6/6
UASM 4559																					
ct (i)	√			√					√		√	√						√		–	5 Y 7/5
ct (ii)	√			√					√		√		√					√		–	10 YR 7/6
UASM 4560																					
ct (i)	√			√			√				√		√					√		–	6 Y 7.5/4
ct (ii)	√			√		√							√					√		–	5 Y 7.5/6
ct (iii)		√		√					√			√						√		–	10 YR 6/6
UASM 4561																					
ct (i)	√			√					√				√					√		–	5 Y 7.5/5
ct (ii)			√	√			√				√		√					√		–	5 Y 7.5/6
ct (iii)		√		√		√						√					√			–	2.5 Y 6/8
UASM 4562																					
ct (i)		√		√			√				√	√						√		–	5 Y 7.5/4
ct (ii)		√		√	√			√			√		√				√			–	10 YR 6.5/6
UASM 4563																					
ct (i)		√		√					√					√				√		–	5 Y 7/4
ct (ii)		√		√	√			√			√		√				√			–	2.5 Y 6/6
UASM 4564																					
ct (i)			√	√					√		√	√						√		–	5 Y 7.5/6
ct (ii)	√			√		√							√				√			–	2.5 Y 6.5/6
UASM 4565																					
ct (i)		√		√					√		√	√						√		±	5 Y 6/4
ct (ii)	√			√		√							√				√			–	10 YR 6/8
UASM Q1		√		√		√			√			√	√					√		–	2.5 Y 7/6
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)			√		√				√		√						√			–	7.5 YR 6/8
L. gummosus UASM 402 (ATCC 29489)	√			√		√										√		√		–	5 Y 8/2

[a] ct, Colony type.

Type species: *Lysobacter enzymogenes.*

Comments: Two strains, each belonging to a different species in this genus, have been the subjects of considerable interest. Strain UASM 3C (= ATCC 29479) of *L. antibioticus* produces the antibiotic myxin (4, 19–21, 27, Cook et al., U. S. Patent 3,609,153, 1971), and important proteolytic enzymes are produced by *L. enzymogenes* strain UASM 495 (= ATCC 29487) (1, 10, 14, 24, 38–42) and by strain UASM AL-1 (= ATCC 27796) (12, 13, 15–17; Huang and Ensign, Bacteriol. Proc., P22, p. 127, 1971). In addition, ultrastructure studies have shown the presence in strain UASM AL-1 of rhapidosomes (25) and the absence of a ruthenium red-stainable acid mucopolysaccharide outer layer in contrast to three fruiting myxobacters studied. Sohre studied intracellular hydrolytic enzymes in UASM

TABLE 4. *Colonial characteristics of Lysobacter strains on SAA after 5 days of incubation*

Strain	Form			Surface		Edge					Elevation						Optical properties			Brown water-soluble pigment	Color (Munsell notation [6])
	Circular	≈ Circular	Irregular	Smooth	Rough	Entire	Undulate	Lobate	Erose	Filamentous	Effuse	Flat	Raised	Convex	Pulvinate	Umbonate	Transparent	Translucent	Opaque		
L. antibioticus																					
UASM 3C (ATCC 29479)	√			√	√				√		√			√			√		√	+	7.5 YR 4/6
UASM 66	√			√					√				√					√		−	2.5 Y 7/5
UASM L17 (ATCC 29480)	√			√					√			√						√		±	2.5 YR 6/4
UASM Q9		√		√		√			√				√						√	±	5 YR 6/6
UASM Q15		√		√					√				√					√		±	5 YR 6/6
UASM 4045 (ATCC 29481)	√			√		√			√				√						√	+	5 YR 5/6
UASM 4169	√			√					√				√					√		+	2.5 YR 4/6
UASM 4551	√			√		√			√				√						√	+	2.5 YR 4.5/6
UASM 4572		√		√		√			√			√							√	+	2.5 YR 4/8
UASM 4574	√			√					√				√						√	+	1.5 YR 3/8
UASM 4578		√		√						√		√						√		+	5 YR 4/6
UASM 4593	√			√					√				√						√	±	2.5 YR 4/8
UASM 4598	√			√					√			√							√	+	2.5 YR 4/6
UASM 8I		√		√		√								√					√	+	2.5 YR 5/5
UASM 10I	√			√		√							√						√	±	5 YR 5/6
UASM 12I	√			√		√							√						√	±	2.5 YR 4/8
L. brunescens																					
UASM D (ATCC 29482)			√		√		√				√		√				√			±	10 YR 4/6
UASM 2 (ATCC 29483)			√		√			√			√			√			√			±	10 YR 4/6
UASM 6 (ATCC 29484)			√		√				√		√						√			±	2.5 Y 4.5/6
UASM 4541			√		√				√		√			√				√		±	7.5 YR 4/8
UASM CB1	√			√		√			√				√					√		+	10 YR 5/8
UASM CB2	√			√		√			√				√					√		±	10 YR 5/8
UASM CB4			√		√					√	√					√	√			±	10 YR 6/8
UASM CB5			√		√					√	√						√			±	10 YR 6/8
UASM CB6			√		√					√	√					√	√			±	10 YR 5/8
UASM CB7			√		√					√	√					√	√			±	10 YR 5/8
L. enzymogenes																					
UASM 495 (ATCC 29487)			√	√					√		√		√					√		−	2.5 Y 6.5/6
UASM AL-1 (ATCC 27796)																					
ct" (i)			√	√					√		√		√					√		−	5 Y 7.5/4
ct (ii)			√	√		√								√				√	√	−	5 Y 7.5/4
ct (iii)			√	√			√						√					√		−	5 Y 8/4
ATCC 21123		√		√					√		√			√				√		−	2.5 Y 7.5/6
UASM 18L																					
ct (y) (ATCC 29485)		√			√				√		√			√				√		−	10 YR 6.5/5
ct (w) (ATCC 29486)			√	√	√				√		√			√					√	−	2.5 Y 7/4
UASM 4553																					
ct (i)		√		√		√								√				√		−	5 Y 8/4
ct (ii)			√		√		√				√		√					√		−	7.5 YR 5.5/6
UASM 4554		√		√					√		√		√					√		−	10 YR 6/6

TABLE 4—*Continued*

Strain	Form			Surface		Edge					Elevation						Optical properties			Brown water-soluble pigment	Color (Munsell notation [6])
	Circular	≈ Circular	Irregular	Smooth	Rough	Entire	Undulate	Lobate	Erose	Filamentous	Effuse	Flat	Raised	Convex	Pulvinate	Umbonate	Transparent	Translucent	Opaque		
UASM 4555																					
ct (i)	√			√		√								√					√	–	7.5 Y 8/4
ct (ii)	√			√		√								√					√	–	5 Y 7.5/4
UASM 4556																					
ct (i)		√		√					√				√						√	–	3 Y 7/4
ct (ii)	√				√				√			√						√		–	7.5 YR 6/6
UASM 4557																					
ct (i)		√			√		√				√			√					√	–	5 Y 8/4
ct (ii)	√				√		√						√					√		–	10 YR 6/6
UASM 4558																					
ct (i)		√		√			√						√						√	–	5 Y 7.5/4
ct (ii)	√				√		√						√					√		–	10 YR 7/6
UASM 4559																					
ct (i)		√			√		√						√						√	–	2.5 Y 7.5/4
ct (ii)		√			√		√						√					√		–	10 YR 6.5/5
UASM 4560																					
ct (i)		√			√		√						√						√	–	10 YR 7/6
ct (ii)	√			√		√								√					√	–	2.5 Y 7.5/6
ct (iii)	√				√				√			√					√			–	10 YR 6/7
UASM 4561																					
ct (i)	√				√				√					√					√	–	5 Y 8.5/4
ct (ii)		√			√		√				√			√					√	–	5 Y 7.5/5
ct (iii)	√			√					√		√		√					√		–	10 YR 7/6
UASM 4562																					
ct (i)		√			√		√						√						√	–	5 Y 7/4
ct (ii)	√			√					√		√		√					√		–	8.4 YR 6/6
UASM 4563																					
ct (i)		√			√				√					√					√	–	5 Y 7.5/4
ct (ii)		√			√		√							√					√	–	2.5 Y 6.5/6
UASM 4564																					
ct (i)			√		√				√					√					√	–	5 Y 7.5/4
ct (ii)		√		√					√				√					√		–	2.5 Y 7/6
UASM 4565																					
ct (i)		√			√		√						√						√	–	4 Y 7/4
ct (ii)		√			√	√							√					√		–	7.5 YR 6/7
UASM Q1	√			√		√							√					√	√	–	2.5 Y 8/6
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)			√		√				√		√					√	√			–	7.5 YR 6/6
L. gummosus UASM 402 (ATCC 29489)	√			√		√									√				√	–	5 Y 8/2

[a] ct, Colony type.

AL-1 (I. Sohre, Ph.D. thesis, University of Karlsruhe, Karlsruhe, Federal Republic of Germany, 1971), and she also reported on the cell cycle in this strain (37). Sendecki et al. investigated the ribosomes of strain UASM 495 (29).

One of the outstanding characteristics of lysobacters is their ability to hydrolyze chitin. Indeed, isolation of these organisms is facilitated by providing chitin as a suspension, in ground mushrooms, or in autoclaved yeast cells (see Materials and Methods).

Soriano's creamy-white, mucoid *Flexibacter albuminosus* and *F. aureus* (33) may belong in *Lysobacter*; however, neither their use of chitin, CMC, and alginate nor their lytic abilities were tested by Soriano, and no cultures of these organisms are known to be available; thus it is impossible to assess them further.

