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Klebsiella ornithinolytica sp. nov., Formerly Known as Ornithine-positive *Klebsiella oxytoca*

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Abstract. The name *Klebsiella ornithinolytica* sp. nov. is proposed for a group of *Klebsiella* strains referred to previously as NIH Group 12 at the National Institute of Health, Tokyo. The members of this species are Gram-negative, encapsulated, nonmotile rods with the general characteristics of the family *Enterobacteriaceae* and of the genus *Klebsiella*. They give positive results in tests for indole production, Voges-Proskauer, citrate utilization, lysine and ornithine decarboxylases, urease, β-galactosidase, malonate utilization, growth in KCN, and esculin hydrolysis, and they produce acid and gas from D-glucose, and acid from L-arabinose, cellobiose, lactose, melibiose, raffinose, rhamnose, sucrose, trehalose, D-xylose, adonitol, D-arabitol, myo-inositol, sorbitol, arbutin, salicin, α-methyl-D-glucoside, and mucate. They give negative results for arginine dihydrolase, phenylalanine deaminase, gelatin liquefaction, Tween 80 hydrolysis, DNase, pectinase, and acid production from D-arabinose, melezitose, and dulcitol. They can grow at 4°C and 42°C, and do not produce any pigment. DNA relatedness of eight strains of *Klebsiella ornithinolytica* to three strains including the type strain of this species averaged 88% in reaction at 75°C. DNA relatedness to the already recognized *Klebsiella* species in *Enterobacteriaceae* was 1 to 20%. Phenotypic and DNA relatedness data also indicated that a group of organisms referred to as Enteric Group 47 or *Klebsiella* Group 47 at the Centers for Disease Control (Atlanta, Georgia) was identical with *K. ornithinolytica*. The type strain of *K. ornithinolytica* is NIH 90-72 (JCM 6096).

Two cultures, first received at the National Institute of Health, Tokyo, in 1972 as ornithine-positive strains of *Klebsiella oxytoca*, were later found to differ from *K. oxytoca* in giving negative reactions in tests for gelatinase and pectinase, and in failing to produce a soluble brown pigment on triple sugar iron agar slopes which are characteristic features of *K. oxytoca*. Subsequently, these organisms were referred to as NIH Group 12. About the same time, at the Centers for Disease Control (Atlanta, Georgia), similar strains at first called Enteric Group 47 and later changed to *Klebsiella* group 47 were examined [3]. Subsequently, further cultures of NIH Group 12 were received, and a collection of 20 strains at this laboratory was submitted to intensive biochemical and DNA hybridization studies. The results suggest that strains of NIH Group 12 and *Klebsiella* Group 47 represent a single new species in the genus *Klebsiella* for which the name *Klebsiella ornithinolytica* is proposed. In this paper, we describe the biochemical and genetic characteristic of *K. ornithinolytica*.

Materials and Methods

Bacterial strains. In total, 21 strains comprising 20 of NIH Group 12 and one of *Klebsiella* Group 47 were studied (Table 1). The strains employed in DNA studies are listed in Tables 3 and 4. All strains were maintained at room temperature in semisolid medium containing 0.3% yeast extract, 1.0% Bacto-Casitone, 0.5% NaCl, and 0.3% agar (pH 7.0).

Phenotypic characterization. In addition to morphology and Gram reaction, 115 phenotypic features were tested. They included 73 characteristics listed in Table 2, assimilation of carbon compounds, and susceptibility to penicillin, ampicillin, carbenicillin, cephaloridin, gentamicin, kanamycin, streptomycin, tetracycline, chloramphenicol, colistin, nalidixic acid, and sulfadiazine. The following 29 carbon compounds were used for assimilation tests: aconitate, D-α-alanine, L-α-alanine, 5-aminovalerate, L-arginine, benzoate, caprate, caproate, capry-

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Table 1. *K. ornithinolytica* strains studies

Strain	Source
90-72[a]	Urine
109-72	Pus
218-74	Urine
981-74	Throat
1008-74	Sputum
295-77	Urine
1219-77	Sputum
328-78	Food
1094-79	Stool
132-81	Urine
1002-81	Food
491-82	Sputum
40-84	Throat
581-84	Food
932-84	Urine
221-85	Urine
828-85	Urine
1215-85	Stool
118-86	Sputum
1001-86	Throat
7478-76[b]	CDC

[a] Type strain; JCM 6096.
[b] *Klebsiella* group 47; CDC, Centers for Disease Control, Atlanta, Georgia.

late, fumarate, L-glutamate, glutarate, heptanonate, L-histidine, DL-3-hydroxybutyrate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, itaconate, DL-lactate, D-malate, L-proline, propionate, putrescine, quinate, L-serine, suberate, L-threonine, L-tyrosine, and valerate.

