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Levinea, a New Genus of the Family *Enterobacteriaceae*

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A new genus of the family *Enterobacteriaceae* is proposed on the basis of its unique biochemical and serological properties. The genus, *Levinea* (named in honor of Max Levine), is composed of two different species, for which the names *Levinea amalonatica* and *Levinea malonatica* are proposed. *L. amalonatica* is designated as the type species. The type strain of *L. amalonatica* is 982_3 (=ATCC 25405) and the type strain of *L. malonatica* is 179_1 (=ATCC 25408). Information is given which distinguishes the proposed new genus from certain species of *Enterobacter* and *Citrobacter*, the genera which it most closely resembles. The similarity of *L. amalonatica* to organisms in the proposed new genus *Padlewskia* Macierewicz (9) is discussed.

The 1966 report of the *Enterobacteriaceae* Subcommittee to the International Committee on Nomenclature of Bacteria noted that the members present reaffirmed the statement that "... biochemically defined groups of the *Enterobacteriaceae* can be regarded as genera" (1). Since 1965 we recovered a number of strains of gram-negative bacteria from hospitalized patients which belong to the family *Enterobacteriaceae* but which distinctly differ from other genera of this family by a number of biochemical properties. They are also thus far serologically unrelated to the other genera. In the present paper the characteristics of this new genus of bacteria, which consists of two species, are described.

MATERIALS AND METHODS

Strains. One-hundred-and-eight strains were isolated from human urine, nose, sputum, and fecal samples submitted to the laboratory for investigation.

Media and Methods. The media and methods used to characterize the strains were basically those recommended in the 1958 Report of the International Subcommittee on the Taxonomy of the *Enterobacteriaceae* as revised by Ewing (5) and Edwards and Ewing (4). Sodium alginate medium (2) was also included.

RESULTS

Levinea gen. nov. *Levinea*, here proposed as a new genus of the family *Enterobacteriaceae*, has the following characteristics: all strains consist of gram-negative, motile rods with peritrichous flagella (Leifson's method, reference 8), as shown in Fig. 1 and 2, produce indole, give positive reactions in the methyl red test, utilize citrate (Simmon's) as a sole source of carbon, reduce nitrates to nitrites, and produce arginine dihydrolase and ornithine decarboxylase; they uniformly fail to produce acetylmethylcarbinol or oxidase, use sodium alginate as a sole carbon source, or produce H_2S in triple sugar iron agar, iron gelatin agar, or in cysteine blood agar as indicated by lead acetate paper; they do not produce phenylalanine deaminase or lysine decarboxylase (Table 1); glucose, rhamnose, arabinose, cellobiose, fructose, and sorbitol are fermented, and inositol, raffinose, and erythritol are not. Colonies produced by organisms in this genus resemble in general those produced by other members of the family *Enterobacteriaceae*. The colonies are grayish, 2 to 3 mm (average) in diameter, convex, opaque, and round with entire edges. The type species is *Levinea amalonatica*.

The generic name *Levinea* is proposed for these microorganisms to honor the late Max Levine, who made major contributions in the field of enteric bacteriology.

Levinea amalonatica sp. nov. *L. amalonatica*, in addition to possessing the characters of the genus as cited above, does not utilize malonate or adonitol, usually produces small amounts of urease in 1 to 6 days, usually ferments lactose in 1 to 4 days, and usually utilizes mucate; although all strains utilize glycerol, gas may or may not be produced; rare strains liquefy gelatin in 18 days; all strains grow in the