TABLE 5. *Colonial characteristics of Lysobacter strains on PCA after 5 days of incubation*

Strain	Form			Surface		Edge				Elevation					Optical Properties			Brown water-soluble pigment	Color (Munsell notation [6])
	Circular	≈ Circular	Irregular	Smooth	Rough	Entire	Undulate	Erose	Filamentous	Effuse	Raised	Convex	Pulvinate	Umbonate	Transparent	Translucent	Opaque		
L. antibioticus																			
UASM 3C (ATCC 29479)		√		√				√				√					√	++	10 YR 4/3
UASM 66	√			√				√			√					√		−	2.5 Y 6/4
UASM L17 (ATCC 29480)	√			√				√			√						√	+	10 YR 4/4
UASM Q9	√			√		√						√					√	+	10 YR 5/6
UASM Q15	√			√		√					√						√	++	7.5 YR 3/2
UASM 4045 (ATCC 29481)		√		√		√						√					√	++	1 Y 4/3
UASM 4169	√			√		√					√						√	+	6 YR 2/4
UASM 4551	√			√		√						√					√	++	6 YR 2/4
UASM 4572		√		√		√						√					√	++	7.5 YR 2/4
UASM 4574	√			√		√						√					√	+	5 YR 2/5
UASM 4578		√		√			√					√					√	+	7.5 YR 2/4
UASM 4593	√			√				√				√					√	±	5 YR 3/4
UASM 4598	√			√			√				√						√	±	7.5 YR 3/4
UASM 8I	√			√		√						√					√	++	10 YR 3/2
UASM 10I	√			√		√						√					√	++	7.5 YR 2.5/3
UASM 12I	√			√		√						√					√	++	10 YR 3/4
L. brunescens																			
UASM D (ATCC 29482)			√		√		√			√				√		√		+	2.5 Y 3/4
UASM 2 (ATCC 29483)			√		√		√			√				√		√		+	2.5 Y 3/4
UASM 6 (ATCC 29484)			√		√		√			√				√		√		+	2.5 Y 3/4
UASM 4541			√		√			√		√		√				√		±	10 YR 4/6
UASM CB1	√			√		√					√					√		++	10 YR 3/6
UASM CB2	√			√		√						√				√		++	1 Y 4/6
UASM CB4	√			√		√						√				√		++	10 YR 4/6
UASM CB5	√			√		√						√				√		++	10 YR 4/6
UASM CB6		√		√					√		√					√		++	10 YR 4/4
UASM CB7		√		√					√		√					√		++	2.5 Y 4/4
L. enzymogenes																			
UASM 495 (ATCC 29487)			√	√				√		√		√				√		−	2.5 Y 5/4
UASM AL-1 (ATCC 27796)																			
ct[a] (i)			√	√		√						√				√		+	2.5 Y 5/6
ct (ii)			√	√		√						√				√		+	5 Y 7/6
ct (iii)			√	√		√						√				√		+	2.5 Y 5/6
ATCC 21123		√		√		√						√			√			−	2.5 Y 5/6
UASM 18L																			
ct (y) (ATCC 29485)		√		√		√						√				√		−	2.5 Y 6/6
ct (w) (ATCC 29486)		√		√			√					√					√	−	5 Y 6.5/6
UASM 4553																			
ct (i)	√			√				√				√					√	−	2.5 Y 6/6
ct (ii)	√			√		√						√				√		−	2.5 Y 6/6
UASM 4554	√			√			√					√					√	−	2.5 Y 6/6
UASM 4555																			
ct (i)	√			√		√						√					√	±	5 Y 6/6
ct (ii)	√			√		√						√					√	−	5 Y 7/4
UASM 4556																			
ct (i)	√			√		√						√					√	−	5 Y 6.5/6
ct (ii)	√				√			√			√					√		−	2.5 Y 6/6

TABLE 5—*Continued*

Strain	Form			Surface		Edge				Elevation					Optical Properties			Brown water-soluble pigment	Color (Munsell notation [6])
	Circular	≈ Circular	Irregular	Smooth	Rough	Entire	Undulate	Erose	Filamentous	Effuse	Raised	Convex	Pulvinate	Umbonate	Transparent	Translucent	Opaque		
UASM 4557																			
ct (i)	√			√		√						√					√	±	5 Y 6.5/5
ct (ii)	√			√		√						√					√	−	2.5 Y 6/6
UASM 4558																			
ct (i)	√			√		√						√					√	±	5 Y 5.5/4
ct (ii)	√			√				√			√						√	−	2.5 Y 6/6
UASM 4559																			
ct (i)	√			√		√						√					√	−	4 Y 7/6
ct (ii)		√		√			√					√				√		−	3 Y 6/6
UASM 4560																			
ct (i)		√		√				√						√			√	+	2.5 Y 3/4
ct (ii)		√		√				√						√			√	+	4 Y 4/6
ct (iii)	√			√		√					√					√		−	2.5 Y 6/6
UASM 4561																			
ct (i)	√			√				√				√					√	−	2.5 Y 6/6
ct (ii)		√		√				√				√					√	−	5 Y 6/6
ct (iii)	√			√		√						√				√		−	2.5 Y 6/8
UASM 4562																			
ct (i)		√		√				√			√					√		−	5 Y 6/6
ct (ii)	√				√			√			√					√		−	2.5 Y 5/6
UASM 4563																			
ct (i)	√				√	√						√					√	−	5 Y 6/6
ct (ii)		√		√			√					√					√	−	2.5 Y 6/6
UASM 4564																			
ct (i)	√			√				√			√				√			−	5 Y 6/6
ct (ii)	√			√		√						√				√		−	2.5 Y 6/6
UASM 4565																			
ct (i)		√		√			√					√					√	−	5 Y 7/6
ct (ii)		√		√		√						√				√		−	1.5 Y 6/6
UASM Q1	√			√				√			√					√		−	2.5 Y 6/6
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)		√		√		√						√				√		−	2.5,Y 6/6
L. gummosus UASM 402 (ATCC 29489)	√			√		√							√				√	−	5 Y 7/4

[a] ct, Colony type.

The 46 isolates studied here fell into four groups on the basis of colony color and other characteristics (Table 12). These groups are here regarded as separate species, as follows.

1a. *Lysobacter enzymogenes* subsp. *enzymogenes* sp. nov. and subsp. nov. (en.zy.mo'ge.nes. Gr. noun *zyme* leaven; M.L. noun *enzymum* enzyme; Gr.v. *gennaio* to produce; M.L. adj. *enzymogenes* enzyme-producing).

Flexible rods 0.3 to 0.5 by 4 to 50 μm (Fig. 3).

Two distinct colony types are known: a dirty-white mucoid colony and a yellowish nonmucoid one. The mucoid colony produces nonmucoid mutants, but the yellowish, nonmucoid colony type does not produce revertants to the dirty white, mucoid type. The following colony descriptions cover the whole range of colony types observed. The types are identical in other properties.

On CCA, 5-day-old colonies are dark cream; circular to irregular; usually with a smooth, but occasionally rough, surface; edge may be entire, undulate, lobate or erose; elevation effuse, flat, or raised, occasionally convex; transparent or translucent; no brown water-soluble pigment is produced.

On SAA, 5-day-old colonies are dark cream; circular to irregular; with a smooth or rough surface; edge may be entire, undulate or erose; elevation effuse, raised, or convex, occasionally

TABLE 6. *Physiology of Lysobacter strains: growth requirements*

Strain	% NaCl causing inhibition (14)[a]		Preferred atmosphere (7)	Growth temp (6)		Initial pH for growth (5)
	Partial	Complete		Range	Optimum	
L. antibioticus						
UASM 3C (ATCC 29479)	2	3	Air or 10% O_2	10–40	25	5–>10
UASM 66	1	3	10% O_2	<23–>37	26–30	5–>10
UASM L17 (ATCC 29480)	1	3	Air	4–>37	26–30	4.5–>10
UASM Q9	1	3	Air	2–>37	23–30	5–>10
UASM Q15	1	3	Air	<23–>37	26–33	5–>10
UASM 4045 (ATCC 29481)	1	3	Air or 10% O_2	4–>37	33	5–>10
UASM 4169	1	3	Air	4–37	30	6–>10
UASM 4551	1	3	Air	<23–>33	26–30	6–>10
UASM 4572	1	3	Air or 10% O_2	<23–37	30	6–>10
UASM 4574	1	3	Air or 10% O_2	4–>33	30	6–>10
UASM 4578	1	3	Air or 10% O_2	4–37	27	6–>10
UASM 4593	1	3	Air or 10% O_2	4–37	30	6–>10
UASM 4598	1	3	Air or 10% O_2	<23–37	23–33	6–>10
UASM 8I	1	3	Air	4–>37	26	5.5–>10
UASM 10I	1	3	Air or 10% O_2	4–>37	26	6–>10
UASM 12I	1	3	Air or 10% O_2	<23–>37	30–33	5–>10
L. brunescens						
UASM D (ATCC 29482)	1	2	10% O_2	12–>35	30–33	<5–>10
UASM 2 (ATCC 29483)	1	2	Air	12–45	35	<5–>10
UASM 6 (ATCC 29484)	<1	1	Air	12–>40	40	6–>10
UASM 4541	1	2	Air	10–50	40	<5–>10
UASM CB1	1	2	Air or 10% O_2	<23–>37	37	6–>10
UASM CB2	1	2	10% O_2	4–37	37	6–>10
UASM CB4	1	2	Air	4–44	30	6–>10
UASM CB5	1	2	Air	4–44	30	5.5–>10
UASM CB6	1	2	Air	4–>37	30	5–>10
UASM CB7	1	2	Air	<23–>37	30	5.5–>10
L. enzymogenes						
UASM 495 (ATCC 29487)	1	3	Air	10–35	35	5–>10
UASM AL-1 (ATCC 27796)	1	3	Air or 10% O_2	5–35	30	6–>10
ATCC 21123	3	>3	Air	10–35	30–35	5–>10
UASM 18L (ATCC 29485/6)	2	>3	Air	10–40	30	5–>10
UASM 4553	2	>3	Air or 10% O_2	10–35	25	5–>10
UASM 4554	2	>3	Air or 10% O_2	10–40	35	5–>10
UASM 4555	1	3	Air or 10% O_2	10–40	25	5–>10
UASM 4556	1	3	Air or 10% O_2	5–35	30–35	5–>10
UASM 4557	1	3	Air or 10% O_2	5–35	30	6–>10
UASM 4558	1	3	Air or 10% O_2	5–40	30–35	6–>10
UASM 4559	1	3	Air or 10% O_2	10–40	25–35	6–>10
UASM 4560	1	>3	Air or 10% O_2	10–40	25–35	7–>10
UASM 4561	1	3	Air or 10% O_2	10–40	25–35	6–>10
UASM 4562	1	3	Air or 10% O_2	10–40	20–30	6–>10
UASM 4563	1	3	Air or 10% O_2	10–40	30	6–>10
UASM 4564	1	3	Air or 10% O_2	10–40	25	6–>10
UASM 4565	1	>3	Air or 10% O_2	10–40	25–35	7–>10
UASM Q1	1	>3	Air	4–>37	30	4.5–>10
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)	1	3	Air or 10% O_2	8–35	30	5–>10
L. gummosus UASM 402 (ATCC 29489)	2	3	Air	10–40	20	6–>10

[a] Numbers in parentheses are days of incubation.

