Identification of Ruiter's Strains, Isolated from Brownd Marinated Herring, as Members of *Erwinia herbicola*

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Deoxyribonucleic acid:ribosomal ribonucleic acid hybridizations, determinations of the guanine plus cytosine content of the deoxyribonucleic acid, and numerical analysis of phenotypic features were used to identify Ruiter's strains, isolated from browned marinated herring, as members of *Erwinia herbicola*.

In 1963 Ruiter (15) isolated from cut onions bacteria which caused browning of marinated herring in a marinade factory in the Netherlands. These bacteria were aerobic, oxidase-negative, nonmotile rods which contained catalase, produced acid from glucose, and grew on citrate; they were unable to produce H_2S or indole or to reduce nitrate. Ruiter proposed that they probably belonged in *Achromobacter anitratum* (Schaub and Hauber) Brisou.

Bacterium anitratum was first described by Schaub and Hauber (16) as a group of gram-negative, strictly aerobic, short rods unable to reduce nitrate. They were isolated from clinical material of human source. Brisou (3) placed *Bacterium anitratum* in the genus *Achromobacter* for ecological and biological reasons. In 1954 Brisou and Prévot (5) created the genus name *Acinetobacter* for the nonmotile achromobacters, and Brisou (4) later proposed *Acinetobacter anitratum* as the type species. This species is now known as *Acinetobacter calcoaceticus* (1).

The taxonomic position of Ruiter's strains, sometimes named *Achromobacter* sp., remained uncertain up to now. In the National Collection of Industrial Bacteria catalogue of strains (2) they are listed as unidentified bacteria. We report here on a specific identification of these strains based on numerical analysis of phenotypic features, deoxyribonucleic acid (DNA):ribosomal ribonucleic acid (rRNA) hybridizations, and guanine plus cytosine (G+C) content of the DNA.

MATERIALS AND METHODS

Bacterial strains. The organisms used in this study are listed in Table 1. *Bergey's Manual of Determinative Bacteriology* (6) does not give a type strain for *Erwinia herbicola*. However, *Enterobacter agglomerans* and *Erwinia herbicola* were regarded as synonyms by Ewing and Fife (13), who proposed NCTC 9381 as the neotype strain of *Enterobacter agglomerans*. We included this strain as well as 30

other strains of *E. herbicola* from five different culture collections and from different sources. All of these strains, except G144, formed one tight phenotypic cluster (Fig. 1). Some representative strains of this cluster have been checked in the API 20E system at 37°C; the strains were identified by the API computer service as *E. herbicola*, "excellent identification."

Methods. DNA:rRNA hybridizations were carried out as described previously (9,10,12). DNA base compositions, expressed as moles percent G+C, were determined with a recording thermal spectrophotometer (11). Phenotypic characterization of the strains was effected using the API 20E and API 50 *Enterobacteriaceae* systems (API System S.A., Montalieu Vercieu, France). For the computer-assisted numerical analysis, the unweighted pair group method was used, with a simple matching coefficient S_{SM} (17).

FIG. 1. Dendrogram of simple matching similarity coefficients (S_{SM}), grouped by the unweighted-pair, group-clustering method, showing the relationship of Ruiter's strains with strains of various *Erwinia* species.

TABLE 1. *List of strains used*

Name according to <i>Bergey's Manual</i> ^a	Strain no.	Origin ^b
	Ruiter 1 = NCIB 9744	1 ^c
	Ruiter 3 = NCIB 9746	1 ^c
	Ruiter 4 = NCIB 9747	1 ^a
	Ruiter 5 = NCIB 9748	2 ^d
	Ruiter 7 = NCIB 10845	2 ^d
<i>Erwinia herbicola</i> subsp. <i>herbicola</i>	D11	3
	G 138	3
	G 139	3
	G 140	3
	G 141	3
	G 142	3
	G 143	3
	G 144	3
	G 145	3
	G 146	3
	G 150	3
	G 151	3
	G 152	3
	G 153	3
	G 154	3
	G 155	3
	G 156	3
	G 157	3
	NCTC 708	4
	NCTC 818	4
	NCTC 892	4
	NCTC 827	4
	NCTC 9381	4
	PA	3
	NCIB 9680	1
	IPO 287	5
	IPO 445	5
	NCPPB 2035	6
	NCPPB 2036	6
	NCPPB 102	6
	JM 1	3
<i>Erwinia herbicola</i> subsp. <i>anas</i>	Y1	3
<i>Erwinia uredovora</i>	NCPPB 802	6
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	NCPPB 1280	6
	NCPPB 546	6
<i>Erwinia chrysanthemi</i>	NCPPB 453	6
<i>Erwinia cypripedii</i>	NCPPB 750	6
<i>Erwinia nigrifluens</i>	NCPPB 565	6
<i>Erwinia amylovora</i>	NCPPB 683	6
<i>Escherichia coli</i>	B	7
<i>Serratia marcescens</i>	ATCC 10759	8
<i>Salmonella typhimurium</i>	1	7
<i>Enterobacter aerogenes</i>	NCTC 10006	4

^a Reference 6.

