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Leclercia adecarboxylata Gen. Nov., Comb. Nov., Formerly Known as *Escherichia adecarboxylata*

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Abstract. The name *Leclercia adecarboxylata* is proposed for a group of the family Enterobacteriaceae previously known as *Escherichia adecarboxylata*. *Leclercia adecarboxylata* can be phenotypically differentiated from all other species of Enterobacteriaceae. The members of this species are positive for motility, indole production, methyl red, growth in the presence of KCN, malonate, beta-galactosidase, beta-xylosidase, esculin hydrolysis, gas production from D-glucose, and acid production from D-cellobiose, D-lactose, melibiose, L-rhamnose, adonitol, D-arabitol, dulcitol, and salicin; the strains were negative for Voges-Proskauer, citrate (Simmons), H_2S (Kligler), lysine and ornithine decarboxylases, arginine dihydrolase, phenylalanine deaminase, gelatinase, DNase, Tween-80 hydrolysis, and acid production from myoinositol and alpha-methyl-D-glucoside. Fermentation of D-raffinose, D-sucrose, and D-sorbitol is variable with strains. DNA relatedness of 11 strains of *L. adecarboxylata* to three strains including the type strain of this species averaged 80% in reactions at 65°C. DNA relatedness to other species in Enterobacteriaceae was 2%–32%, indicating that this species was placed in a new genus *Leclercia* gen. nov. The type strain of *L. adecarboxylata* is ATCC 23216.

Escherichia adecarboxylata was the name proposed by Leclerc [14] in 1962 for a group of yellow-pigmented organisms resembling *Escherichia coli* in the IMViC test. Ewing and Fife [6] described *E. adecarboxylata* as a synonym of *Enterobacter agglomerans* and included it in biogroup C3 of the latter. On the other hand, *E. agglomerans* is very heterogeneous as shown by Brenner [1] in DNA hybridization studies and by Farmer et al. [8] in biochemical reactions. Since that makes precise identification of *E. agglomerans* difficult, yellow-pigmented cultures that are negative for lysine and ornithine decarboxylases and arginine dihydrolase are tentatively identified as *E. agglomerans*, and *E. adecarboxylata* has received little attention. However, *E. adecarboxylata* is a legitimate and valid species standing in nomenclature and the name is conserved in the Approved Lists of Bacterial Names [19]. Most of strains of *E. adecarboxylata* that were first studied by Leclerc were isolated from foods, but these authors have found that the species is not rare among clinical isolates.

Between 1970 and 1983, the Enterobacteriology Laboratory at the National Institute of Health in Tokyo received a large number of strains that were initially determined to be *Enterobacter agglomerans* by a commercial packed identification system or a conventional method, or that did not belong to any described species of Enterobacteriaceae. Those strains have been carefully characterized again by conventional biochemical methods. The application of the computer-associated identification bases on a probability matrix has resulted in the identification of 86 strains as *Escherichia adecarboxylata* with a value of 0.01–0.95 of likelihood and more than 0.9 of the identification score. Extensive biochemical tests and DNA hybridization studies on these strains substantiated the computer identification and indicated that *E. adecarboxylata* belongs to a new genus in the family Enterobacteriaceae for which a generic name *Leclercia* is proposed. In this report, *Leclercia adecarboxylata* is characterized biochemically and genetically.

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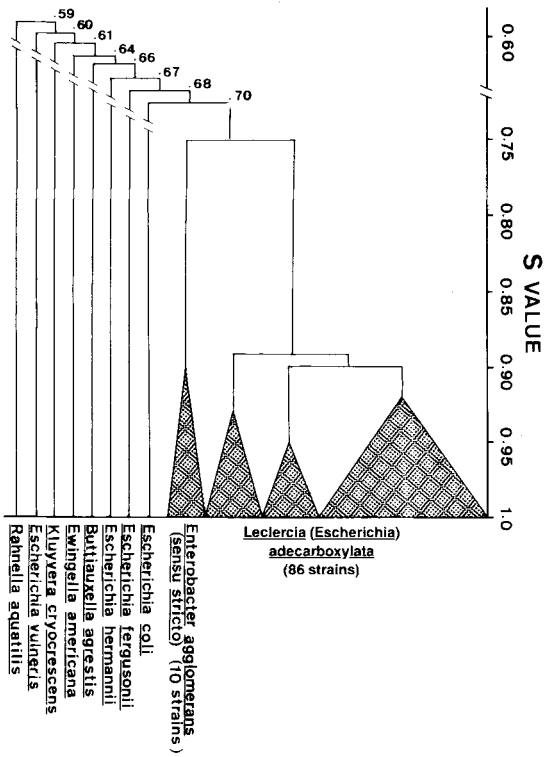

Fig. 1. Numerical analysis of phenotypic characteristics of *Escherichia adecarboxylata* and other species in Enterobacteriaceae.