TABLE 7. *Physiology of Lysobacter strains: antibiotic susceptibilities*[a]

Strain	% SLS at which growth is		Chloramphenicol (30 μg)	Streptomycin (10 μg)	Penicillin G (10 U)	Polymyxin B (300 U)		Actinomycin D
	Reduced	Inhibited				Kirby-Bauer	Author's scheme	
L. antibioticus								
UASM 3C (ATCC 29479)	>0.01	0.1	R	R	R	I	S	—
UASM 66	>0.01	0.1	S	S	R	I	S	R
UASM L17 (ATCC 29480)	>0.01	0.1	R	R	R	I	S	R
UASM Q9	>0.01	>0.1	R	R	R	I	S	R
UASM Q15	>0.01	0.1	R	R	R	R	S	R
UASM 4045 (ATCC 29481)	>0.01	0.1	R	R	R	R	S	R
UASM 4169	>0.01	0.1	R	R	R	I	S	R
UASM 4551	>0.01	0.1	R	R	R	I	S	R
UASM 4572	>0.01	0.1	R	I	R	I	S	R
UASM 4574	>0.01	0.1	R	R	R	I	S	R
UASM 4578	>0.01	0.1	R	R	R	R	S	R
UASM 4593	>0.01	0.1	R	R	R	I	S	R
UASM 4598	>0.01	0.1	R	I	R	I	S	R
UASM 8I	>0.01	0.1	R	R	R	R	I	R
UASM 10I	>0.01	0.1	R	R	R	I	S	R
UASM 12I	>0.01	0.1	R	R	R	I	S	R
L. brunescens								
UASM D (ATCC 29482)	0.01	0.1	S	S	S	I	S	S
UASM 2 (ATCC 29483)	0.01	0.1	S	S	S	I	S	S
UASM 6 (ATCC 29484)	0.01	0.1	S	S	I	I	S	S
UASM 4541	>0.01	0.1	S	I	I	I	S	S
UASM CB1	0.01	0.1	S	I	S	—	—	S
UASM CB2	0.01	0.1	S	S	S	—	—	S
UASM CB4	>0.01	0.1	S	S	S	—	—	S
UASM CB5	>0.01	0.1	S	S	S	—	—	S
UASM CB6	>0.01	0.1	S	I	S	—	—	S
UASM CB7	>0.01	0.1	S	R	S	—	—	S
L. enzymogenes								
UASM 495 (ATCC 29487)	0.01	0.1	S	R	R	I	S	I
UASM AL-1 (ATCC 27796)	0.01	>0.1	R	R	R	R	S	I
ATCC 21123	>0.01	>0.1	I	R	R	R	S	—
UASM 18L (ATCC 29485/6)	>0.01	0.1	I	R	R	R	S	—
UASM 4553	>0.01	>0.1	R	R	R	I	S	R
UASM 4554	>0.01	0.1	S	R	R	R	S	I
UASM 4555	>0.01	>0.1	S	R	R	R	S	I
UASM 4556	>0.01	>0.1	R	R	R	I	S	R
UASM 4557	>0.01	>0.1	I	R	R	I	S	R
UASM 4558	>0.01	>0.1	R	R	R	R	S	I
UASM 4559	>0.01	>0.1	R	R	R	R	S	I
UASM 4560	>0.01	>0.1	S	R	R	I	S	I
UASM 4561	>0.01	>0.1	R	I	R	R	S	I
UASM 4562	>0.01	>0.1	I	R	R	R	S	I
UASM 4563	>0.01	>0.1	I	R	R	R	S	R
UASM 4564	>0.01	0.1	R	R	R	R	S	R
UASM 4565	>0.01	>0.1	I	R	R	I	S	R
UASM Q1	>0.01	>0.1	R	R	R	R	S	R
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)	0.01	0.1	S	R	R	I	S	I
L. gummosus UASM 402 (ATCC 29489)	>0.01	>0.1	R	R	R	I	S	I

[a] All read at 2 days except SLS which was read at 5 days. Abbreviations: S, susceptible; I, intermediate; R, resistant; —, not done. Columns 3 through 6 are scored by the Kirby-Bauer scheme (3). Column 7 is scored on the author's scheme based on the behavior of control organisms *Escherichia coli, Pseudomonas aeruginosa, Arthrobacter* sp., *Serratia marcescens,* and *Bacillus subtilis* (see Table 1). Column 8 is scored in comparison to control organisms *E. coli* and *B. subtilis:* S, more susceptible than *E. coli* but not so susceptible as *B. subtilis;* R, same as or less susceptibility than *E. coli,* i.e., resistant. Early and late observations were made at 2 and 3 days, respectively.

TABLE 8. *Physiology of Lysobacter strains: antimicrobial lytic action*[a]

Strain	Bacteria							Fungi				Alga
	Escherichia coli	*Pseudomonas aeruginosa*	*Arthrobacter* sp.	*Serratia marcescens*	*Bacillus subtilis*	UASM 4432 (actinomycete)	UASM 4441 (actinomycete)	*Rhizopus* sp.	*Penicillium notatum*	*Sclerotinia sclerotiorum*	Yeast	*Chlorella* sp.
L. antibioticus												
UASM 3C (ATCC 29479)	L	—	L	L	L	?	L	—	L	?	L	L
UASM 66	?	—	L	—	L	—	?	—	—	L	—	L
UASM L17 (ATCC 29480)	?	?	L	?	L	—	—	?	—	L	L	L
UASM Q9	—	—	L	L	?	—	?	?	?	L	L	L
UASM Q15	?	—	L	?	L	—	—	?	?	L	L	L
UASM 4045 (ATCC 29481)	?	—	—	?	?	—	—	?	?	L	L	L
UASM 4169	—	—	L	?	?	—	?	?	?	L	L	L
UASM 4551	—	—	?	L	?	—	—	?	?	L	?	L
UASM 4572	—	—	—	—	?	—	—	?	?	L	?	L
UASM 4574	?	—	L	?	?	—	—	—	?	L	—	L
UASM 4578	?	—	—	—	?	—	—	—	—	L	—	L
UASM 4593	?	—	L	—	?	—	—	?	—	L	—	L
UASM 4598	—	—	—	—	?	—	—	—	—	L	—	L
UASM 8I	?	—	—	—	—	—	—	?	—	L	—	L
UASM 10I	—	—	L	—	L	—	?	—	?	L	L	L
UASM 12I	?	—	—	—	L	—	?	—	?	L	L	L
L. brunescens												
UASM D (ATCC 29482)	L	L	L	—	L	?	L	L	L	L	L	L
UASM 2 (ATCC 29483)	L	L	L	—	L	—	L	—	L	L	L	L
UASM 6 (ATCC 29484)	—	—	L	—	L	—	L	—	—	—	L	L
UASM 4541	L	L	L	—	L	—	L	?	L	—	L	L
UASM CB1	?	?	L	L	—	—	—	—	—	L	L	L
UASM CB2	L	?	?	L	—	—	—	—	?	L	L	L
UASM CB4	L	?	?	L	—	—	—	—	—	?	L	?
UASM CB5	L	—	—	—	—	—	—	—	—	—	L	L
UASM CB6	L	—	—	—	—	—	—	L	—	—	L	L
UASM CB7	L	L	—	L	—	—	—	—	—	—	L	L
L. enzymogenes												
UASM 495 (ATCC 29487)	—	?	L	—	L	L	L	L	L	—	?	L
UASM AL-1 (ATCC 27796)	L	?	L	—	L	L	L	L	L	L	L	L
ATCC 21123	—	—	L	L	L	L	L	L	L	L	L	L
UASM 18L (ATCC 29485/6)	—	?	L	L	L	L	L	—	L	L	L	L
UASM 4553	—	—	L	L	L	L	L	L	L	L	L	L
UASM 4554	—	—	L	L	L	L	L	L	L	L	L	L
UASM 4555	L	—	L	L	L	L	L	L	L	L	L	L
UASM 4556	L	?	L	L	L	L	L	L	L	L	L	L
UASM 4557	L	—	L	L	L	L	L	L	L	L	L	L
UASM 4558	—	—	L	L	L	L	L	L	L	?	L	L
UASM 4559	L	—	L	L	L	L	L	L	L	?	L	L
UASM 4560	—	?	L	L	L	L	L	L	L	L	L	L
UASM 4561	L	?	L	L	L	L	L	L	L	L	L	?
UASM 4562	L	?	L	?	L	L	L	L	L	L	L	—
UASM 4563	—	?	L	L	L	L	L	L	L	L	L	L
UASM 4564	—	?	L	—	L	L	L	L	L	L	L	—

TABLE 8—*Continued*

Strain	Bacteria							Fungi				Alga
	Escherichia coli	*Pseudomonas aeruginosa*	*Arthrobacter* sp.	*Serratia marcescens*	*Bacillus subtilis*	UASM 4432 (actinomycete)	UASM 4441 (actinomycete)	*Rhizopus* sp.	*Penicillium notatum*	*Sclerotinia sclerotiorum*	Yeast	*Chlorella* sp.
UASM 4565	—	—	L	L	L	L	L	L	L	L	L	L
UASM Q1	L	—	L	L	?	—	—	L	L	L	L	L
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)	—	—	L	—	L	L	L	L	L	–	L	
L. gummosus UASM 402 (ATCC 29489)	—	—	L	—	L	L	L	?	L	L	L	L

" Results were read after 11 days except for the yeast, whose results were read at 9 days. Maximum lytic activity in each test noted as: L, good lysis; ?, possible lysis; –, no lysis.

flat; translucent or opaque; no brown, water-soluble pigment is produced.