Biochemical tests were carried out by the methods described by Ewing [2] and Cowan [1]. Growth on IsoSensi test agar (Oxoid) was examined for 7 days for yellow pigmentation. Gluconate agar slopes incubated for 7 days at room temperature were also observed for the production of soluble brown pigment as described by Korth et al. [6]. The method described by Killian and Bülow was used for β-xylosidase [5]. M70 minimal medium without growth factor [9] and supplemented with 0.1% carbon compound was used for the carbon source utilization tests; the results were read after incubation for 4 days. Antimicrobial susceptibility tests were performed by the Kirby-Bauer method with Sensi-discs (BBL).

Extraction of DNA. The cells from a culture that was in the mid-log phase growth in brain heart infusion broth at 30°C were centrifuged and washed with saline–EDTA (0.15 *M* NaCl and 0.1 *M* ethylenediaminetetraacetate). The packed cells were resuspended in a volume of the same solution equal to about one-tenth the volume of the original culture. Lysis of the cells was accomplished by adding sodium lauryl sulfate (SLS) to give a final concentration of 1% (vol/vol) in a 60°C water bath. An equal volume of water-saturated phenol was added to the cell lysate, and the resulting aqueous layer, separated by centrifugation at 12,000 *g* for 10 min at 5°C, was carefully collected. Two volumes of cold 95% ethanol were added to the aqueous phase. The precipitated DNA was then spooled onto a glass stirring rod and dissolved in 10 ml of SSC (0.15 *M* NaCl and 0.015 *M* trisodium citrate). RNase was added to the DNA solution, and the mixture was incubated at 37°C for 30 min followed by addition of an equal volume of water-saturated phenol to extract the DNA again. After repeating the precipitation with ethanol, dissolution in SSC, and extraction with phenol several times, the DNA was finally dissolved in SSC. Purity and concentration of the DNA preparation were assayed spectrophotometrically as described previously [7].

G + C content of DNA. The guanine plus cytocine content of DNA from eight strains of *K. ornithinolytica* was determined by the melting temperature method of Owen et al. [8].

DNA hybridization. DNA hybridization was carried out by methods described previously [7]. DNAs from three strains of *K. ornithinolytica*, and one each of *K. planticola*, *K. pneumoniae*, *K. oxytoca*, and *K. terrigena* were radiolabeled by the in vitro nick translation method with a commercial kit (product code TRK.625 and N.5500A, Amersham, England). The relatedness of labeled DNA from these eight strains to unlabeled DNA from nine other strains of *K. ornithinolytica* and stock DNA from 41 strains of *Enterobacteriaceae* listed in Tables 3 and 4 was determined on nitrocellulose membrane filters by the technique described by Johnson [4]. Reaction was carried out both at 65°C, where optimum reassociation occurs, and at 75°C, where only closely related sequences reassociate.

Results and Discussion

Phenotypic characterization. Table 2 shows the results given by the 20 strains of NIH Group 12 and of one strain of *Klebsiella* Group 47 to 73 tests that are commonly used for the identification of *Enterobacteriaceae*.

All 21 strains were Gram-negative, encapsulated, nonmotile rods. They utilized the following substrates as sole sources of carbon: aconitate, D-α-alanine, L-α-alanine, 5-aminovalerate, caproate, fumarate, L-glutamate, L-histidine, DL-3-hydroxybutyrate, *p*-hydroxybenzoate, DL-lactate, D-malate, L-proline, putrescine, quinate, and L-serine, but none of them utilized benzoate, caprate, *m*-hydroxybenzoate, itaconate, propionate, suberate, L-threonine, L-tyrosine, or valerate. Assimilation of caprylate and heptanonate varied with different strains.

All strains were sensitive to cephaloridine, chloramphenicol, colistin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfadiazine, and tetracycline, but resistant to penicillin and carbenicillin. Susceptibility to ampicillin varied with different strains.

Base composition of DNA. The G + C content of DNA from seven strains of *K. ornithinolytica* ranged from 57 to 58 mol%.