TABLE 1. *Biochemical reactions of Levinea amalonatica and Levinea malonatica*

Test or substrate	*L. amalonatica* type strain (ATCC 25405)	*L. amalonatica* (50 strains)		*L. malonatica* type strain (ATCC 25408)	*L. malonatica* (58 strains)	
		Reaction[a]	Per cent positive		Reaction[a]	Per cent positive
Indole[b]	+[c]	+	100	+	+	100
Methyl red[b]	+	+	100	+	+	100
Voges-Proskauer[b]	–	–	0	–	–	0
Citrate (Simmon's)	+	+	100	+	+	100
Hydrogen sulfide (TSI)	–	–	0	–	–	0
Urease	+	+(1–6)[i]	90	+	+(1–6) or –	77.6
Motility	+	+	100	+	+	100
Peritrichous flagella	+	+	100	+	+	100
Phenylalanine deaminase	–	–	0	–	–	0
Lysine decarboxylase	–	–	0	–	–	0
Arginine dihydrolase	+(3)	+(1–6)	100	+(4)	+(1–5)	100
Ornithine decarboxylase	+	+	100	+	+	100
KCN (growth)	+	+	100	+(4)	+(1–5) or –	75.9
Gelatin (Kohn's 22 C) liquefaction	–	–	8[d]	–	–	0
Nitrate reduction	+	+	100	+	+	100
Malonate utilization	–	–	0	+	+(1–2)	100
Glucose	AG	AG	100	AG	AG	100[e]
Lactose	AG(2)	AG(1–14)	92[g]	AG(4)	AG(1–6) or –	84.5[h]
Sucrose	–	–	0	–	–	8.6
Adonitol	–	–	0	AG	AG	100
Inositol	–	–	0	–	–	0
Rhamnose	AG	AG	100	AG	AG	100
Arabinose	AG	AG	100[k]	AG	AG	100
Raffinose	–	–	0	–	–	0
Glycerol acid	+(2)	+(1–4)	100	+	+	100
Gas	+(2)	+ or –	76	+	+	100
Cellobiose	AG	AG	100	AG	AG	100
Mannitol	AG	AG	100	AG	AG	98.3
Erythritol	–	–	0	–	–	0
Dulcitol	–	–	0	–	d[j]	44.8
Salicin	AG(6)	AG(1–6)	98[g]	AG(4)	AG(1–7)	98.3[f]
Aesculin	A	A	100	A	A	100
Fructose	AG	AG	100	AG	AG	100
Sorbitol	AG	AG	100	AG	AG	100
Organic acids						
Mucate	+	+	96	–	+ or –	75.9
Tartrate	–	–	0	–	–	0
Citrate	–	–	0	–	–	0
Sodium alginate	–	–	0	–	–	0
Oxidase	–	–	0	–	–	0

[a] All tests except gelatin liquefaction and oxidase observed for 14 days; gelatin kept for 21 days.
[b] Similar reactions obtained at 22 and 37 C.
[c] Reaction occurs in 1 or 2 days.
[d] Positive in 18 days.
[e] Two strains were anaerogenic.
[f] Three of the fermenting strains were anaerogenic.
[g] Seven of the fermenting strains were anaerogenic.
[h] Eleven of the fermenting strains were anaerogenic.
[i] Numbers within parentheses indicate days required for reaction to occur.
[j] Different biochemical types.
[k] One strain positive in 5 days.

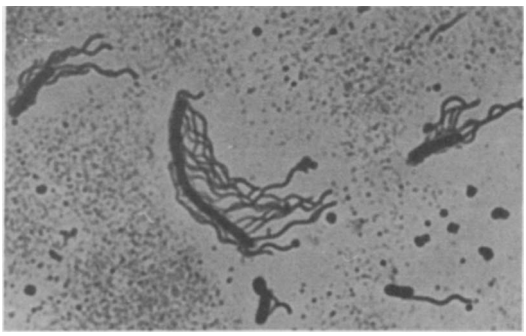

Fig. 1. *Peritrichous cells of Levinea amalonatica ATCC 25405. Although most of the cells are multitrichous, a few have a single, lateral flagellum. Twenty-four-hour broth culture at 26 C. Staining by Leifson's method. ×1,200.*

presence of KCN and do not ferment sucrose or dulcitol. Additional characteristics of this species are given in Table 1. The majority of the strains of this species were isolated from human feces. The type strain of *L. amalonatica* is 982_3 (= ATCC 25405). It was isolated from a human fecal sample and gives the reactions as shown in Table 1.