On PCA, 5-day-old colonies are deep yellow cream; more or less circular; usually with a smooth surface; edge may be entire, undulate or erose; elevation mainly convex, sometimes raised or umbonate; translucent or opaque; some water-soluble pigment is produced.

Older cultures on most media produce copious, dark brown, water-soluble pigment.

Growth of 15 of 18 strains is reduced by 1% NaCl, and growth of 12 of 18 is inhibited by 3% NaCl; preferred atmosphere, air or 10% O_2; temperature range for growth, 5 to 40°C; temperature for optimum growth, 25 to 35°C.

Two of 18 strains utilize urea as N source.

Growth of 16 of 18 strains is not reduced by 0.01% sodium lauryl sulfate (SLS), and only three of 18 strains are inhibited completely by 0.1% SLS. Resistant to 10 μg of streptomycin and to 10 U of penicillin, susceptible to 300 U of polymyxin B, resistant or intermediate in response to actinomycin D, and variable in response to 30 μg of chloramphenicol.

Ninety percent or more of the isolates lyse gram-positive bacteria (including actinomycetes), filamentous fungi, a yeast, and a green alga. Less than 50% of the isolates lyse gram-negative bacteria.

Attack on glucose is oxidative, and in 1 strain of 18 it is also fermentative. Acid is produced from glucose, cellobiose, sucrose, and lactose. Fifteen of 18 strains produce acid from glycerol and from mannitol.

Lipase activity is shown on Tweens 20, 40, 60, and 80.

Hydrolyzes alginate, pectate, and CMC but not starch.

Liquefies gelatin; peptonizes milk in 1 to 2 days; growth and NH_3 are produced from casein, Penassay, Casitone, and Casamino Acids broths; grows well on 0.2% tryptone agar; 17 of 18 strains show beta-, and one strain shows alpha-hemolysis.

Three of 18 strains produce H_2S; colorless colonies are produced on MacConkey agar; pink colonies are produced on EMB: the citrate test is positive.

Does not reduce NO_3^- or NO_2^-.

Habitat: Soil.

The G+C content of the DNA ranges from 69.0 to 70.1 mol%; that of the type strain is 69.0 mol% (T_m).

Type strain: UASM 495 (= ATCC 29487).

1b. *Lysobacter enzymogenes* subsp. *cookii* Christensen subsp. nov. (coo'ki.i. M.L. gen.n. *cookii* of Cook; named for F. D. Cook, the microbiologist who first isolated lysobacters and who recognized their lytic and antibiotic potentials).

Description as for *L. enzymogenes* subsp. *enzymogenes,* with the following exceptions.

Colonies on CCA are creamy brown and irregular with rough surfaces.

Colonies on SAA are creamy brown, irregular, and transparent.

Colonies on PCA are creamy brown with no water-soluble pigment.

The production of brown water-soluble pigment is not so marked, although it was observed on three media.

Preferred atmosphere is air; temperature range for growth, 8 to 35°C; temperature for optimum growth, 20°C; pH range for growth, 6 to >10.

Utilizes urea as N source.

Growth is reduced by 0.01% SLS and com-

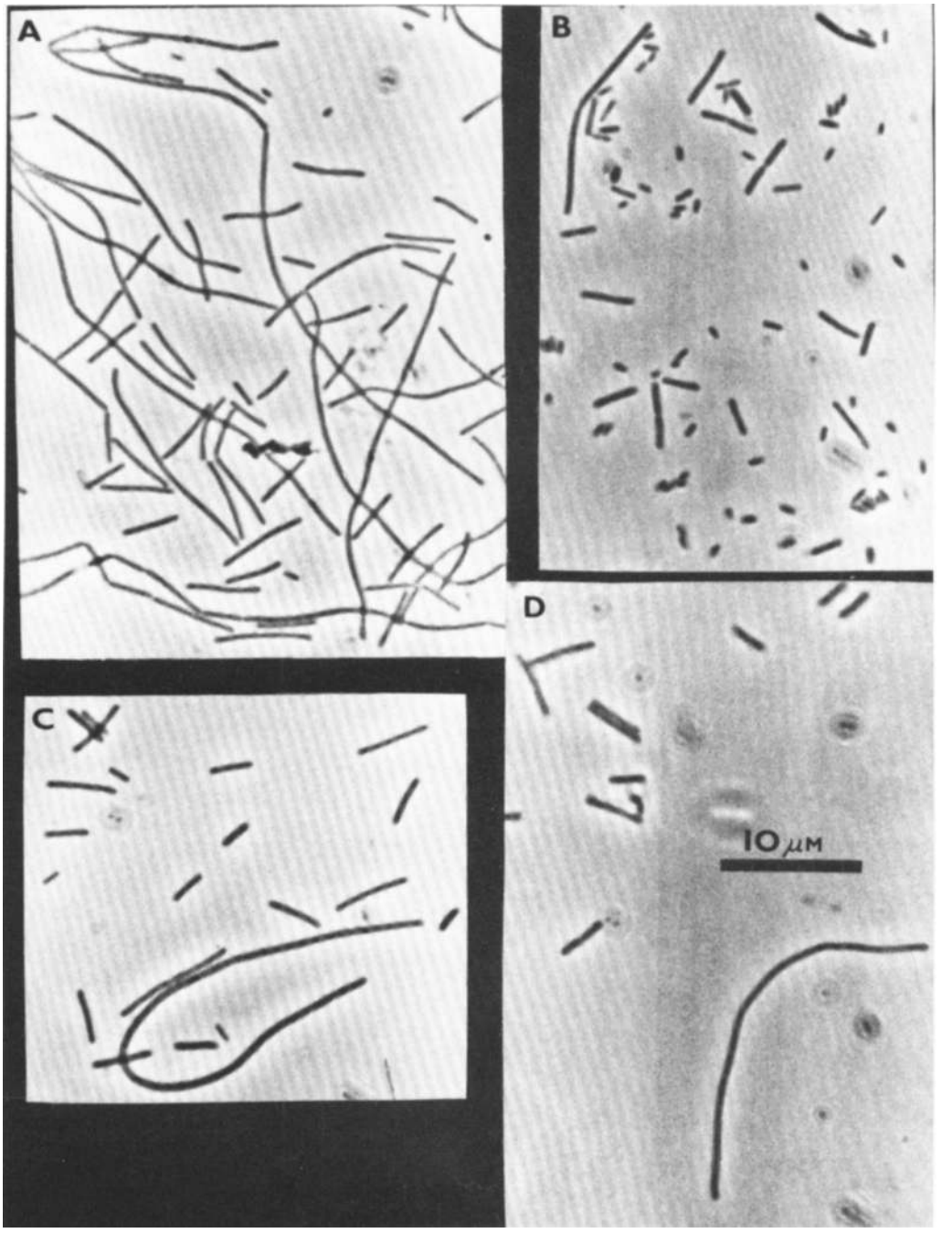

FIG. 3. *Cells of Lysobacter enzymogenes from skim acetate broth cultures. (A) UASM 495 (= ATCC 29487), 44 h; (B) ATCC 21123, 44 h; (C) UASM 18 LW (= ATCC 29485), 44 h; (D) L. enzymogenes subsp. cookii UASM 13B (= ATCC 29488), 60 h. All micrographs are at the same magnification.*

pletely inhibited by 0.1% SLS. Intermediate reaction to actinomycin D; susceptible to 30 μg of chloramphenicol.

Does not lyse gram-negative bacteria.

Does not produce acid from glycerol or mannitol.

Hydrolyzes starch but not alginate.

Alpha-hemolytic.