Materials and Methods

Bacterial strains. A total of 86 strains consisting of 58 isolates from clinical specimens including blood, sputum, urine, stool, and wounds; 27 from food, water, and the environment; and ATCC 23216, which is a type strain of *E. adecarboxylata*, were studied. The strains of *E. adecarboxylata* employed in DNA studies are listed in Table 2. The 86 strains from which phenotypic and source data were obtained are not listed individually. All strains were maintained in semisolid nutrient agar at room temperature.

Phenotypic characterization. Besides morphology, motility, and gram reaction, 30 biochemical tests were used for the preliminary characterization of cultures. They included: oxidase, H_2S (Kligler), Voges-Proskauer, indole, citrate (Simmons), urease (Christensen), phenylalanine deaminase, lysine and ornithine decarboxylases, arginine dihydrolase, Tween-80 hydrolysis, beta-galactosidase, beta-xylosidase, esculin hydrolysis, and acid production from L-arabinose, D-cellobiose, D-melibiose, D-raffinose, L-rhamnose, D-sucrose, D-trehalose, D-xylose, adonitol, D-arabitol, dulcitol, D-mannitol, D-sorbitol, myoinositol, alpha-methyl-D-glucoside, and salicin.

For the numerical analysis of phenotypic characteristics of the 86 strains selected subsequently, an additional 200 features were tested: flagellation; colony morphology; growth at 4° and 42°C; yellow pigmentation; citrate (Christensen and Kauffmann and Petersen); *d*- and *l*-tartrate (Kauffmann and Petersen); nitrate reduction to nitrite; growth in the presence of KCN; deoxyribonuclease; gelatinase; tetrathionate reductase; pectinase; beta-glucuronidase; gas production from D-glucose; acid production from D-arabinose, D-fucose, L-fucose, D-galactose, beta-gentibiose, D-lactose, D-levulose, D-lyxose, D-maltose, D-mannose, D-melezitose, ribose, L-sorbose, D-tagatose, D-trehalose, D-turanose, L-lyxose, L-arabitol, erythritol, glycerol, xylitol, amygdalin, arbutin, gluconate, 2-ketogluconate, 5-ketogluconate, and mucate; susceptibility to penicillin, ampicillin, carbenicillin, cephaloridin, gentamicin, kanamycin, streptomycin, tetracycline, chloramphenicol, colistin, nalidixic acid, and sulfadiazine; and assimilation of 49 each of carbohydrates, amino acids, and organic acids.

Methods used for biochemical testing were those described by Edwards and Ewing [5] and Cowan [4]. Beta-xylosidase and beta-glucuronidase were tested using a method described by Killian and Bulow [12]. Pigmentation was determined by growth on Isosensi test agar (Oxoid) after seven days. Susceptibility testing was performed by the Kirby-Bauer method with Sensi-discs (BBL). API 50CH, API 50AA, and API 50AO strips (API System) were used for assimilation tests. All tests were carried out at 35°C and results were read after 48 h of incubation unless otherwise stated.

After the additional 200 features were tested, all phenotypic data were coded in binary notation and computations were performed. The similarity between each pair of strains was calculated by Jaccard coefficient. The coefficients were subsequently clustered by single linkage analysis. For the numerical analysis of phenotypic characteristics of the 86 *E. adecarboxylata* strains, ten strains of *E. agglomerans* and eight of other species of Enterobacteriaceae listed in Fig. 1 were also involved.

DNA hybridization and base composition. DNA hybridization was carried out by methods described previously [13]. Unlabeled DNA was extracted and purified as described by Marmur [15] and Marmur and Doty [16]. DNA from three strains of *E. adecarboxylata* and one of *E. agglomerans* were radiolabeled by the in vitro nick translation method using a commercial reagent kit (catalog no. NEN-005, New England Nuclear). The relatedness of labeled DNA from the four strains to unlabeled DNA from 11 strains of *E. adecarboxylata* and stock DNA from 18 strains of listed species of Enterobacteriaceae (Table 2) was determined on nitrocellulose membrane filters by using the technique described by Johnson [11].