^b 1, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; 2, A. Ruiter, TNO, Ijmuiden, The Netherlands; 3, D. C. Graham, Department of Agriculture and Fisheries, Edinburgh, Scotland; 4, National Collection of Type Cultures, Colindale Avenue, London; 5, Instituut voor Plantenziektenkundig Onderzoek, Wageningen, The Netherlands; 6, National Collection of Plant Pathogenic Bacteria, Harpenden, Hertfordshire, England; 7, Departmental Collection, Laboratorium voor Microbiologie, Rijksuniversiteit, Gent, Belgium; 8, American Type Culture Collection, Rockville, Md.; 9, International Collection of Phytopathogenic Bacteria, University of California, Davis; 10, Cell Biology Research Institute, Canada Department of Agriculture, Ottawa, Canada.

^c Received as "presumably *Achromobacter* sp."^d Received unnamed.^e Renamed *Janthinobacterium lividum* (10).

TABLE 1 (continued)

Name according to Bergey's Manual ^a	Strain no.	Origin ^b
<i>Klebsiella pneumoniae</i>	N4B	7
<i>Agrobacterium tumefaciens</i>	ICPB TT111	9
<i>Pseudomonas fluorescens</i>	ATCC 13525	8
<i>Xanthomonas campestris</i>	ICPB T11	9
<i>Chromobacterium lividum</i> ^a	NCTC 9796	4
<i>Bordetella bronchiseptica</i>	NCTC 8761	4
<i>Bacillus subtilis</i>	BQ2	7
<i>Arthrobacter globiformis</i>	CBRI 21010	10

TABLE 2. Results of DNA:rRNA hybridizations and of G+C determinations^a

Organism for DNA preparation	Strain no.	G+C Content of DNA (mol %)	Hybridizations	
			$T_{m(e)}$	Percent binding ^b
<i>Escherichia coli</i>	B	52.2	81	0.15
Ruiter's strain	NCIB 9744	53.0	77	0.17
Ruiter's strain	NCIB 9746	53.5	75.5	0.13
Ruiter's strain	NCIB 9747	53.7	ND ^c	ND
Ruiter's strain	NCIB 9748	53.7	77	0.11
Ruiter's strain	NCIB 10845	51.8	76	0.15
<i>Salmonella typhimurium</i>	1	55.2	79	0.18
<i>Serratia marcescens</i>	ATCC 10759	58.2	78	0.18
<i>Erwinia amylovora</i>	NCPPB 683	54.0	76.5	0.24
<i>Erwinia herbicola</i> subsp. <i>herbicola</i>	NCTC 9381	55.9	77	0.14
<i>Enterobacter aerogenes</i>	NCTC 10006	53.8	77	0.17
<i>Klebsiella pneumoniae</i>	N4B	58.6	77	0.17
<i>Agrobacterium tumefaciens</i>	ICPB TT111	60.6	63.5	0.07
<i>Pseudomonas fluorescens</i>	ATCC 13525	60.2	64	0.09
<i>Xanthomonas campestris</i>	ICPB T11	64.3	66	0.04
<i>Janthinobacterium lividum</i>	NCTC 9796	65.5	65	0.15
<i>Bordetella bronchiseptica</i>	NCTC 8761	69.5	66	0.07
<i>Bacillus subtilis</i>	BQ2	47.7	58	0.16
<i>Arthrobacter globiformis</i>	CBRI 21010		56	0.07

^a The methods used have been described previously (9, 10, 12). Hybridizations were performed with ¹⁴C-labeled rRNA from *E. coli* B.

^b Percent binding is the amount (micrograms) of rRNA bound to 100 µg of DNA.

^c ND, Not determined.

RESULTS AND DISCUSSION

The DNA base composition of Ruiter's organisms is 51.8 to 53.7 mol% G+C (see Table 2). De Ley (7) determined the DNA base composition of a group of numerically analyzed acinetobacters. *Acinetobacter anitratum* strains constituted a homogeneous group with G+C values between 40.7 and 42.8 mol%. Obviously Ruiter's strains cannot belong in *Acinetobacter calcoaceticus* (syn.: *A. anitratum*).