TABLE 9. *Biochemical reactions of Lysobacter strains: sugars, alcohols, lipids, polysaccharides*[a]

Strain	OF test with glucose (15)[b]		Acid production from: (17)					Lipase activity (5)				Polysaccharide hydrolysis					
															Starch		
	Oxidative	Fermentative	Cellobiose	Sucrose	Lactose	Glycerol	Mannitol	Tween 20	Tween 40	Tween 60	Tween 80	Alginate (30)	Pectate (30)	Agar (gelase) (5)	SYS (4)	NBS (25)	Potato (8)
L. antibioticus																	
UASM 3C (ATCC 29479)	+	−	+	−	−	−	−	−	±	±	+	−	−g	−	−	−g	−
UASM 66	+	+ slow	+	−	−	−	−	+	±	+	+	+				−	
UASM L17 (ATCC 29480)	+	−	+	−	−	−	−	+	±	+	+	−	−g			−	
UASM Q9	+	−	+	+	+	+	+	+	+	+	+	+	−g −g			−	
UASM Q15	+	+ slow	+ weak	−	−	−	−	+	+	+	+	−	−g			−	
UASM 4045 (ATCC 29481)	+	+ slow	+	−	−	−	−	+	+	+	+	−	−g			−	
UASM 4169	+	+ slow	+	−	−	−	−	−	±	±	+	−	−g			−	
UASM 4551	+	+	+	−	−	−	−	−	±	+	+	−	−g			−	
UASM 4572	+	+ slow	+	−	−	−	−	+	±	+	+	−	−g			−	
UASM 4574	+	+	+ weak	−	−	−	−	+	+	+	+	−	−g			−	
UASM 4578	+	−	+	−	−	−	−	+	+	+	+	−	−g			−	
UASM 4593	+	+ slow	+	−	−	−	−	+	+	+	+	−	−g			−	
UASM 4598	+	+ slow	+	−	+	−	−	+	±	+	+	−	−g			−	
UASM 8I	+	−	+	−	−	−	−	−	+	±	+	+	−g			−	
UASM 10I	+	+	+	−	−	−	−	+	+	+	+	−	−g			−	
UASM 12I	+	+ slow	+	−	−	−	−	+	+	+	+	+	−g			−	
L. brunescens																	
UASM D (ATCC 29482)	+	−	−	−	−	−	−	−	−	±	−	−	+	+	+	+	+
UASM 2 (ATCC 29483)	+	−	−	−	−	−	−	−	±	±	+	−	+	+	+	+	+
UASM 6 (ATCC 29484)	−	−	−	−	−	−	−	−	−	±	−	−	+	+	+	+	+
UASM 4541	+	−	−	−	−	−	−	−	±	+	+	−	+	+	+	+	+

UASM CB1	+	−	−	−	−	−	−	−			−	−	−			+	
UASM CB2	+	−	−	−	−	−	−	−			−	−	+			+	
UASM CB4	+	−	−	−	−	−	−	−			−	−	+			+ slow	
UASM CB5	+	+ slow	−	−	−	−	−	−			−	−	+			+ slow	
UASM CB6	+	+ slow	−	−	−	−	−	−			−	−	+			+ slow	
UASM CB7	+ slow	+ slow	−	−	−	−	−	−			−	−	+			+ slow	
L. enzymogenes																	
UASM 495 (ATCC 29488)	+	−	+	+	+	+	+	+	+	+	+	+	+		−	+ slow	−
UASM AL-1 (ATCC 27796)	−≠	−	+ weak	−	+ weak	−	−	+	+	+	+	+	+	−	−	−	−
ATCC 21123	+	−	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−
UASM 18L (ATCC 29485/6)	+	−	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−
UASM 4553	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4554	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4555	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4556	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4557	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4558	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4559	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4560	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4561	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4562	+	−	+	+	+	+	+					+		−	−	−	−
UASM 4563	+	−	+	+	+	+	+					+		+ slow	−	−	−
UASM 4564	+	−	+	+	+	+	+					+		+ slow	−	−	−
UASM 4565	+	−	+	+	+	+	+					+		−	−	−	−
UASM Q1	+	+	+	+	+	−	−	+	+	+	+	+	+			−	
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)	+	−	+	+	+	−	−	+	+	+	+	−	+	+	−	−	+
L. gummosus UASM 402 (ATCC 29489)	+	−	+ weak	+ weak	+	−	−	+	+	+	+	−	+	−	−	−	−

[a] Abbreviations and symbols. OF (acid production tests): +, yellow color due to lowering of pH; −, no change from greenish-blue color; ≠, growth but no pH change. Lipase activity: +, crystals formed; −, no crystals observed; blank, not done. Alginate, pectate: +, liquefaction; −, no liquefaction; g, growth but no liquefaction; blank, not done. Agar (gelase): +, unstained gelase field left around colony after flooding with I-KI; −, normal yellow color throughout agar. Starch (SYS = salts yeast-extract starch, potato): +, yellow clear field left around colony after flooding with I-KI; −, no field, agar purple showing unchanged starch present. Starch (NBS = nutrient broth starch): +, iodine spot test for starch negative; −, iodine test positive; g, growth but no hydrolysis.

[b] Numbers in parentheses are days of incubation.

TABLE 10. *Biochemical reactions of Lysobacter strains: protein hydrolysis and miscellaneous reactions*[a]

Strain	Milk peptonization[b]	Hemolysis of sheep RBC (12)[c]	Growth and production of ammonia in:				H_2S production (7)	Growth on:		Reduction of nitrates	
			Penassay broth (11)		Salts-Casamino Acids broth (7)						
			Growth	$\rightarrow NH_3$	Growth	$\rightarrow NH_3$		MacConkey (2)	EMB (2)	$NO_3^- \rightarrow NO_2^-$ (11)	$NO_2^- \rightarrow$ gas (11)
L. antibioticus											
UASM 3C (ATCC 29479)	1[c]	α	+	−	+	−	−	−	+	+	−
UASM 66	1	β					−	−	+	+	−
UASM L17 (ATCC 29480)	1	β					−	−	+	−	−
UASM Q9	1	β					−	+	+	+	−
UASM Q15	1	β					−	−	+	−	−
UASM 4045 (ATCC 29481)	1	β					−	−	+	−	−
UASM 4169	1	β					−	−	+	−	−
UASM 4551	1	α					−	−	+	−	−
UASM 4572	1	α					−	−	+	−	−
UASM 4574	1	α					−	−	+	−	−
UASM 4578	1	α					−	−	+	−	−
UASM 4593	1	α					−	−	+	−	−
UASM 4598	1	α					−	−	+	−	−
UASM 8I	1	β					±	+	+	+	+
UASM 10I	1	β					−	−	+	−	−
UASM 12I	1	β					−	−	+	−	−
L. brunescens											
UASM D (ATCC 29482)	1	γ	+	−	+	−	+	−	−	−	−
UASM 2 (ATCC 29483)	1	γ	+	−	+	−	−	−	−	−	−
UASM 6 (ATCC 29484)	1	−	+	−	+	−	+	−	−	−	−
UASM 4541	1	γ	+	−	+	−	+	−	−	−	−
UASM CB1	1	γ					+	−	±	−	−
UASM CB2	1	γ					+	−	−	−	−
UASM CB4	1	γ					+	−	−	−	−
UASM CB5	1	γ					+	−	−	−	−
UASM CB6	1	γ					+	−	−	−	−
UASM CB7	1	γ					+	−	−	−	−
L. enzymogenes											
UASM 495 (ATCC 29487)	1	β	+	+	+	+	+	+	+	−	−
UASM AL-1 (ATCC 27796)	2	β	+	−	+	+	−	+	+	−	−
ATCC 21123	1	β	+	+	+	+	+	+	+	−	−
UASM 18L (ATCC 29485/6)	1	β	+	+	+	+	−	+	+	−	−
UASM 4553	1	β	+	+	+	+	−			−	−
UASM 4554	2	β	+	+	+	+	−			−	−
UASM 4555	2	β	+	+	+	+	−			−	−
UASM 4556	2	β	+	+	+	+	−			−	−
UASM 4557	2	β	+	+	+	+	−			−	−
UASM 4558	3	β	+	+	+	+	−			−	−
UASM 4559	2	β	+	+	+	+	−			−	−
UASM 4560	1	β	+	+	+	+	−			−	−
UASM 4561	2	β	+	+	+	+	−			−	−
UASM 4562	2	β	+	+	+	+	−			−	−

TABLE 10—*Continued*

Strain	Milk peptonization[b]	Hemolysis of sheep RBC (12)[c]	Growth and production of ammonia in: Penassay broth (11) Growth	Penassay broth (11) →NH_3	Salts-Casamino Acids broth (7) Growth	Salts-Casamino Acids broth (7) →NH_3	H_2S production (7)	Growth on: MacConkey (2)	Growth on: EMB (2)	Reduction of nitrates: $NO_3^- \rightarrow NO_2^-$ (11)	Reduction of nitrates: $NO_2^- \rightarrow$ gas (11)
UASM 4563	1	β	+	+	+	+	−			−	−
UASM 4564	2	β	+	+	+	+	−			−	−
UASM 4565	1	β	+	+	+	+	−			−	−
UASM Q1	1	γ					+	+	+	−	−
L. enzymogenes subsp. *cookii* UASM 13B (ATCC 29488)	2	α	+	+	+	+	−	−	+	−	−
L. gummosus UASM 402 (ATCC 29489)	1	α	+	+	+	−	−	−	+	−	−

[a] Symbols. Blank, not done. Hemolysis: RBC, erythrocytes; γ, growth but no hemolysis; −, no growth; β, true hemolysis; α, partial hemolysis. MacConkey: +, growth as small, cream-colored colonies; −, no growth. EMB: +, growth as pink colonies; ±, small, colorless colonies; −, no growth. Reduction of nitrates: ($NO_3^- \rightarrow NO_2^-$ column) +, no NO_3^-, no NH_3, neutral reaction, NO_2^- present; −, NO_3^- present; ($NO_2^- \rightarrow$ gas column): +, no NO_2^-, no NO_3^-, no NH_3, neutral reaction, may have bubbles; −, NO_2^- present.

[b] Number of days for complete clearing.

[c] Numbers in parentheses are days of incubation.

TABLE 11. *DNA base ratios of selected Lysobacter strains*

Strain	mol% G+C
L. antibioticus	
UASM 3C (= ATCC 29479)	62[a], 69.2
UASM L17 (= ATCC 29480)	68.6
UASM 4045 (= ATCC 29481)	66.2
L. brunescens	
UASM 2 (= ATCC 29483)	66.8, 67.9
UASM D (= ATCC 29482)	67.6, 67.8
L. enzymogenes	
UASM 495 (= ATCC 29487)	69.0, 69.0
UASM AL-1 (= ATCC 27796)	69.2
ATCC 21123	70.1
L. enzymogenes subsp. *cookii* UASM 13B (= ATCC 29488)	65.4
L. gummosus UASM 402 (= ATCC 29489)	65.7

[a] Datum from M. Mandel, 1961, personal communication to F. D. Cook. All other determinations were made in this laboratory.

No growth on MacConkey agar.

Habitat: Soil.

