Erwinia aphidicola*, a new species isolated from pea aphid, *Acyrtosiphon pisum

Hosami Harada,^{*,†} Hiroshi Oyaizu,¹ Yoshimasa Kosako,² and Hajime Ishikawa

Department of Biological Sciences, Graduate School of Science, ¹Department of Applied Biological Chemistry, Graduate School of Agriculture and Agricultural Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
²Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (Riken), Wako 351-01, Japan

(Received October 17, 1997; Accepted December 26, 1997)

Five strains of Gram-negative, oxidase-negative, facultatively anaerobic, fermentative, motile, rod-shaped bacterium with the general characteristics of the family Enterobacteriaceae were isolated from the gut of multiple specimens of the pea aphid. All the strains caused aphid mortality when ingested by insects via a synthetic diet. The results of biochemical tests showed that these strains are most related to *Erwinia herbicola* and *Pantoea agglomerans*. According to DNA-DNA hybridization, the five strains showed more than 96% relatedness to each other, indicating that these organisms are members of a single species. These strains were most closely related to *Erwinia herbicola* (22% DNA relatedness). Phenotypic differentiation of these strains from *Erwinia herbicola*, which was also detected from aphid gut, was based on negative reactions in tests of yellow pigment production, gelatin liquefaction, acid production from inulin, starch and dulcitol, and positive acid production from melibiose, inositol, cellobiose and glycerol. On the basis of these data, the name *Erwinia aphidicola* is proposed for the new organism. The type strain is strain X 001 (=IAM 14479).

Key Words—*Erwinia aphidicola*; insect gut; pea aphid

In a previous study, aerobic bacteria were isolated from aphid gut in order to investigate insect-bacteria interaction, and seven groups were identified as major constituents of the gut flora of the pea aphid, *Acyrtosiphon pisum* (Harada et al., 1996). Based on the molecular phylogenetic analyses of 16S rDNA, three of the seven bacterial groups were identified as members of the family Enterobacteriaceae, and tentatively named bacterium T, W and X. DNA-DNA hybridization testing and a phylogenetic tree of 16S rDNA indicated that bacterium T was identical to *Erwinia herbicola* (Harada et al., 1996).

Bacterium X was detected from almost all the healthy specimens, and was the largest in number among the seven bacterial groups which constituted the major flora of the insect gut (Harada and Ishikawa, 1993; Harada et al., 1996). In enterobacterial groups we examined, only bacterium X, and *Er. herbicola* successfully infected the gut of the insects that had

been kept aseptically (Harada and Ishikawa, 1997). Bacterium X grew very well in aphid gut and caused aphid mortality in the laboratory, whereas *Er. herbicola* seemed to have no effect on insect health.

In this study, the five strains of predominant bacterial species in aphid gut were examined biochemically and by DNA-DNA hybridization. Our results indicated that these bacteria belong to a new species in the genus *Erwinia*, for which the name *Erwinia aphidicola* is proposed.

Materials and Methods

Bacterial strains. Strains X 001, X 081, X 151, X 171 and X 181 were isolated from the guts of healthy apterous aphids as previously described (Harada et al., 1996). Whole bodies of the insect, which were surface-sterilized by dipping into 70% ethanol for 5 min, were washed in sterilized Carlson's solution (0.7% NaCl (w/v), 0.02% KCl (w/v), 0.02% CaCl₂ · 2H₂O (w/v), 0.01% MgCl₂ · 6H₂O (w/v), 0.02% NaH₂PO₄ (w/v), 0.012% NaHCO₃ (w/v) and 0.8% glucose (w/v)), and lightly homogenized in the same solution. The homogenate was spread on L broth agar and incubated overnight at 28°C to form colonies of the gut bacteria.

* Address reprint requests to: Dr. Hosami Harada, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

† Present address: Bio-oriented Technology Research Advancement Institution, Nisshin, Ohmiya 331, Japan.

Table 1. Levels of intraspecies DNA-DNA relatedness of *Erwinia aphidicola* to selected members of the Enterobacteriaceae.