The specific epithet *amalonaticus* (Greek prefix *a,* not; Modern Latin adjective *malonaticus* pertaining to malonate) is a Modern Latin adjective meaning "not pertaining to malonate."

Levinea malonatica sp. nov. The name *Levinea malonatica* is proposed for the organisms in the genus *Levinea* which utilize malonate and adonitol, may or may not produce small amounts of urease or grow in KCN, and which may or may not ferment lactose or dulcitol and utilize mucate. A few strains ferment sucrose, but all strains ferment adonitol and produce gas as well as acid from glycerol.

Isolated approximately the same number of times from various sources, such as human feces, urine, nose, throat, and sputum. Found as pathogens in a number of urinary-tract infections and present in wounds on three occasions. Regarded as a usual inhabitant of the human intestinal tract.

The type strain of *L. malonatica* is 179_1 (= ATCC 25408). This strain was isolated from a human throat; its description is given in Table 1.

The specific epithet *malonaticus* is a Modern Latin adjective meaning "pertaining to malonate."

DISCUSSION

The reactions described for *Levinea* (Table 1) differ from those reported for hitherto described genera on the same set of characteristics although they do resemble somewhat those of certain species of *Enterobacter* and *Citrobacter.* Some of the reactions which can be used to differentiate these genera are shown in Table 2. The proposed new genus, *Levinea,* differs from both *Enterobacter* and *Citrobacter* in that *Levinea* produces indole and gives a positive methyl red test but does not produce acetylmethylcarbinol. *Levinea* also differs from *Enterobacter cloacae* in that gelatin is not liquefied (8% of the strains of *L. amalonatica* liquefied gelatin after 18 days of incubation), sucrose and raffinose are not fermented, and gas is usually produced from glycerol. Citric acid is not utilized by levineae, whereas *E. cloacae* in most instances does utilize this substrate. Aesculin is uniformly hydrolyzed by strains of the new genus, whereas hydrolysis is variable for strains of *Enterobacter cloacae. L. amalonatica* never utilized malonate while *E. cloacae* usually gives a positive reaction. *L. malonatica* differs from *E. cloacae* in that the

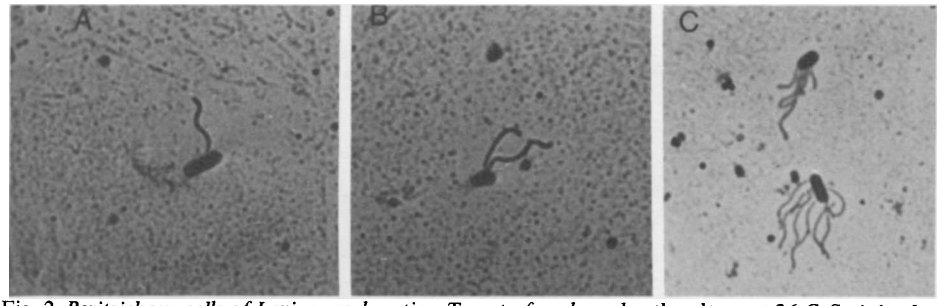

Fig. 2. *Peritrichous cells of Levinea malonatica. Twenty-four-hour broth culture at 26 C. Staining by Leifson's method. ×1,200. (A and B) ATCC 25408. The cells of this strain generally have a single, lateral flagellum (A); occasionally two or more flagella are evident (B). (C) ATCC 25410. The cells of this strain are predominantly multitrichous.*

TABLE 2. *Some differentiating characteristics of Levinea amalonatica, Levinea malonatica, Enterobacter cloacae, and Citrobacter freundii*