The G+C content of the DNA of the one strain so far assigned to this subspecies is 65.4 mol% (T_m).

Type strain: UASM 13B (= ATCC 29488).

2. *Lysobacter antibioticus* sp. nov. (an.ti.bi.o'ti.cus. Gr. pref. *anti* against; Gr.n. *bius* life; M.L. adj. *antibioticus* against life, antibiotic).

Flexible rods 0.4 by 4 to 40 μm (Fig. 4).

On CCA, 5-day-old colonies are pinkish brown; circular to irregular; with a smooth surface; entire, undulate, erose, or filamentous edge; effuse, flat, raised, or convex elevation; translucent or opaque; and some or much, brown, water-soluble pigment is produced.

On SAA, 5-day-old colonies are pinkish brown; more or less circular; with a smooth surface; edge may be entire, erose, or filamentous; elevation may be effuse, flat, raised, or convex; translucent or opaque; some or much, brown, water-soluble pigment is produced.

On PCA, 5-day-old colonies are orange brown; more or less circular; with a smooth surface; edge entire, undulate, or erose; elevation raised or convex; mostly opaque; much, brown, water-soluble pigment is produced.

Older cultures on most media produce copious, brown, water-soluble pigment, and deep red crystals of the antibiotic myxin may be observed within the highly mucoid colonies.

Growth of 15 of 16 strains was reduced by 1% NaCl and inhibited by 3% NaCl; preferred atmosphere, air or 10% O_2; temperature range for growth, 2 to 40°C; temperature for optimum growth, 25 to 33°C.

Only one strain uses urea as N source.

TABLE 12. *Differential characteristics of species of the genus Lysobacter*

Characteristic	*L. antibioticus* N = 16[a]	*L. brunescens* N = 10	*L. enzymogenes* N = 19	*L. gummosus* N = 1
Colonies				
Type of growth	Sloppy, mucoid	Spreading, thin	Sloppy, mucoid	Pulvinate, gummy
Smooth surface	+	−	+	+
Opaque	+	−	+	+
Color	Pink to brownish pink	Yellow to chocolate	Cream to creamy brown	Pale yellowish-gray
Water-soluble pigment	+	+	+	−
Broth culture viscous	+	−	+	++++
Hydrolysis of CMC	+	−	+	+
Hydrolysis of alginate	−[4b]	−	+[1]	−
Hydrolysis of pectate	−	+[1]	+	+
Hydrolysis of starch	−	+	−[2]	−
Acid from alcohols	−[1]	−	+[4]	−
Citrate as sole C source	+	−	+	+
Urea as N source	−[1]	−	−[3]	+
Growth on MacConkey	−[2]	−	+	−
Growth on EMB	+	−	+	+
Hemolysis	α or β	γ	β[2]	α
H_2S produced	−	+[1]	−	−
Actinomycin D	Resistant	Susceptible	Resistant or intermediate	Intermediate
Penicillin G	Resistant	Susceptible or intermediate	Resistant	Resistant

[a] N, Number of strains examined.
[b] Superscripts are numbers of strains giving opposite or different result.

Growth is not reduced by 0.01% SLS, but 15 of 16 strains are inhibited by 0.1% SLS; resistant to 30 μg of chloramphenicol, 10 U of penicillin, and to actinomycin D; and susceptible to 300 U of polymyxin B; 13 of 16 strains are resistant to 10 μg of streptomycin.

Eleven of 16 strains lyse one or more gram-positive bacteria; only one strain lyses a gram-negative bacterium and an actinomycete; all strains lyse one of the three filamentous fungi tested, and all lyse a green alga; 8 of 16 strains lyse a yeast.

Attack on glucose is oxidative, and 11 of the 16 strains also show fermentation. Acid is produced from glucose and cellobiose; 2 of 16 strains produce acid from lactose; 1 of 16 strains produces acid from sucrose, glycerol, and mannitol.

Lipase activity is shown by 12 of 16 strains on Tween 20, and there is a variable amount of activity from all strains on Tweens, 40, 60, and 80.

Hydrolyzes CMC but not pectate or starch. Four of 16 strains hydrolyze alginate.

Liquefies gelatin; peptonizes milk in 24 h; grows and produces NH_3 on casein and Casitone broths; 1 strain of 16 grows but does not produce NH_3 in Penassay and Casamino Acids broths; grows well on 0.2% tryptone agar; 7 of 16 strains are alpha-hemolytic, and 9 of 16 are beta-hemolytic.

H_2S is not produced; 2 of 16 strains show growth (with colorless colonies) on MacConkey agar; pink colonies produced on EMB; citrate test is positive.

Four of 16 strains reduce NO_3^- to NO_2^-, and one of these strains further denitrifies NO_2^- to a gas.

Habitat: Soil.

The G+C content of the DNA ranges from 66.2 to 69.2 mol%; that of the type strain is 69.2 mol% (T_m).

Type strain: UASM 3C (= ATCC 29479).

3. *Lysobacter gummosus* sp. nov. (gum.mo'sus. L. adj. *gummosus* slime (gum)-producing).

Short rods, 0.4 by 2.0 μm, which glide in a series of short jerks; the cells are too short to flex (Fig. 5).

On CCA, 5-day-old colonies are pale yellowish-gray and circular with a smooth surface and entire edge; pulvinate; translucent; no water-soluble pigment is produced; gummy.

On SAA, 5-day-old colonies are pale yellowish-gray and circular with a smooth surface and entire edge; pulvinate; opaque; no water-soluble pigment is produced; very gummy.

On PCA, 5-day-old colonies are yellow-gray and circular with a smooth surface and entire edge; pulvinate; opaque; no water-soluble pigment is produced; very gummy.

Older cultures are intensely gummy and do not produce a water-soluble pigment. In very old cultures, the viscosity of the colony may de-

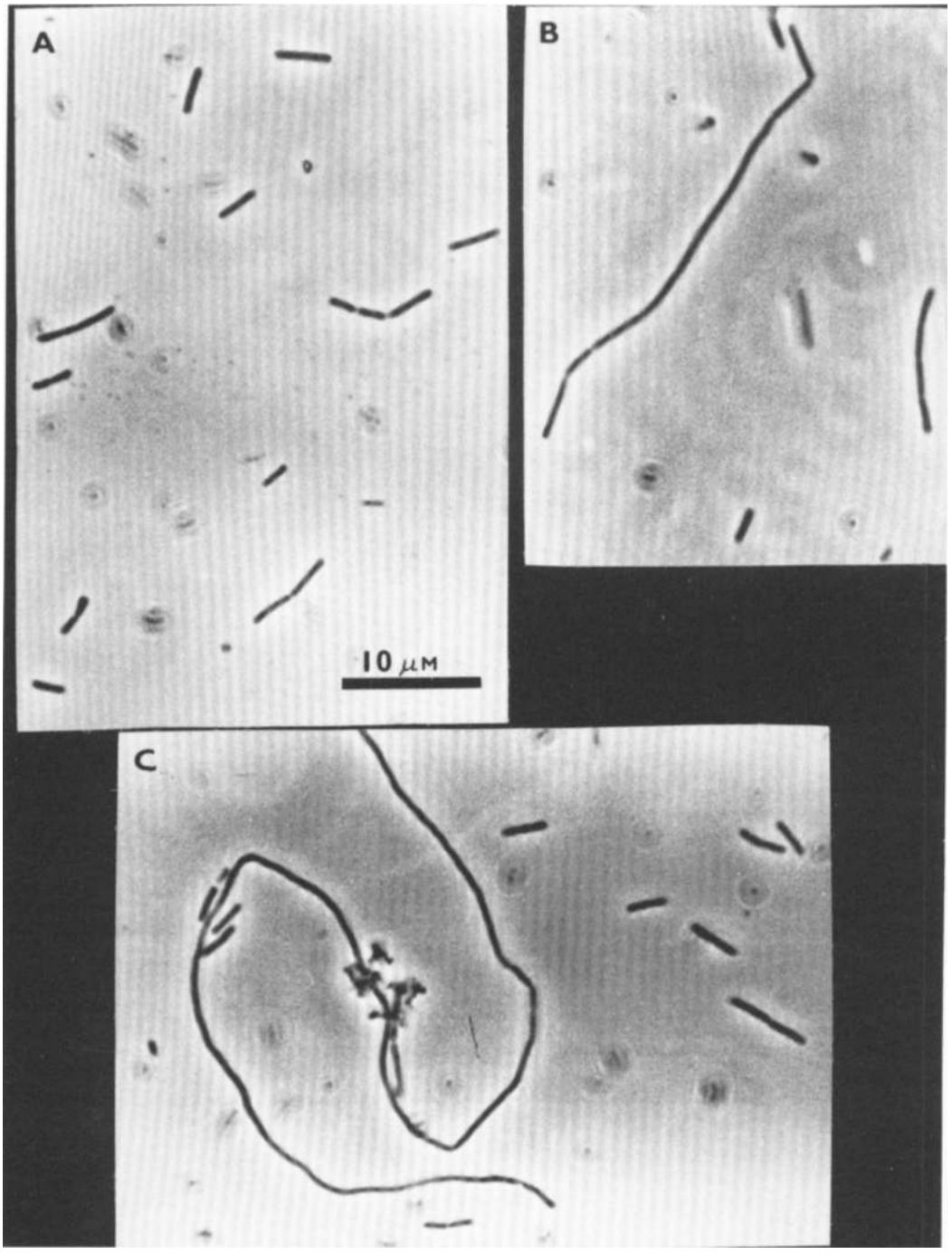

FIG. 4. *Cells of Lysobacter antibioticus from skim acetate broth cultures. (A) UASM 3C (= ATCC 29479), 66 h; (B) UASM 4593, 32 h; (C) UASM 4578, 72 h. All micrographs are at the same magnification.*

crease dramatically, and viable cells can still be recovered from this thin slime.