The base composition of DNA from 11 *E. adecarboxylata* strains was determined from the thermal denaturation temperature by the method described by Owen et al. [17].

Results

Phenotypic characterization. Table 1 presents characteristics of the 86 strains that are commonly applied to classify Enterobacteriaceae.

All strains were gram-negative, motile rods with peritrichous flagellation and utilized the following substrates as a sole carbon source: DL-lactate, L-malate, pyruvate, 2-ketoglutarate, D-alpha-alanine, L-alpha-alanine, L-serin, L-histidine, L-aspartate, L-glutamate, L-proline, glucosamine, L-arabinose, ribose, D-xylose, mucate, D-galactose,

Table 1. Phenotypic characteristics of *Leclercia* (*Escherichia*) *adecarboxylata*[a]

Test (substrate)	% Positive of 85 strain[b]	Reaction for type strain[c]
Motility (37°C)	98.8	+
Growth at 4°C (14 days)	49.4	+
at 42°C	0	−
Pigmentation (yellow) (7 days)	14.1	+
Oxidase	0	−
Indole	100	+
Methyl-red	100	+
Voges-Proskauer	0	−
Citrate (Simmons)	0	−
Citrate (Christensen)	0	−
Hydrogen sulfide (Kligler)	0	−
Lysine decarboxylase	0	−
Arginine dihydrolase	0	−
Ornithine decarboxylase	0	−
Phenylalnine deaminase	0	−
Urease (Christensen)	10	−
Nitrate to nitrite	98.8	+
Malonate	100	+
Gelatin liquefaction	0	−
Tween-80 hydrolysis	0	−
DNase (25°C)	0	−
KCN, growth in	100	+
D-Tartrate (Kauffmann-Petersen)	0	−
Citrate (Kauffmann-Petersen)	0	−
Acetate	0	−
Pectinase	0	−
Beta-galactosidase	100	+
Beta-xylosidase	100	+
Beta-glucuronidase	0	−
Esculin hydrolysis	100	+
Mucate	65	+
Tetrathionate reductase	54.1	+
D-Glucose, acid	100	+
D-Glucose, gas	98.8	+
Acid from:		
L-Arabinose	100	+
Cellobiose	100	+
Beta-gentibiose	100	+
Lactose	100	+
Maltose	100	+
Melibiose	100	+
Melezitose	0	−
Raffinose	58.8	+
L-Rhamnose	100	+
Sorbose	0	−
Sucrose	51.8	+
Trehalose	100	+
D-Xylose	100	+
Adonitol	97.6	+
D-Arabitol	96.4	+
Dulcitol	87.1	+
Erythritol	0	−
Myoinositol	0	−
D-Mannitol	100	+
D-Sorbitol	9.4	−
Arbutin	100	+
Alpha-methyl-D-glucoside	0	−
Salicin	100	+
Gluconate	100	+
2-Ketogluconate	100	+
5-Ketogluconate	0	−

[a] All the strains tested are positive for acid production from D-galactose, D-levulose, D-mannose, and ribose; and negative for D-arabinose, L-xylose, methylmannoside, methylxyloside, amygdalin, xylitol, D-turanose, D-tagatose, D-lyxose, D-fucose, L-fucose, and L-arabitol.

[b] The value given is for 48 h of incubation at 35°C, unless otherwise indicated.

[c] +, positive reaction within 48 h; and −, negative reaction after four days.

D-glucose, D-levulose, D-mannose, L-rhamnose, D-mannitol, arbutin, esculin, salicin, cellobiose, D-maltose, D-melibiose, D-trehalose, gluconate, and 2-ketogluconate.