Source of unlabeled DNA ^a		Relative binding ratio ^b
<i>Erwinia aphidicola</i>	X 001 ^T	100
<i>Er. aphidicola</i>	X 081	96
<i>Er. aphidicola</i>	X 151	107
<i>Er. aphidicola</i>	X 171	108
<i>Er. aphidicola</i>	X 181	115
<i>Er. herbicola</i>	JCM 7000 ^T	22
<i>Er. ananas</i>	JCM 6986 ^T	20
<i>Er. carotovora</i>	IAM 12633	12
<i>Pantoea agglomerans</i>	JMC 1236 ^T	15
<i>Enterobacter asburiae</i>	JCM 6051 ^T	15
<i>En. sakazakii</i>	JCM 1233 ^T	13
<i>En. intermedius</i>	JCM 1238 ^T	12
<i>En. gergoviae</i>	JCM 1234 ^T	6
<i>En. amnigenus</i>	JCM 1237 ^T	13
<i>En. aerogenes</i>	JCM 1235 ^T	14
<i>Serratia rubidaea</i>	JCM 1240 ^T	17
<i>S. liquefaciens</i>	JCM 1245 ^T	15
<i>S. ficaria</i>	JCM 1241 ^T	18
<i>S. marcescens</i>	JCM 1239 ^T	15
<i>Klebsiella pneumoniae</i>	JCM 1662 ^T	10
<i>K. oxytoca</i>	JCM 1665 ^T	10
<i>K. planticola</i>	JCM 7251 ^T	14
<i>K. ornithinolytica</i>	JCM 6096 ^T	12

^aJCM, Japan Collection of Microorganisms; IAM, Institute of Applied Microbiology.

^bRelative binding ratios were calculated as follows: (fluorescence of heterologous DNA/fluorescence of homologous DNA) × 100. The relative binding ratios are averages from three experiments.

Each of the five strains was established from a distinct individual aphid. These strains were maintained on either a medium containing 1% Casitone (Difco Laboratories, Detroit, MI, U.S.A.), 0.3% yeast extract, 0.5% NaCl and 0.7% agar or nutrient agar, and grown at 28°C. The eighteen reference strains used in this study are listed in Table 1.

Phenotypic characteristics. API 20E strips (bioMerieux-Vitek Japan, Ltd., Tokyo, Japan) were used for preliminary characterizations; API 50CH strips (bioMerieux-Vitek Japan, Ltd., Tokyo, Japan) were used for fermentation tests. All of these strips were used as directed by the manufacturer. Preparations were incubated at 28°C for 2 days, and the results were recorded. Additional tests were performed for citrate and malonate utilization according to standard procedures (Cowan, 1974; Ewing, 1986). Cellular morphology, motility and flagella morphology were determined using cells grown on LB nutrient agar containing 0.5% yeast extract, 1% peptone and 0.5% NaCl.

DNA-DNA hybridization. To prepare the genomic DNA of bacterial strains, cells were grown in nutrient broth at 30°C for 24 h with shaking. DNA was prepared by the method of Marmur (1961). DNA aliquots

of strain X 001^T were labeled with photobiotin, and hybridization studies were done by a method previously described (Ezaki et al., 1989). The fluorescence was read using a fluorometer (CS-9300PC Scanning Densitometer; Shimadzu, Kyoto, Japan).

Results

Isolation of gut aerobic bacteria from pea aphid

It was observed that the whole-body extract of surface-sterilized pea aphid contains many groups of bacteria (Harada et al., 1996). To eliminate the contamination by bacteria which adhered to the surface of the aphid body, we performed preparatory experiments. At first, we dipped aphids into a culture of *Escherichia coli* XL1-blue, which are resistant to tetracycline, and then divided the aphids into 5 groups (each group contained five specimens). Each group of aphids was dipped into 70% ethanol for 0, 2, 5, 10 or 15 min, and then washed in sterilized Carlson's solution. Surface-sterilized aphids were homogenized individually in LB liquid medium containing 20 µg/ml tetracycline, and the homogenate was incubated for 24 h at 30°C with shaking. No bacterial growth was detected from any of the aphid homogenates except the control group that had not been treated with 70% ethanol. Further experiments revealed that treatment with 70% ethanol for longer than 15 min eliminates not only the bacteria which adhered to the surface but also those which settled in the gut. For this reason, we employed the treatment with 70% ethanol for 5 min to sterilize the surface of the aphids. In addition, it was shown that the extract of digestive tracts surgically isolated from aphids also contains the same kinds of bacteria as those isolated from the whole body, which was confirmed by RFLP analysis of 16S rDNA (data not shown). Thus, the bacteria isolated from the whole-body extract of surface-sterilized aphids were supposed to be derived from their digestive tracts. Strains X 001^T, X 081, X 151, X 171 and X 181 were the predominant group in the aphid gut bacterial flora (Harada et al., 1996).