Growth is reduced by 2% NaCl and inhibited by 3% NaCl; the preferred atmosphere is air; the temperature range for growth is 10 to 40°C; optimum growth occurs at 20°C; the pH range for growth is 6 to >10.

Urea is used as N source.

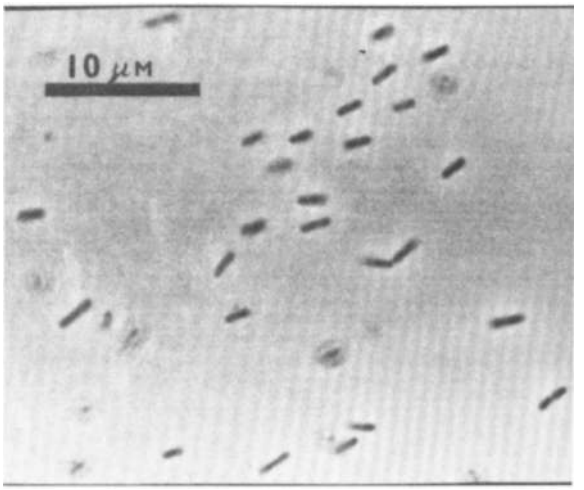

FIG. 5. *Cells of Lysobacter gummosus from skim acetate broth culture, UASM 402 (= ATCC 29489), 44 h.*

Growth is not reduced by 0.01% SLS and is not inhibited by 0.1% SLS. Resistant to 30 μg of chloramphenicol, 10 μg of streptomycin, and 10 U of penicillin; susceptible to 300 U of polymyxin B; intermediately susceptible to actinomycin D.

Lyses gram-positive (including actinomycetes) but not gram-negative bacteria, filamentous fungi, a yeast, and an alga.

Attacks glucose oxidatively. Acid is produced from glucose, cellobiose, sucrose, and lactose, but not from glycerol or mannitol.

Shows lipase activity on Tweens 20, 40, 60, and 80.

Hydrolyzes pectate and CMC but not alginate or starch.

Liquefies gelatin; peptonizes milk in 24 h; grows and produces NH_3 on casein, Penassay, and Casitone broths; grows but does not produce NH_3 on Casamino Acids broth; grows well on 0.2% tryptone agar; alpha-hemolytic.

H_2S is not produced; the oxidase test is equivocal; no growth is produced on MacConkey agar; pink colonies occur on EMB; the citrate test is positive.

Does not reduce NO_3^- or NO_2^-.

Habitat: Soil.

The G+C content of the DNA of the one strain so far assigned to this species is 65.7 mol% (T_m).

Type strain: UASM 402 (= ATCC 29489).

4. *Lysobacter brunescens* sp. nov. (bru.nes'cens. L.v. *brunesco* to become dark brown; L. part. adj. *brunescens* becoming dark brown).

Flexible rods 0.2 to 0.5 by 7.0 to 70.0 μm (Fig. 6).

On CCA, 5-day-old colonies are brownish-yellow and irregular with a rough surface; the edge may be undulate, lobate, erose, or filamentous; the elevation is effuse; the center is convex or umbonate; transparent; variable amounts of a brown, water-soluble pigment are produced.

On SAA, 5-day-old colonies are yellow-brown and circular to irregular; rough or smooth surface; edge may be entire, undulate, lobate, erose, or filamentous; the elevation is effuse, sometimes raised; the centers are often raised, convex, or umbonate; transparent or translucent; variable amounts of a brown, water-soluble pigment are produced.

On PCA, 5-day-old colonies are deep yellow-brown and circular to irregular; smooth or rough surface; the edge may be entire, undulate, erose, or filamentous; the elevation is effuse, raised, or convex; the center may sometimes be umbonate; translucent; typically, a dark brown, water-soluble pigment is copiously produced.

Older cultures on all media produce copious, dark-brown, water-soluble pigment.

Growth of 9 or 10 strains is reduced by 1% NaCl and inhibited by 2% NaCl; the preferred atmosphere is air or 10% O_2; the temperature range for growth is 4 to 50°C; for optimum growth, it is 30 to 40°C.

Eight of 10 strains utilize asparaginate as N source. Urea is not used as N source.

Growth of 5 of 10 strains is not reduced by 0.01% SLS; all strains are inhibited by 0.1% SLS. Susceptible to 30 μg of chloramphenicol, 300 U of polymyxin B, and to actinomycin D; 8 of 10 strains are susceptible to 10 U of penicillin G; 6 of 10 strains are susceptible to 10 μg of streptomycin.

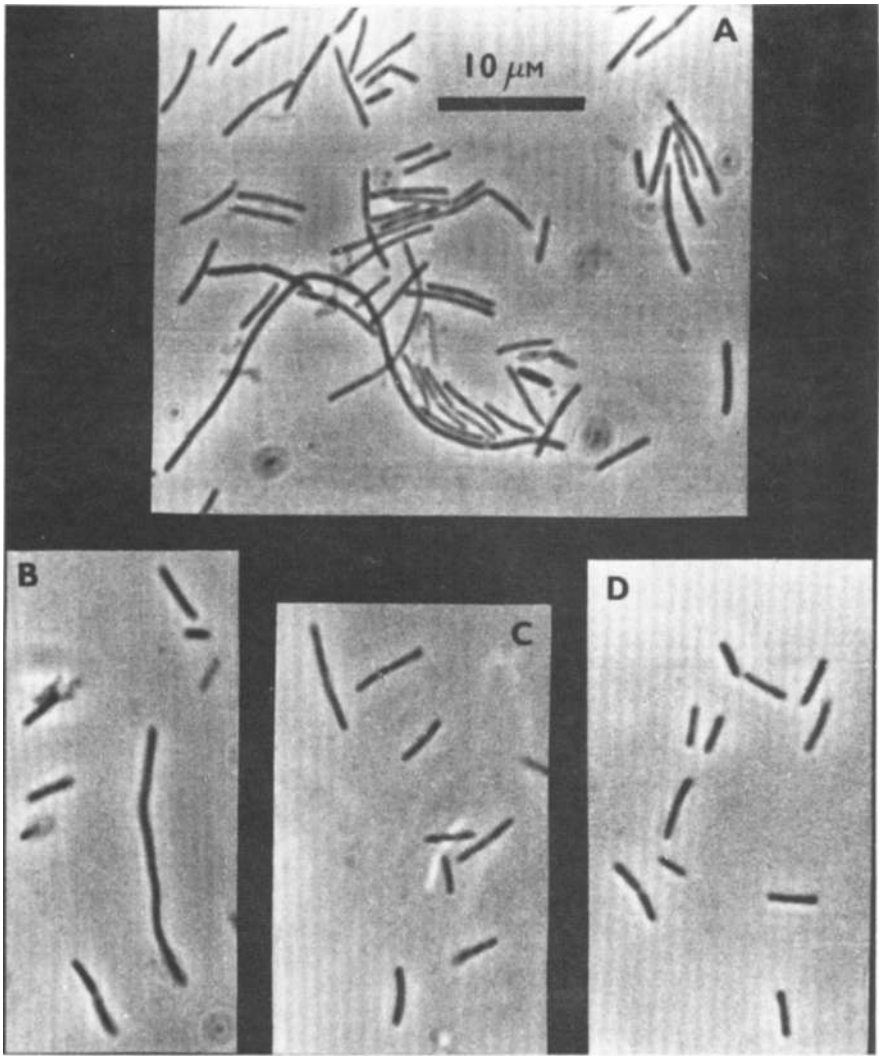

FIG. 6. *Cells of Lysobacter brunescens from skim acetate broth cultures. (A) UASM 2 (= ATCC 29483), 60 h; (B) UASM D (= ATCC 29482), 60 h; (C) UASM 4541, 44 h; (D) UASM CB5, 30 h. All micrographs are at the same magnification.*

Ninety percent or more of the isolates lyse gram-negative bacteria, a yeast, and an alga; 40 to 50% of the isolates lyse gram-positive bacteria (including actinomycetes) and filamentous fungi.

Attacks glucose oxidatively; 3 of 10 strains also attack glucose fermentatively. Nine of 10 strains produce acid from glucose. No acid is produced from cellobiose, sucrose, lactose, glycerol, or mannitol.

Lipase activity: There is no activity on Tween 20. Two of 4 strains are positive on Tween 40; 4 of 4 are positive on Tween 60; and 2 of 10 are positive on Tween 80.

Hydrolyzes starch; 9 of 10 strains hydrolyze pectate. There is no action on alginate or CMC.

Liquefies gelatin; peptonizes milk in 24 h; grows and produces NH_3 in casein and Casitone broths; four of four strains grow but do not produce NH_3 in Penassay and Casamino Acids broths; grows well on 0.2% tryptone agar; nonhemolytic.

Nine of 10 strains produce H_2S; no growth occurs on MacConkey agar; only 1 of 10 strains grows on EMB, producing small, colorless colonies; the citrate test is negative.

Does not reduce NO_3^- or NO_2^-.

Habitat: Fresh water.

TABLE 13. *Characteristics useful in differentiating the genera Cytophaga and Lysobacter and the order Myxobacterales*[a]

Differential character	*Cytophaga*	*Lysobacter*	*Myxobacterales*
DNA base ratio (mol% G+C)	29–42	65–70	67–71
Fruiting bodies	−	−	+
Colony color	Yellow, orange, or red	White, cream, yellow, brown, pink, or red	Gray, yellow, orange, brown, pink, red, purple, or nearly black
Brown, water-soluble pigment production	In one pink sp. only	In all except one whitish-yellow sp.	±
Optical properties of colony	Mainly transparent	Translucent or opaque except for one yellow-brown sp.	Transparent, translucent, or opaque
Degradation of filter paper	±	−	±
Degradation of agar	±	−	±
Degradation of chitin	One yellow sp. only	+	±
Antimicrobial lytic action	A few strains	All species	Most species
Actinomycin D	Susceptible	Three of the four spp. are resistant	Susceptible

[a] Symbols: +, all species positive; −, all species negative; ±, some species or some strains positive.