Test or substrate	*Levinea amalonatica*[a]	*Levinea malonatica*[a]	*Enterobacter cloacae*[b]	*Citrobacter freundii*[c]
Indole	+[d,e]	+[d,e]	–	–
Methyl red	+[d,e]	+[d,e]	–	–
Voges-Proskauer	–[d,e]	–[d,e]	+	–
Hydrogen sulfide (TSI)	–	–	–	+(1–7) or –
Urease	+(1–6)[f]	+(1–6) or –	+ or –	+(1–7) or –
Arginine dihydrolase	+(1–6)	+(1–5)	+	+(1–7) or –
Ornithine decarboxylase	+	+	+	– or +(1–7)
KCN (growth)	+	+(1–5) or –	+	+
Gelatin (Kohn's, 22 C)	–	–	+(1–>14)[g]	–
Malonate utilization	–	+	+ or –	– or +(1–7)
Lactose	AG(1–14)[h]	AG(1–6)[i] or –	AG	AG(1–>14)
Sucrose	–	–	AG	– or AG(1–>14)
Inositol	–	–	– or +(1–7)	–
Raffinose	–	–	AG	– or AG
Glycerol acid	+ or –	+	– or +(1–14)	+ or –
Gas	+ or –	+	– or +(1–14)	+ or –
Cellobiose acid	+	+	+	+(1–>14)
Gas	+	+	+	– or +(1–14)
Adonitol	–	AG	– or AG	–
Dulcitol	–	d[j]	– or +	+ or –
Salicin	AG(1–6)	AG(1–7)	AG(1–7)	– or AG(1–>14)
Aesculin	+	+	– or +	–
Organic acids				
Mucate	+	+ or –	+ or –	+
D-Tartrate	–	–	– or +	+(7–14)
Citrate	–	–	+ or –	+(1–7)

[a] All tests except gelatin observed for 14 days; gelatin kept for 21 days.
[b] According to Fife et al. (6).
[c] According to Davis and Ewing (3).
[d] Reaction occurs in 1 or 2 days.
[e] Similar reactions obtained at 37 and 22 C.
[f] Numbers within parentheses indicate days required for reaction to take place.
[g] Seventy-six per cent liquefy gelatin in 8 to 14 days.
[h] Seven strains produced no gas.
[i] Eleven strains produced no gas (excluding anaerogenic strains).
[j] Different biochemical types.

former ferments adonitol, whereas the latter is most often negative.

The significant characteristics of the levineae which distinguish them from the citrobacters (particularly *Citrobacter freundii*) are the lack of H_2S production in triple sugar iron agar, the ability to hydrolyze aesculin, and the inability to utilize citrate and D-tartrate (Kauffmann and Petersen's medium, reference 7). Levineae always produce arginine dihydrolase, and ornithine decarboxylase, whereas citrobacters vary in the production of these enzymes. Salicin fermentation and gas formation in cellobiose by strains of the proposed genus were constant as opposed to an occasionally positive reaction by *Citrobacter*, and *L. malonatica* produces acid and gas from adonitol whereas *C. freundii* does not. As for the other species, *L. amalonatica* does not ferment dulcitol, and *C. freundii* usually ferments this substrate.

The biochemical reactions of *C. freundii* as described by Davis and Ewing (3) broaden the genus *Citrobacter* to include a number of strains which fail to produce H_2S in triple sugar iron agar, give positive indole reactions, etc. It is possible that some of the strains described therein are the same as those described in this paper as *L. amalonatica*. A comparison of the characteristics of *L. malonatica* and *L. amalonatica* with one another and with *C. freundii* as

originally described, as well as the rather large number of "aberrant" strains, led the authors to the conclusion that the levineae indeed comprise a new genus bearing a close resemblance to one another in their biochemical reactions.

Levineae also share a number of biochemical characteristics with "atypical *Enterobacter cloacae*" described by Young et al. (Bacteriol. Proc., p. 106, 1968) and further discussed by Washington et al. (10). Although the latter authors believed that the number of biochemical reactions shared with *E. cloacae* should qualify the new group as a separate species within the genus *Enterobacter*, further tests performed by the authors since the report by Young et al. demonstrate differences that would require a redefinition of *Enterobacter* to include them. It does not seem suitable to us, therefore, to broaden the generic description to include reactions that would not allow distinct differentiation.