None of the 86 strains could utilize the following substrates as a sole carbon source: propionate, butyrate, isobutyrate, *n*-valerate, *n*-caproate, heptanonate, caprylate, peragonate, caprate, oxalate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, glycolate, DL-hydroxybutyrate, D-tartrate, mesotartrate, levulinate, citraconate, itaconate, mesaconate, aconitate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *o*-hydroxybenzoate, D-mandalate, L-mandalate, phthalate, isophthalate, triphthalate, glycin, L-leucine, L-isoleucin, L-norleucine, L-valine, DL-norvaline, DL-2-aminobutyrate, L-threonine, L-cysteine, L-methionate, L-phenylalanine, L-tyrosine, D-tryptophane, L-tryptophane, trigonelline, L-ornithine, L-lysin, L-citrulline, L-arginine, DL-kynurenine, putrescine, creatine, beta-alanine, DL-3-amino butyrate, DL-4-amino butyrate, DL-5-amino butyrate, 2-amino benzoate, 3-amino benzoate, 4-amino benzoate, urea, acetamide, sarcosine, ethylamine, butylamine, amylamine, ethanolamine, benzylamine, diaminobutane, spermine, histamine, tryptamine, erythritol, D-arabitol, L-xylose, L-sorbose, myoinositol, methylmannoside, methylxyloside, alpha-methyl-D-glucoside, D-melezitose, xylitol, D-turanose, D-tagatose, D-fucose, L-fucose, L-arabitol, and 5-ketogluconate.

Most of the 86 strains assimilated the following substrates: succinate, D-malate, adonitol, dulcitol, D-lactose, beta-gentibiose, and D-arabitol. Assimi-

Table 2. Intra- and extra-species DNA relatedness of *Leclercia*

Strain		Labeled DNA		
		ATCC 23216	301	886-76
L. adecarboxylata	ATCC 23216	100	100	82.3
886-76		85.8	100	100
301		86.4	100	78.7
1523		66.2	89.2	78.9
302		77.4	94.4	93.9
3015		82.6	86.3	72.3
171		87.2	100	80.6
363		64.2	72.8	62.1
E 396		83.0	84.3	75.2
884-76		78.8	80.5	78.7
887-76		75.2	80.6	77.4
E. agglomerans	ATCC 27155	32.0	27.6	30.5
B. agrestis	JCM 1090	12.4	9.5	8.7
C. lapagei	CDC 485-76	7.8	10.6	9.0
C. davisae	CDC 3278-77	9.3	7.4	11.3
C. neteri	ATCC 33856	11.5	8.3	12.4
C. freundii	ATCC 8090	13.8	15.6	11.6
C. amalonaticus	ATCC 25405	12.4	10.4	12.5
C. diversus	ATCC 27156	28.5	30.4	27.6
E. coli	ATCC 11775	26.2	29.7	31.3
E. hermannii	ATCC 33650	8.6	12.4	9.7
E. vulneris	CDC 875-72	7.5	9.2	8.0
E. furgusonii	CDC 1016-74	9.3	14.2	8.1
E. amnigenus	JCM 1237	4.6	7.8	5.4
E. intermedium	ATCC 33110	5.7	8.5	8.1
E. cloacae	ATCC 13047	5.3	4.6	3.4
E. taylorae	CDC 2126-81	11.3	12.5	12.2
E. americana	ATCC 33852	4.5	3.3	2.8
M. wiscinsensis	CDC 3065-75	3.4	4.9	2.1
R. aquatilis	ATCC 33071	3.2	2.0	5.3
Y. regensburgei	JCM 2403	6.9	7.5	6.8

lation of fumarate, DL-glycerate, phenylacetate, D-sorbitol, amygdalin, D-sucrose, D-raffinose, and D-lyxose was variable with strains. Few strains utilized *l*-tartrate.

All strains were susceptible to ampicillin, carbenicillin, cephaloridine, gentamicin, kanamycin, colistin, and nalidixic acid, but resistant to penicillin. With few exceptions, the 86 strains were also susceptible to streptomycin, tetracycline, chloramphenicol, and sulfadiazin.

Of a total 230 features tested for numerical analysis of phenotypic characters, 98 were found to be significant and subsequent computation was performed with these number of features. The results of the computation and clustering by single linkage analysis are shown in Fig. 1. The 86 strains of *E. adecarboxylata* formed a cluster (cluster 1) at a 90% level of similarity and the cluster was further divided into three phenons at a similarity level of 92%–95%. Cluster 2 consisted of eight cultures of *E. agglomerans*. The similarity value between clusters 1 and 2 was less than 75%. Also, similarities between cluster 1 and other species compared were less than 70%.

DNA hybridization. DNA from three strains of *E. adecarboxylata* that were representatives of the three phenons mentioned above were radiolabeled and tested for relatedness to those from other strains of each phenon and to those from representatives of other species of Enterobacteriaceae. The results are presented in Table 2. The degree of reassociation between each of labeled DNAs from the three strains (ATCC 23216, NIH 358-72, and NIH 886-76) and unlabeled DNAs from other strains of cluster 1 ranged from 64.2% to 100% at 65°C. In reactions at 75°C, labeled 358-72 DNA showed an average of 85% relatedness (range 71%–99%). When relatedness between DNA from *E. adecarboxylata* strains and DNA from the type strain of *E. agglomerans* (ATCC 27155) was tested, the degree of reassociation was 9.2%–30.4% at 65°C. On the other hand, ATCC 23216 exhibited 3.2%–32.0% relative binding with other species tested.