The G+C content of the DNA ranges from 66.8 to 67.9 mol%; that of the type strain ranges from 67.6 to 67.8 mol% (T_m).

Type strain: UASM D (= ATCC 29482).

Assignment of an unidentified, gliding, polysaccharolytic isolate to the genus *Cytophaga,* to the genus *Lysobacter,* or to the order *Myxobacterales* can be achieved on the basis of two fundamental characteristics: DNA base ratio and fruiting-body formation. Since these are not usually the first items of information gleaned about an unidentified isolate, other characteristics useful in differentiating between the above-mentioned taxa are listed in Table 13. This table relies heavily on colony characteristics because of the paucity of biochemical data on *Cytophaga* and *Myxobacterales.*

The degradation of polysaccharides is important in all three groups but is of only minor help in differentiating between these groups. Some species or strains of *Cytophaga,* of *Lysobacter,* and of myxobacteria can hydrolyze CMC, starch, and/or chitin. Alginate and pectate are degraded by some cytophagas and by some lysobacters but are not known to be degraded by myxobacters (only three strains have been tested [P. Christensen, unpublished results]). However, the polysaccharase potential is of value in the identification of individual species within the genera *Cytophaga* and *Lysobacter* and within the order *Myxobacterales.*

We have recently found that the colonial morphologies of some strains of three *Lysobacter* species appear to change slightly after the cells have been freeze-dried. In addition, it has come to our attention that strains of *L. enzymogenes* do not exhibit as strong a microbial lytic action and do not maintain as high a level of certain proteolytic enzymes after they have been maintained in the laboratory on skim milk media for some time.

As a result of these observations, the following recommendations are made: (i) those workers who need *Lysobacter* cultures with antimicrobial lytic action and who find that cultures received from the American Type Culture Collection are unsuitable in this regard should isolate their own strains directly from soil by the simple method outlined in Materials and Methods (this is by far the best way to insure high activity); and (ii) workers should maintain strains of *Lysobacter* on CCA (8), on which medium the organisms do not appear to lose their polysaccharolytic and proteolytic potential.

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REPRINT REQUESTS

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LITERATURE CITED

1. **Allen, L. C., and D. R. Whitaker.** 1972. The β-lytic protease of myxobacter 495: aspects of its specificity. Proc. Can. Fed. Biol. Soc. **15**:#31.
2. **American Type Culture Collection.** 1974. Catalogue of strains (bacteria), 11th ed. American Type Culture Collection, Rockville, Md.
3. **Bailey, W. R., and E. G. Scott.** 1970. Diagnostic microbiology, 3rd ed. Mosby, St. Louis, Mo.
4. **Behki, R. M., and S. M. Lesley.** 1972. Deoxyribonucleic

acid degradation and the lethal effect by myxin in *Escherichia coli*. J. Bacteriol. **109**:250–261.
5. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology. Williams and Wilkins Co., Baltimore.
6. **Christensen, P. J.** 1974. A new approach to the description of colony colour of cytophagas and their allies. Can. J. Microbiol. **20**:937–942.
7. **Christensen, P. J.** 1977. Synonymy of *Flavobacterium pectinovorum* Dorey with *Cytophaga johnsonae* Stanier. Int. J. Syst. Bacteriol. **27**:122–132.
8. **Christensen, P. J., and F. D. Cook.** 1972. The isolation and enumeration of cytophagas. Can. J. Microbiol. **18**:1933–1940.
9. **Daft, M. J., and W. D. P. Stewart.** 1971. Bacterial pathogens of fresh-water blue-green algae. New Phytol. **70**:819–829.
10. **Damoglou, A. P., L. C. Allen, C. Roy, and D. R. Whitaker.** 1972. The β-lytic protease of myxobacter 495: aspects of its structure. Proc. Can. Fed. Biol. Soc. **15**:#27.
11. **Dworkin, M., and S. M. Gibson.** 1964. A system for studying microbial morphogenesis: rapid formation of microcysts in *Myxococcus xanthus*. Science **146**:243–244.
12. **Ensign, J. C., and R. S. Wolfe.** 1965. Lysis of bacterial cell walls by an enzyme isolated from a myxobacter. J. Bacteriol. **90**:395–402.
13. **Ensign, J. C., and R. S. Wolfe.** 1966. Characterization of a small proteolytic enzyme which lyses bacterial cell walls. J. Bacteriol. **91**:524–534.
14. **Gillespie, D. C., and F. D. Cook.** 1965. Extracellular enzymes from strains of *Sorangium*. Can. J. Microbiol. **11**:109–118.
15. **Hedges, A., and R. S. Wolfe.** 1974. Extracellular enzyme from myxobacter AL-1 that exhibits both B-1, 4-glucanase and chitosanase activities. J. Bacteriol. **120**:844–853.
16. **Jackson, R. L., and G. R. Matsueda.** 1970. Myxobacter AL-1 protease. Methods Enzymol. **19**:591–599.
17. **Jackson, R. L., and R. S. Wolfe.** 1968. Composition, properties and substrate specificities of Myxobacter AL-1 protease. J. Biol. Chem. **243**:879–888.
18. **Katznelson, H., D. C. Gillespie, and F. D. Cook.** 1964. Studies on the relationships between nematodes and other soil micro-organisms. III. Lytic action of soil myxobacters on certain species of nematodes. Can. J. Microbiol. **10**:699–704.
19. **Lesley, S. M., and R. M. Behki.** 1967. Mode of action of myxin on *E. coli*. J. Bacteriol. **94**:1837–1845.
20. **Lesley, S. M., and R. M. Behki.** 1971. Recovery of metabolic activity in *E. coli* following limited exposure to myxin. Can. J. Microbiol. **17**:1327–1333.
21. **Lesley, S. M., R. M. Behki, and D. C. Gillespie.** 1967. Production of radioactive myxin. Can. J. Microbiol. **13**:1251–1257.
22. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of DNA from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
23. **Martin, S. M., and V. So.** 1969. Solubilization of autoclaved feathers and wool by myxobacteria. Can. J. Microbiol. **15**:1393–1397.
24. **Matheson, A. T., and K. Mikulik.** 1972. The influence of growth conditions and isolation procedures on the proteolytic breakdown of ribosomal proteins from Myxobacter 495. Can. J. Microbiol. **18**:355–359.
25. **Pate, J. L., J. L. Johnson, and E. J. Ordal.** 1967. The fine structure of *Chondrococcus columnaris*. II. Structure and formation of rhapidosomes. J. Cell. Biol. **35**:15–35.
26. **Pate, J. L., and E. J. Ordal.** 1967. The fine structure of *Chondrococcus columnaris*. I. Structure and formation of mesosomes. J. Cell. Biol. **35**:1–14.
27. **Peterson, E. A., D. C. Gillespie, and F. D. Cook.** 1966. A wide-spectrum antibiotic produced by a species of *Sorangium*. Can. J. Microbiol. **12**:221–230.
28. **Peterson, E. A., H. Katznelson, and F. D. Cook.** 1965. The influence of chitin and myxobacters on numbers of actinomyctes in soil. Can. J. Microbiol. **2**:595–596.
29. **Sendecki, W., K. Mikulik, and A. T. Matheson.** 1971. Some properties of ribosomes from Myxobacter 495. Can. J. Biochem. **49**:1333–1339.
30. **Shilo, M.** 1970. Lysis of blue-green algae by myxobacter. J. Bacteriol. **104**:453–461.
31. **Sierra, G.** 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substances. Antonie van Leeuwenhoek J. Microbiol. Serol. **23**:15–22.
32. **Smit, M., and A. G. Clark.** 1971. The observation of myxobacterial fruiting bodies. J. Appl. Bacteriol. **34**:399–401.
33. **Soriano, S.** 1945. El nuevo orden Flexibacteriales y la clasificación de los órdenes de las bacterias. Rev. Argent. Agron. **12**:120–140.
34. **Stanier, R. Y.** 1957. Order VIII. *Myxobacterales* Jahn 1915, p. 854–891. *In* R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), Bergey's manual of determinative bacteriology, 7th ed. The Williams and Wilkins Co., Baltimore.
35. **Stewart, J. R., and R. M. Brown.** 1969. *Cytophaga* that kills or lyses algae. Science **164**:1523–1524.
36. **Stewart, J. R., and R. M. Brown.** 1971. Algicidal nonfruiting myxobacteria with high G+C ratios. Arch. Mikrobiol. **80**:176–190.
37. **Tan, I., W. Hartmann, U. Guntermann, A. Hüttermann, and H. Kühlwein.** 1974. Studies on the cell cycle of Myxobacter AL-1. I. Size fractionation of exponentially growing cells by zonal centrifugation. Arch. Mikrobiol. **100**:389–396.
38. **Whitaker, D. R.** 1965. Lytic enzymes of *Sorangium* sp.: isolation and enzymatic properties of the α- and β-proteases. Can. J. Biochem. **43**:1935–1954.
39. **Whitaker, D. R.** 1967. Simplified procedures for production and isolation of the bacteriolytic proteases of *Sorangium* sp. Can. J. Biochem. **45**:991–993.
40. **Whitaker, D. R.** 1970. The α-lytic protease of a myxobacterium. Methods Enzymol. **19**:599–613.
41. **Whitaker, D. R., F. D. Cook, and D. C. Gillespie.** 1965. Lytic enzymes of *Sorangium* sp. Some aspects of enzyme production in submerged culture. Can. J. Biochem. **43**:1927–1933.
42. **Whitaker, D. R., C. Roy, C. S. Tsai, and L. Jurášek.** 1965. Lytic enzymes of *Sorangium* sp. A comparison of the proteolytic properties of the α- and β-lytic proteases. Can. J. Biochem. **43**:1961–1970.