Previously we have shown (10,12) that the similarity between rRNA cistrons, expressed as the midpoint temperature, $T_{m(e)}$, of the thermal elution of the heterologous DNA:rRNA hybrids, appeared to be a good criterion for the identification of organisms and for the classification of

taxa at generic and suprageneric levels. Upon hybridization with [¹⁴C]rRNA of *Escherichia coli* B, the rRNA cistrons of Ruiter's strains fell within those of the *Enterobacteriaceae*. The similarity with the rRNA cistrons of other taxa is much lower (Table 2). The $T_{m(e)}$ of hybrids between DNA of Ruiter's strains and reference rRNA from *Agrobacterium tumefaciens* is also low (12). We concluded that Ruiter's strains belong in the family *Enterobacteriaceae*, but the limited resolution of the method used did not allow generic identification.

To pinpoint the exact taxonomic position of Ruiter's strains, we performed a phenotypic characterization using the API 20E and API 50 *Enterobacteriaceae* test systems. The tests were carried out at 28°C because Ruiter's strains do

not grow at 37°C. Thirty-one authentic strains of *E. herbicola* subsp. *herbicola*, one of *E. herbicola* subsp. *ananas*, one of *E. uredovora*, one of *E. nigrifluens*, two of *E. carotovora* subsp. *carotovora*, one of *E. cypripedii*, and one of *E. chrysanthemi* were also included. All of the *E. herbicola* strains and all of Ruiter's strains produced acid from L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, mannitol, *N*-

acetylglucosamine, maltose, and D-gluconate. None of these strains produced acid from erythritol, L-xylose, methyl-D-xyloside, L-sorbose, dulcitol, inulin, amylose, starch, or glycogen; they did not produce H₂S, deoxyribonuclease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, oxidase, or gelatinase. The results of the remaining tests are given in Table 3.

TABLE 3. *Some phenotypic properties of strains of Erwinia herbicola subsp. herbicola and of Ruiter's strains*^a

Character	<i>Erwinia herbicola</i> ^b	Ruiter's strains				
		NCIB 9744	NCIB 9746	NCIB 9747	NCIB 9748	NCIB 10845
Acid production from:						
Glycerol	d	+	+	+	+	+
D-Arabinose	d	—	—	+	—	+
Adonitol	—	—	—	—	—	—
D-Galactose	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+
Meso-inositol	+	+	+	+	+	—
Sorbitol	d	—	—	—	—	+
Methyl-D-mannoside	—	—	—	—	—	—
Methyl-D-glucoside	—	—	—	—	—	—
Amygdalin	—	+	+	+	+	+
Arbutin	+	+	+	+	+	+
Salicin	+	+	+	+	+	+
D-Cellobiose	d	+	+	+	+	+
Lactose	d	+	+	+	+	+
D-Melibiose	d	+	+	+	+	+
Sucrose	+	—	—	+	—	—
D-Trehalose	+	—	—	+	—	—
D-Melezitose	—	—	—	—	—	—
D-Raffinose	—	—	—	—	—	—
Dextrin	—	—	—	—	—	—
Galactarate	d	—	—	+	—	—
Use of esculin	d	+	+	+	+	+
Utilization as carbon source:						
Malonate	d	+	+	+	+	+
Acetate	—	—	—	—	—	—
Citrate (Simmons)	d	+	+	+	+	—
Citrate (Christensen)	+	+	+	+	+	+
Presence of:						
Lipase	d	—	—	—	—	—
Tetrathionate reductase	d	+	—	+	—	—
β-Galactosidase	d	+	+	+	+	+
Urease	—	—	—	—	—	—
Tryptophan deaminase	—	—	—	—	—	—
Indole production	—	—	—	—	—	—
Acetoin production	+	+	+	+	+	+
Methyl red	—	—	—	—	—	—
Nitrate reduction	+	—	—	—	—	—

^a Properties were determined in API 20E and API 50 *Enterobacteriaceae* tests after 48 h at 28°C. +, More than 90% of the strains were positive; —, more than 90% of the strains were negative; d, 11 to 89% of the strains were positive.

^b Thirty strains; see Fig. 1.

A numerical analysis of all of the strains tested was carried out using the unweighted pair group clustering method (Fig. 1). On this basis, Ruiter's strains were identified as members of *E. herbicola* subsp. *herbicola*. The known DNA base range of *E. herbicola* extends from 52 to 59 mol% G+C (8, 18); the G+C values of Ruiter's strains fall within this range. Many *E. herbicola* strains have been isolated from plant surfaces (14). Ruiter's isolates (15) originated from cut and rotting onions.

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REPRINT REQUESTS

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