Table 2. Phenotypic characteristics of *Klebsiella ornithinolytica*[a]

Test (Substrate)	% positive of 20 strains[b]	Reaction for CDC 2478-76[c]	Reaction for type strain
Motility (35°C)	0	−	−
Growth at:			
4°C (14 days)	95	+	+
42°C	95	+	+
Pigmentation (7 days):			
yellow, insoluble	0	−	−
brown, soluble (TSI)	0	−	−
Encapsulation	100	+	+
Oxidase	0	−	−
Catalase	100	+	+
Indole (24 h)	100	+	+
Voges-Proskauer	100	+	+
Citrate:			
Simmons (4 days)	100	+	+
Christensen (4 days)	100	+	+
H_2S (Kligler)	0	−	−
Lysine decarboxylase	100	+	+
Arginine dihydrolase	0	−	−
Ornithine decarboxylase	100	+	+
Phenylalanine deaminase	0	−	−
Urease (Christensen)	100	+	+
Gelatin liquefaction	0	−	−
Tween-80 hydrolysis	0	−	−
DNase (25°C) (4 days)	0	−	−
Pectinase (4 days)	0	−	−
D-Tartrate: Kauffmann-Petersen	0	−	−
Acetate (4 days)	100	+	+
Malonate	100	+	+
Nitrate to nitrite	100	+	+
KCN, growth in	95	+	+
β-galactosidase	100	+	+
β-xylosidase	100	+	+
D-glucose, acid	100	+	+
D-glucose, gas	100	+	+
Acid from:			
L-Arabinose	100	+	+
D-Cellobiose	100	+	+
D-Lactose	100	+	+
D-Maltose	100	+	+
D-Mannose	100	+	+
D-Melibiose	100	+	+
D-Melezitose	0	−	−
D-Raffinose	100	+	+
L-Rhamnose	100	+	+
D-Sorbose	100	+	+
D-Sucrose	100	+	+
D-Trehalose	100	+	+
D-Xylose	100	+	+
Adonitol	100	+	+
D-Arabitol	100	+	+
Dulcitol	0	−	−
Erythritol	0	−	−
Glycerol	100	+	+
Myo-Inositol	100	+	+
D-Mannitol	100	+	+
D-Sorbitol	100	+	+
Arbutin	100	+	+
α-Methyl-D-glucoside	100	+	+

Table 2—*Continued*

Test (Substrate)	% positive of 20 strains[b]	Reaction for CDC 2478-76[c]	Reaction for type strain
Salicin	100	+	+
Gluconate	100	+	+
2-Ketogluconate	100	+	+
5-Ketogluconate	100	+	+
Mucate	100	+	+
Esculin hydrolysis	100	+	+

[a] All strain tested are positive for acid production from L-fucose, D-galactose, β-gentiobiose, D-levulose, ribose, and D-turanose; and negative for D-arabinose, D-fucose, D-lyxose, D-tagatose, L-xylose, L-arabitol, and xylitol.
[b] The values given are for 48 h of incubation at 35°C unless otherwise indicated.
[c] *Klebsiella* group 47 at CDC.

Table 3. Relatedness of DNA among *Klebsiella ornithinolytica* and other *Klebsiella* species

		K. ornithinolytica						*K. pneumoniae*		*K. oxytoca*		*K. planticola*		*K. terrigena*	
		90–72		218–74		132–81		ATCC	13883	ATCC	13812	ICPB	3072	ATCC	33257
Source of unlabeled DNA		60°C	75°C	60°C	75°C	60°C	75°C	60°C	75°C	60°C	75°C	60°C	75°C	60°C	75°C
K. ornithinolytica															
NIH group 12	90-72[b]	100[a]	100	100	95	95	96	52	39	76	41	30	18	24	20
	218-74	93	94	100	100	86	83	68	38	44	37	28	19	34	19
	981-74	87	87	89	79	85	80	60	39	48	29	38	18	30	15
	132-81	69	69	83	81	100	100	52	40	49	34	25	24	28	27
	1002-81	87	86	93	95	80	78	45	31	49	41	31	23	34	21
	491-82	85	81	85	84	87	87	67	45	58	38	25	20	26	15
	40-84	96	92	90	89	94	95	72	48	59	44	28	13	25	10
	1215-85	91	90	92	88	88	85	69	46	55	36	33	16	31	18
CDC *Klebsiella* group 47															
	7478-76	83	80	85	85	95	90	60	38	50	28	40	23	35	24
K. pneumoniae	ATCC 13883[b]	25	18	37	16	28	16	100	100	NT	NT	NT	NT	NT	NT
K. oxytoca	ATCC 13182[b]	51	33	78	42	65	48	NT	NT	100	100	NT	NT	NT	NT
K. planticola	ATCC 33531[b]	26	12	30	21	29	11	NT	NT	NT	NT	NT	NT	NT	NT
	ICPB 3072	27	15	42	30	39	28	NT	NT	NT	NT	100	100	NT	NT
	412-82	34	18	40	19	38	13	NT	NT	NT	NT	NT	NT	NT	NT
	414-82	49	25	42	22	45	24	NT	NT	NT	NT	NT	NT	NT	NT
K. terrigena	ATCC 33257[b]	25	18	45	28	35	21	NT	NT	NT	NT	NT	NT	100	100