Phenotypic characterization

The morphological and biochemical characteristics of the predominant strains in aphid gut are shown in Tables 2 and 3. These strains exhibited the biochemical characteristics of the family Enterobacteriaceae. They were Gram-negative, oxidase-negative, catalase-positive, facultatively anaerobic, fermentative, rod-shaped bacteria which were motile by means of peritrichous flagella. The colonies were ivory, and did not produce the yellow pigment which has been observed in *Er. herbicola*. The strains grew in nutrient medium from 6°C to 40°C, but not above 42°C. Opti-

Table 2. Phenotypic characteristics of *Erwinia aphidicola*.

Characteristic	% of positive strains (n=5)	Strain X 001 ^T
Pigment production	0	— ^a
Motility	100	+
Growth at 37°C	100	+
Growth at 42°C	0	—
Voges-Proskauer test	100	+
ONPG (β-galactosidase) ^b	100	+
Oxidase activity	0	—
Indole production	0	—
Nitrate reduction	100	+
Gelatin liquefaction	0	—
Tryptophan deaminase activity	0	—
H ₂ S produced from sodium thiosulfate	0	—
Arginine dihydrolase activity	0	—
Lysine decarboxylase activity	0	—
Ornithine decarboxylase activity	0	—
Deoxyribonuclease activity	0	—
Urease activity	0	—
Utilization of		
Citrate (Simmons)	100	+
Malonate	100	+

^a +, positive reaction; —, negative reaction.

^b ONPG, o-nitrophenyl-β-D-galactopyranoside.

mal growth was observed between 25°C and 35°C. No growth factors were required since the cells were able to grow in M9 medium.

DNA-DNA hybridization

The identification probability, which was calculated based on the percent positive of the morphological and biochemical characteristics of the predominant strains in aphid gut, indicated their close relationship to *Er. herbicola* (Lelliot and Dickey, 1984) and *Pantoea agglomerans* (Gavini et al., 1989). We further performed DNA-DNA hybridization tests with these species and other phenotypically related species listed in Table 1. It turned out that *Er. herbicola* (JCM 7000^T) was 22% related to strain X 001^T. The level of relatedness of *P. agglomerans* (JCM 1236^T) was 15%. All the other members of the Enterobacteriaceae tested were less than 20% related to strain X 001^T. Unlabeled DNAs from the four other predominant strains in aphid gut had over 96% relatedness to strain X 001^T (Table 1).

Discussion

The five strains we investigated were shown to be essentially identical phenotypically and highly related to each other as determined by DNA-DNA hybridization, leaving no doubt that they are members of a single species.

All these strains were isolated as the predominant

Table 3. Acid production from various carbohydrates by *Erwinia aphidicola*.

Acid production from	% of positive strains (n=5)	Strain X 001 ^T
Glycerol	100	+ ^a
Erythritol	100	+
D-Arabinose	0	—
L-Arabinose	100	+
D-Ribose	100	+
D-Xylose	100	+
L-Xylose	0	—
Adonitol	100	W
β-Methyl-D-xyloside	0	—
D-Galactose	100	+
D-Glucose	100	+
D-Fructose	100	+
D-Mannose	100	+
L-Sorbose	0	—
L-Rhamnose	100	+
Dulcitol	0	—
Inositol	100	+
D-Mannitol	100	+
D-Sorbitol	40	W
α-Methyl-D-mannoside	0	—
α-Methyl-D-glucoside	0	—
N-Acetyl-D-glucosamine	100	+
Amygdalin	100	+
Arbutin	100	+
Esculin	100	+
Salicin	100	+
Cellobiose	100	+
Maltose	100	+
Lactose	20	—
Melibiose	100	+
Sucrose	100	+
Trehalose	100	+
Inulin	0	—
Melezitose	0	—
Raffinose	100	+
Starch	0	—
Glycogen	0	—
D-Xylitol	100	+
β-Gentiobiose	100	+
D-Turanose	0	—
D-Lyxose	100	+
D-Tagatose	0	—
D-Fucose	100	+
L-Fucose	0	—
D-Arabitol	0	—
L-Arabitol	0	—
D-Gluconate	100	+
2-Keto-D-gluconate	100	+
5-Keto-D-gluconate	100	+