Base composition of DNA. The G + C contents of DNA from 11 strains of *E. adecarboxylata* ranged from 52.4 to 54.8 mol%.

Discussion

Phenotypic and DNA relatedness data mentioned above indicate that *Escherichia adecarboxylata* is a separate taxon not only from *Enterobacter agglomerans* in a strict sense [18], but also from other taxa of the family Enterobacteriaceae. It is distinguishable from other species by using ordinary biochemical tests, as shown in Table 3. As mentioned in the previous section, the name *Escherichia adecarboxylata* is preserved with the type strain in the Approved Lists of Bacterial Names, but it is clear from the DNA relatedness data that this species is assigned to a new genus, but not to genera *Escherichia* nor *Enterobacter*.

For the classification of recently reported species *Escherichia hermannii* and *Escherichia vulneris*, Brenner et al. [2, 3] chose the genus *Es-*

cherichia because of the relative similarity of their phenotypic characteristics to those of *Escherichia coli*, though those two species showed less than 50% relatedness of DNA and a new genus was considered for each of them. A similar choice was considered for the classification of *Enterobacter sakazakii* by Farmer et al. [7], who emphasized that they hesitate to create a new genus for a single species that can be readily identified within the confines of an existing genus. Gavini et al. [9, 10] also reported that *E. adecarboxylata* is an independent species of *E. coli, E. agglomerans, E. sakazakii* and *Rahnella aquatilis*. However, they did not decide to create a new genus for this species.

There is a similar problem to that in those species mentioned above on the classification of *E. adecarboxylata*. It is indole and methyl red positive and Voges-Proskauer and citrate negative. It ferments lactose with gas production. These characteristics are the same as those of *E. coli*, and *E. adecarboxylata* may be confused with the latter species in medical and food microbiology laboratories. Teramoto and Sakazaki [20] reported frequent isolation of *E. adecarboxylata* from food and environment materials. In their study, 36% of coliform organisms giving a ++−− reaction in the IMViC system were *E. adecarboxylata*, while less than 0.5% of those coliforms were identified as *E. hermannii* and *E. fergusonii*. For its relative importance in food microbiology, the present authors decided to assign *E. adecarboxylata* to a new genus, *Leclercia*. The generic name *Leclercia* was formed by adding the Latin ending *ia* to the surname of H. Leclerc, a French bacteriologist. It honors his contribution to enteric bacteriology. He first reported this organism and gave the name *Escherichia adecarboxylata*.

Description of the genus *Leclercia* and *L. adecarboxylata*: The proposed genus *Leclercia* is presently monospecific and the description for the genus is the same as that for species *L. adecarboxylata* comb. nov. A complete description of *L. adecarboxylata* is given in Tables 1–3. The type strain is ATCC 23216.

Although the clinical significance of *L. adecarboxylata* was uncertain, 58 of the 86 strains studied here were isolated from blood (18), sputum (27), urine (5), stool (2), and wounds (2). It may be often found in foods. Since it forms colonies resembling *E. coli* on MacConkey medium, desoxycholate–lactose, and eosin–methylene blue agars and gives a ++−− reaction in the IMViC system, it may be confused with *E. coli* or not further investigated.

Table 3. Differential characteristics of *Leclercia adecarboxylata* from other species in Enterobacteriaceae

Test (substrate)	% Positive[a]
Indole	100
Voges-Proskauer	0
Citrate (Simmons)	0
Lysine decarboxylase	0
Arginine dihydrolase	0
Ornithine decarboxylase	0
Malonate	100
Growth in KCN	100
H_2S (Kligler)	0
Gas from D-glucose	98.8
Acid from:	
D-Cellobiose	100
D-Lactose	100
D-Melibiose	100
L-Rhamnose	100
Adonitol	97.6
D-Arabitol	96.4
D-Sorbitol	9.4
Alpha-methyl-D-glucoside	0
Esculin hydrolysis	100
Beta-xylosidase	100
Beta-glucuronidase	0

[a] Percent positive within 48 h at 35°C.

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