[a] Numeral indicates relative binding ratio.
[b] Type strain.

DNA hybridization. The DNA hybridization results are shown in Table 3 and 4. The degree of reassociation between labeled DNA from three strains of *K. ornithinolytica* (90-72, 218-74, and 132-81) and unlabeled DNA from eight other strains of the same species and from one strain of *Klebsiella* Group 47 ranged from 83% to 100% (average 90%) in tests at 60°C, and the relatedness remained high at 75°C (average 88%). All other species tested, including each of the four *Klebsiella* species already recognized, were less closely related (1%–48%) to the strains of *K. ornithinolytica*; the relatedness was, however, closer to *Klebsiella* species than to other species.

Farmer et al. [3] stated, "DNA hybridization data indicated that strains of *Klebsiella* Group 47 are related to *K. planticola*." They considered that the Group 47 may be a biogroup of *K. planticola* that gives positive reactions in tests for indole and

Table 4. Relatedness of DNA from *Klebsiella ornithinolytica* 90-72[a] to DNA from Enterobacteria other than *Klebsiellae*

Source of unlabeled DNA		Relative binding at 60°C
Enterobacter aerogenes	ATCC 13048[a]	20
Enterobacter cloacae	ATCC 13047[a]	15
Enterobacter gergoviae	CDC 604-77[a]	14
Enterobacter intermedium	ATCC 33110[a]	10
Enterobacter amunigenus	ATCC 33072[a]	7
Enterobacter sakazakii	ATCC 25944[a]	8
Enterobacter taylorae	CDC 2126-81	12
Escherichia coli	ATCC 11775[a]	10
Citrobacter freundii	ATCC 8090[a]	12
Citrobacter diversus	ATCC 27156[a]	12
Citrobacter amalonaticus	ATCC 25405[a]	10
Kluyvera ascorbata	CDC 1924-78	20
Kluyvera cryocrescens	CDC 1396-73	11
Leclercia adecarboxylata	ATCC 23216[a]	9
Escherichia hermannii	CDC 412-82	20
Escherichia vulneris	CDC 875-72[a]	15
Hafnia alvei	ATCC 13337[a]	8
Cedecea davisae	CDC 3278-77[a]	10
Cedecea lapagei	CDC 0485-76[a]	10
Edwardsiella tarda	ATCC 15947[a]	10
Serratia marcescens	ATCC 13880[a]	15
Serratia liquefaciens	CDC 1284-57[a]	10
Serratia rubidaea	ATCC 27593[a]	12
Serratia plymuthica	CCM 640[a]	15
Serratia ficalia	CIP 2602	8
Serratia fonticola	ATCC 29844	8
Salmonella choleraesuis	ATCC 13312[a]	10
Moellerella wisconsensis	CDC 3065-75	8
Ewingella americana	CDC 4020-76	5
Leminorella grimontii	CDC 3257-76	5
Leminorella richardii	CDC 598-78	5
Morganella morganii	ATCC 25830[a]	5
Proteus vulgaris	ATCC 13315[a]	3
Proteus mirabilis	ATCC 29906[a]	3
Proteus penneri	CDC 1808-73[a]	5
Providencia rettgeri	CDC 1143	1
Providencia alcalifaciens	ATCC 9886[a]	1
Providencia stuartii	CDC 2894-68	3
Yersinia enterocolitica	CIP 160	5
Yersinia frederiksenii	YE 867	8
Yersinia intermedia	Bottone No. 48	10

[a] Type strain.

ornithine. In the present study, however, DNA relatedness between NIH Group 12 and *K. planticola* was less than 30% in 75°C reaction.