To test further the validity of our proposal of a new genus and to determine the specificity of the antigens within the new genus *Levinea*, antisera were prepared in rabbits (which had no detectable preinoculation antibodies) against a representative strain of each of the two species. Twenty-four heat-killed antigens (12 representatives of each species) were also made for use in performing agglutination tests. These antigens were tested against all *Shigella*, *Salmonella*, and *Escherichia coli* grouping sera (serotypes 1 to 140), and no evidence of antigenic similarity was obtained. Antigens made from representative strains of the various groups of *Salmonella* and *Shigella* as well as from 5 *Klebsiella* sp., 10 *E. coli* serotypes, *Proteus morganii*, *Proteus rettgeri*, *Proteus mirabilis*, and *Proteus vulgaris*, 1 strain of *Enterobacter aerogenes* subsp. *alvei* (6), 6 strains of *E. cloacae*, and 6 strains of *C. freundii*, including 3 which were H_2S negative in triple sugar iron agar, and indole positive (obtained through the courtesy of W. H. Ewing, NCDC, Atlanta, Ga.), were also tested in *Levinea* antisera. No agglutination was obtained with any of these antigens. However, when the antigens prepared from *L. amalonatica* were tested against sera prepared from a strain within this species, 46% of the strains gave a titer level of 640 or higher, 5% of which were 20,480 or above. None of the *L. amalonatica* strains had a titer less than 80, indicating that they share a common group antigen. This antiserum also agglutinated 12% of *L. malonatica* strains to a dilution of 640 or higher. Antiserum to *L. malonatica* was tested against all other strains in the same group, and 31.9% gave titer levels of 640 or higher. There were 6 strains which had a titer level less than 40, and 15 more which had a titer level of only 40 in the antiserum prepared against the representative strain, indicating that *L. malonatica* was less homogenous as a group. This antiserum also gave titers of 640 or higher with 11.3% of the *L. amalonatica* strains although most of the strains gave titers of 160 or less in the *L. malonatica* antisera and a few were negative (<40). These results indicate antigenic relatedness between the two species.

A group of 27 strains of microorganisms, similar to, if not identical with, *L. amalonatica* was described by Macierewicz (9). The name "Padlewskia" was proposed for these strains. However, Macierewicz obtained 4 positive sucrose reactions out of 27, whereas we obtained none for the malonate-negative strains; urease production was reported as negative by Macierewicz using a liquid urea medium "according to Christensen as modified by Hormaeche and Munilla," and we demonstrated urease to be formed in 1 to 6 days (usually 1 to 2), though not so strongly as by *Proteus* species, on Christensen's urea agar slants. There were also differences in the following: 1 or 2 of the 27 strains reported by Macierewicz failed to produce arginine dihydrolase or ferment rhamnose and sorbitol; 1 strain was positive in raffinose. Consistent (100%) results were given by *L. amalonatica*. Furthermore, three of the strains studied by Macierewicz failed to produce gas from glucose and cellobiose, whereas all *L. amalonatica* strains produced gas from these carbohydrates. A reasonable doubt can be entertained as to whether these two groups are indeed identical (the authors were unsuccessful in establishing personal communication) as the strains of *L. amalonatica* have a precise biochemical reaction pattern. This was not accomplished through elimination of those strains which did not conform to the typical reactions, as all strains which gave the IMVIC, phenylalanine, lysine, arginine, and ornithine reactions typical of the proposed genus were included in the study.

ACKNOWLEDGMENT

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ADDENDUM

Since submitting this manuscript, a personal communication was received from W. Frederiksen, Statens Seruminstitut, Copenhagen, Denmark, informing the

authors that he has published a paper describing an organism which appears to be identical to *Levinea malonatica* (Publ. Fac. Univ. J. E. Purkyne, Brne, 1970, K:89–93).

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