^a +, positive reaction; —, negative reaction; W, weak reaction.

species in aphid gut, and had strong affinity to the aphid gut (Harada et al., 1996; Harada and Ishikawa, 1997b). The results of biochemical tests shown in Tables 2 and 3 suggest that the predominant species in aphid gut is most closely related to *Er. herbicola* (Lelliot and Dickey, 1984) and *P. agglomerans* (Gavini et

Table 4. Differential characteristics of *Erwinia* species.^a

Species	Yellow pigment	Hydrogen sulfide production	Indole production	Nitrate reduction	Voages-Proskauer test	Acid production from						
						Inositol	Cellobiose	Lactose	D-Xylose	Raffinose	Melibiose	Maltose
<i>Erwinia aphidicola</i>	— ^c	—	—	+	+	+	+	—	+	+	+	+
<i>Er. herbicola</i> ^b	+	+	—	+	+	—	—	d	+	d	—	+
<i>Er. ananas</i>	+	d	+	—	+	+	+	+	+	+	+	+
<i>Er. carotovora</i>	—	+	—	+	+	d	+	+	+	+	+	d
<i>Er. chrysanthemi</i>	—	+	+	+	+	d	+	d	+	+	+	—
<i>Er. cacticola</i>	—	—	—	+	+	—	—	[—]	d	—	—	—
<i>Er. cypripedii</i>	—	+	—	+	—	+	+	—	+	—	+	+
<i>Er. rhapontici</i>	—	+	—	+	+	+	+	+	d	+	+	+
<i>Er. nigritluens</i>	—	+	—	—	+	+	—	—	+	+	+	—
<i>Er. amylovora</i>	—	—	—	—	+	—	—	—	—	—	—	—
<i>Er. mallotivora</i>	—	—	—	—	+	—	—	—	+	—	—	—
<i>Er. persicinus</i>	—	—	—	+	+	+	+	+	—	+	+	+
<i>Er. quercina</i>	—	+	—	—	+	—	—	—	—	—	—	—
<i>Er. salicis</i>	—	+	—	—	+	+	—	—	—	+	+	—
<i>Er. rubrifaciens</i>	—	+	—	—	—	—	—	—	—	—	—	—

^a Data from reference (Holt et al., 1994) except *Erwinia herbicola*.
^b Data from reference (Lelliot and Dickey, 1984).
^c —, 0–10% positive; [—], 11–25% positive; +, 26–75% positive; [+], 76–89% positive; ++, 90–100% positive. Results for 48 h incubation at 28°C (*Erwinia aphidicola*) or 27°C (other *Erwinia* species).

Table 5. Differential characteristics of *Erwinia aphidicola* and *Pantoea* species.^a