The DNA relatedness results strongly suggest that NIH Group 12 is a single new species within the genus *Klebsiella*, and we propose the name *K. ornithinolytica* for it (*or.ni.thino.ly'ti.ca*, Eng.n.ornithine, an amino acid; Gr.adj. *lyticus* dissolving; M.L.fem.adj. *ornithinolytica*, ornithine dissolving). Phenotypic and DNA relatedness studies also suggest that the CDC group called *Klebsiella* Group 47 may be included in this species.

Description of *Klebsiella ornithinolytica* sp. nov. Strains of *K. ornithinolytica* are Gram-negative, encapsulated, nonmotile rods conforming to the definition of the family *Enterobacteriaceae*. They give positive reactions in the Voges-Proskauer test, in tests for indole production, citrate utilization (Simmons' and Christensen's media), urease (Christensen's agar), lysine and ornithine decarboxylases, utilization of acetate and malonate, growth in KCN broth, reduction of nitrate to nitrite, β-galactosidase, β-xylosidase, esculin hydrolysis, acid and gas production from glucose, and acid from fermentation of L-arabinose, cellobiose, L-fucose, galactose, β-gentiobiose, lactose, levulose, maltose, mannose, malibiose, raffinose, rhamnose, ribose, sorbose, sucrose, trehalose, D-xylose, adonitol, D-arabitol, glycerol, myo-inositol, mannitol, sorbitol, arbutin, α-methyl-D-glucoside, salicin, gluconate, 2-ketogluconate, 5-ketogluconate, and mucate. They give negative reactions in tests for H_2S production (triple sugar iron agar), arginine dihydrolase, phenylalanine deaminase, gelatinase, Tween 80 hydrolysis, DNase (25°C), pectinase, D-tartrate utilization (Kauffmann-Petersen's medium), and fermentation of D-arabinose, D-fucose, lyxose, melezitose, tagatose, L-arabitol, dulcitol, erythritol, and xylitol. They can grow at 4°C as well as at 42°C. They do not produce soluble brown pigment.

The type strain of *K. ornithinolytica* is NIH 90-72 (JCM 6096). It was isolated from a urine specimen from a woman with urinary tract infection. Phenotypic characteristics of the type strain are those of the species, as shown in Table 2. The base composition of its DNA is 57 ± 1 mol%.

Of the 21 strains of this species examined, 17 were isolated from clinical materials, including urine (7), throat swabs (3), sputa (4), stool (2), and pus (1), but their clinical significance is unknown. It may also be found in food.

Differentiation of *K. ornithinolytica* from other *Klebsiella* species. Tests useful in differentiating *K. ornithinolytica* from other *Klebsiella* species are shown in Table 5.

Table 5. Differentiation of *Klebsiella ornithinolytica* from other *Klebsiella* species

Species	Growth at 4°C (14 days)	Growth at 42°C	Soluble brown pigment (7 days)	Indole (24 h)	Ornithine decarboxylase	Gelatin liquefaction (7 days)	Pectinase (4 days)	D-Tartrate (Kauffmann-Petersen)	Fermentation: D-Arabinose	Fermentation: Dulcitol	Fermentation: Melezitose	Fermentation: D-Turanose	Fermentation: L-Arabitol	Fermentation: 5-Ketogluconate	Sole carbon source (14 days): Caprate	Sole carbon source (14 days): DL-3-Hydroxybutyrate	Sole carbon source (14 days): *m*-Hydroxybenzoate	Sole carbon source (14 days): *n*-Caproate
K. ornithinolytica	+	+	−	+	+	−	−	−	−	−	−	+	−	+	−	+	−	+
K. pneumoniae subsp. *pneumoniae*	−	+	−	−	−	−	−	d	−	d	d	−	−	−	d	d	d	−
K. pneumoniae subsp. *ozaenae*	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
K. pneumoniae subsp. *rhinoscleromatis*	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
K. oxytoca	−	+	+	+	−	+	+	+	+	d	+	−	+	+	d	−	+	−
K. planticola	+	+	−	d	−	−	−	+	−	d	−	−	−	+	−	+	−	−
K. terrigena	+	−	−	−	d	−	−	−	−	d	+	−	−	+	+	+	+	−

Results after 48 h incubation unless otherwise indicated.
Symbols: +, 91–100% strains positive; −, 91–100% strains negative; d, 11–90% strains positive.

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