Characteristic	<i>Erwinia aphidicola</i>	<i>Pantoea ananas</i>	<i>P. stewartii</i> subsp. <i>stewartii</i>	<i>P. stewartii</i> subsp. <i>indologenes</i>	<i>P. agglomerans</i>	<i>P. dispersa</i>	<i>P. citrea</i>	<i>P. punctata</i>	<i>P. terrea</i>
Nitrate reduction	+	d	—	—	+	—	+	+	+
Indole production	—	+	—	+	—	—	—	—	—
Citrate utilization	+	+	—	+	d	+	+	+	+
β-Galactosidase	+	+	+	+	+	+	+	—	—
Acid production from									
glycerol	+	+	—	[+]	[—]	d	+	+	+
D-arabitol	—	+	—	+	d	+	[+]	—	—
cellobiose	+	+	—	+	d	+	—	—	—
maltose	+	+	—	+	+	+	+	[—]	[—]
lactose	—	+	—	+	[—]	[—]	+	—	—
α-methyl-D-mannoside	—	[+]	—	—	—	—	—	—	—
arbutin	+	+	—	+	+	—	[—]	—	+
salicin	+	+	—	+	+	[—]	[—]	—	+
raffinose	+	[+]	+	+	—	—	—	d	d
D-turanose	—	—	—	—	—	+	—	—	—
D-fucose	+	—	—	—	—	—	+	+	+
Hydrolysis of esculin	+	d	—	+	+	+	—	[—]	+
Motility	+	+	—	[+]	+	+	—	—	+
Malonate utilization	+	—	—	—	+	—	—	—	—

^a Data from reference (Mergaert et al., 1993).^b —, 0–10% positive; [—], 11–25% positive; d, 26–75% positive; [+], 76–89% positive; +, 90–100% positive. Results are for 48 h incubation at 28°C (*Erwinia aphidicola*) or 30°C (*Pantoea* species).Table 6. Differential characteristics of *Erwinia aphidicola* and phenotypically related members of the family Enterobacteriaceae.^a

Species	Urease activity	Ornithine decarboxylase activity	Motility	Gelatin liquefaction	Utilization of malonate	Acid production from	
						Inositol	Lactose
<i>Erwinia aphidicola</i>	— ^c	—	+	—	+	+	—
<i>Enterobacter asburiae</i>	d	+	—	—	—	—	[+]
<i>En. sakazakii</i>	—	+	+	—	[—]	[+]	+
<i>En. intermedius</i>	—	+	+	—	+	—	+
<i>En. gergoviae</i>	+	+	+	—	+	—	d
<i>En. amnigenus</i>	—	+	+	—	+	—	d
<i>En. aerogenes</i>	—	+	+	—	+	+	+
<i>Serratia rubidaea</i>	—	—	[+]	+	+	[—]	+
<i>S. liquefaciens</i>	—	+	+	+	—	d	—
<i>S. ficaria</i>	—	—	+	+	—	d	[—]
<i>S. marcescens</i>	[—]	+	+	+	—	[+]	[—]
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	+	—	—	—	+	+	+
<i>K. oxytoca</i>	+	—	—	—	+	+	+
<i>K. planticola</i>	+	—	—	—	+	+	+
<i>K. ornithinolytica</i> ^b	+	+	—	—	+	+	+

^a Data from reference (Holt et al., 1994).^b Data from reference (Sakazaki et al., 1989).^c —, 0–10% positive; [—], 11–25% positive; d, 26–75% positive; [+], 76–89% positive; +, 90–100% positive. Results for 48 h incubation at 28°C (*Erwinia aphidicola*) or 36°C (other species).

al., 1989), which are referred to as synonyms. The result that no species related to the predominant species in aphid gut showed a higher DNA-DNA homology index than 22% (Table 1), coupled with the differentiation of biochemical characteristics shown in

Tables 4–6, convinced us that the predominant species in aphid gut is different from other members of the family Enterobacteriaceae, and establishes a new species.

From aphid gut, we also detected another group of

bacterium that produces yellow pigment, which was identified as *Er. herbicola* based on DNA-DNA hybridization (Harada et al., 1996). *Er. herbicola* was smaller in number than the predominant species in the aphid gut. Both of the species seem to have adapted to the insect gut environment since they are able to colonize and grow well in the guts of aphid which have been maintained free of microbes (Harada and Ishikawa, 1997b). In addition to this, these species, in common, produced extracellular polysaccharides when they were grown on media which contains sucrose, trehalose or their component monosaccharides (Harada, H., unpubl.). While the DNA relatedness of the predominant species of aphid gut and *Er. herbicola* indicated in this study was not so high, a close relationship between these species was supported by molecular phylogeny based on the *groE* nucleotide sequences and its deduced amino acid sequences. The predominant species of aphid gut clearly formed a single cluster with *Er. herbicola* and *Er. ananas* (100 and 94% bootstrap values, respectively) (Harada and Ishikawa, 1997a).

The predominant species in aphid gut is distinguished from the members of the genus *Enterobacter* by its negative reaction in ornithine decarboxylase, distinguished from members of the genus *Serratia* by its negative reaction in gelatin liquefaction, and distinguished from members of the genus *Klebsiella* by its negative reaction in urease (Table 6). In considering the phenotypic characteristics, DNA-DNA relatedness and its ecology, the new species should be classified in the genus *Erwinia*. Below we describe the new species *Erwinia aphidicola*.

Description of Erwinia aphidicola sp. nov. *Erwinia aphidicola* (a. phi'di.co.la. L. aphid the aphid; L. suff.-cola dweller; M. L. n. *aphidicola*, aphid dweller). Cells are Gram-negative, oxidase-negative, catalase-positive, fermentative rods (0.5 to 0.6 by 1.6 to 2.0 μ m) and motile by means of peritrichous flagella. All strains grow well on nutrient agar and common laboratory media. Colonies on peptone-yeast extract agar are circular and smooth. These organisms grow well at 25, 30 and 35°C, but not at 42°C. Other biological and nutritional characteristics at 28°C are shown in Tables 2 and 3. The characteristics that differentiate this species from other species are shown in Tables 4–6. The type strain is strain X 001 (=IAM 14479),

which was isolated from aphid gut.

This work was supported in part by a research grant from the Program for Promotion of Basic Research Activities for Innovation Biosciences (ProBRAIN) of the Bio-oriented Technology Research Advancement Institution.

References

- Cowan, S. T. (1974) Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd ed., Cambridge University Press, London.
- Ewing, W. H. (1986) Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th ed., Elsevier, New York.
- Ezaki, T., Hashimoto, Y., and Yabuuchi, E. (1989) Fluorometric deoxyribonucleic acid—Deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.*, **39**, 224–229.
- Gavini, F., Mergaert, J., Beji, A., Mielcarek, C., Izard, D., Kersters, K., and De Ley, J. (1989) Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea*, new genus as *Pantoea agglomerans* new combination and description of *Pantoea dispersa*. *Int. J. Syst. Bacteriol.*, **39**, 337–345.
- Harada, H. and Ishikawa, H. (1993) Gut microbe of aphid closely related to its intracellular symbiont. *BioSystems*, **31**, 185–191.
- Harada, H., Oyaizu, H., and Ishikawa, H. (1996) A consideration about the origin of aphid intracellular symbiont in connection with gut bacterial flora. *J. Gen. Appl. Microbiol.*, **42**, 17–26.
- Harada, H. and Ishikawa, H. (1997a) Phylogenetical relationship based on *groE* genes among phenotypically related *Enterobacter*, *Pantoea*, *Klebsiella*, *Serratia* and *Erwinia* species. *J. Gen. Appl. Microbiol.*, **43**, 355–361.
- Harada, H. and Ishikawa, H. (1997b) Experimental pathogenicity of *Erwinia aphidicola* to pea aphid, *Acyrtosiphon pisum*. *J. Gen. Appl. Microbiol.*, **43**, 363–367.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., and Williams, S. T. (1994) Bergey's Manual of Determinative Bacteriology, 9th ed., Williams & Wilkins, Baltimore.
- Lelliot, R. A. and Dickey, R. S. (1984) Genus VII, *Erwinia*. In Bergey's Manual of Systematic Bacteriology, Vol. 1., ed. by Krieg, N. R. and Holt, J. G., Williams & Wilkins, Baltimore, pp. 469–476.
- Marmur, J. (1961) A procedure for the isolation of deoxyribonucleic acid from microorganism. *J. Mol. Biol.*, **3**, 208–218.
- Mergaert, J., Verdonck, L., and Kersters, K. (1993) Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int. J. Syst. Bacteriol.*, **43**, 162–173.
- Sakazaki, R., Tamura, K., Kosako, Y., and Yoshizaki, E. (1989) *Klebsiella ornithinolytica*, new species, formerly known as ornithine-positive *Klebsiella oxytoca*. *Curr. Microbiol.*, **18**, 201–206.