Frateuria, a New Genus for "*Acetobacter aurantius*"

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The properties and taxonomic positions of 11 strains previously identified as members of "*Acetobacter aurantius* Kondô and Ameyama" (this name is not on the Approved Lists) were reexamined. For each we determined about 100 phenotypic features, the protein gel electropherograms, and the parameters of deoxyribonucleic acid: ^{14}C -ribosomal ribonucleic acid (DNA:[^{14}C]rRNA) hybrids. The 11 strains fell into three taxonomically distinct groups. Strain IFO 3248 was the only one which belonged in *Acetobacter*. Strain IFO 3246 was an atypical *Gluconobacter*. The remaining nine strains formed a tight cluster, with very similar phenotypic features and protein gel electropherograms. Taxonomically, this cluster is quite removed from *Gluconobacter* and *Acetobacter*, and the properties of its DNA:rRNA hybrids suggest that it is closer to *Pseudomonas* Section I (R. E. Buchanan and N. E. Gibbons [ed.], *Bergey's Manual of Determinative Bacteriology*, 8th ed.) and *Xanthomonas*. We propose the name *Frateuria* gen. nov. for this cluster, with *Frateuria aurantia* sp. nov. as the type species and IFO 3245 as the type strain. An extensive phenotypic description and minimal standards of the new genus are given, as is the phenotypic differentiation from *Gluconobacter* and *Acetobacter*.

The name "*Acetobacter aurantium*" (sic) was proposed by Kondô and Ameyama (17) to refer to four strains of acetic acid bacteria isolated from *Lilium auratum* Lindl. Because these strains share diagnostic features with *Gluconobacter* as well as with *Acetobacter*, Asai (3) and Asai et al. (4) judged that it was difficult to assign them to either of these two genera and therefore referred to them as "intermediate" strains. Additional characteristics of these strains were given by Ameyama and Kondô (1, 2). Yamada et al. (24) showed that these strains produce ubiquinone Q8, whereas *Gluconobacter* possesses ubiquinone Q10 and *Acetobacter* possesses ubiquinone Q9. Yamada et al. (25) isolated and characterized six additional polarly flagellated intermediate strains from *Rubus parvifolius*.

The taxonomic position of these strains has been obscure to now. They have not been considered to belong to either *Acetobacter*, because of their polar flagellation and their ubiquinone Q8, or to *Gluconobacter*, because of their oxidation of lactate, their lack of requirement for growth factors, their oxidation of amino acids, and their characteristic ubiquinone Q8, but they were believed to lie somewhere in between these two genera. The problematic taxonomic status of these strains is further illustrated by the complete lack of any reference to them in Sneath and Skerman's list of reference strains of bacteria (22), the *Index Bergeyana* (8), the eighth edition of *Bergey's Manual* (7), and the Ap-

proved Lists of Bacterial Names (21). The name "*Acetobacter aurantius*" thus has no standing in nomenclature. Fourteen "*A. aurantius*" strains have been mentioned in the literature, and 11 of them are available from the culture collection at the Institute for Fermentation (IFO), Osaka, Japan, where they are held as "*A. aurantius*" IFO 3245, 3246, 3247, 3248, 3249, 13328, 13329, 13330, 13331, 13332, and 13333.

As part of a current extensive reinvestigation of the acetic acid bacteria, we examined the "*A. aurantius*" strains by deoxyribonucleic acid:ribosomal ribonucleic acid (DNA:rRNA) hybridizations to establish their taxonomic position at the generic and suprageneric levels (15), by protein gel electrophoresis to reveal the internal fine structure of the taxon (16; K. Kersters and J. De Ley, in M. Goodfellow and R. Board [ed.], *Impact of Modern Methods on Classification and Identification*, in press), and by an extensive phenotypic analysis. On the basis of our data, we propose, in the discussion below, a new genus and species name, *Frateuria aurantia*, for strains IFO 3245, 3247, 3249, and 13328 to 13333. Strain IFO 3246 was found to be a strain of *Gluconobacter*, and strain IFO 3248, a strain of *Acetobacter*.

MATERIALS AND METHODS

Bacterial strains. The 11 strains used in this study were obtained from the Institute of Fermentation, Osaka, Japan (see Table 1).

Nutritional, physiological, and biochemical

TABLE 1. *Organisms used in this study*

Strain received as	Name given in the present paper	Strain no.	Isolated from
" <i>Acetobacter aurantius</i> "	<i>Frateruria aurantia</i>	IFO 3245 (= G-6 = Kondô 67)	<i>Lilium auratum</i> , Japan (17)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 3247 (= Kondô 71 = ATCC 12301)	<i>Lilium auratum</i> , Japan (17)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 3249 (= Kondô 69)	<i>Lilium auratum</i> , Japan (17)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 13328 (= Yamada 5)	Fruit of <i>Rubus parvifolius</i> (raspberry) Japan (25)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 13329 (= Yamada 11)	Fruit of <i>Rubus parvifolius</i> (raspberry) Japan (25)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 13330 (= Yamada 20)	Fruit of <i>Rubus parvifolius</i> (raspberry) Japan (25)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 13331 (= Yamada 28)	Fruit of <i>Rubus parvifolius</i> (raspberry) Japan (25)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 13332 (= Yamada 38)	Fruit of <i>Rubus parvifolius</i> (raspberry) Japan (25)
" <i>A. aurantius</i> "	<i>Frateruria aurantia</i>	IFO 13333 (= Yamada 130)	Fruit of <i>Rubus parvifolius</i> (raspberry) Japan (25)
" <i>A. aurantius</i> "	<i>Gluconobacter</i> sp.	IFO 3246t ^a (= Kondô 70)	<i>Lilium auratum</i> , Japan (17)
" <i>A. aurantius</i> "	<i>Acetobacter</i> sp.	IFO 3248 (= Kondô 68)	<i>Lilium auratum</i> , Japan (17)

^a Strain IFO 3246 displayed two stable colony types (t₁, t₂) upon plating. Only strain IFO 3246t₁ was used, because both types showed identical protein electropherograms.

tests. The cultures were grown either on a medium containing (wt/vol) 5% D-glucose, 1% yeast extract (Oxoid), 3% CaCO₃, and 2.5% agar or on one with 2.5% mannitol, 0.5% yeast extract (Oxoid), 0.3% peptone (Oxoid), and 2.5% agar. The Hoyer media were used as modified by Frateur (14) with either 3% (vol/vol) ethanol, glucose, or mannitol. To test for tolerance towards different temperatures and NaCl, glucose, and ethanol concentrations, a basal medium containing 5% D-glucose and 0.5% yeast extract (Oxoid) was used. Acid production from different carbon sources was detected in the medium of Asai et al. (4). The oxidation and overoxidation of ethanol to acetic acid, and to CO₂ and water, respectively, were tested by the method of Frateur (14). Gelatinase activity was tested on a medium containing (wt/vol) 2% mannitol, 0.1% D-glucose, 0.5% yeast extract (Oxoid), 0.3% peptone (Oxoid), and 12% gelatin. The reduction of nitrates was tested on a medium containing 5% D-glucose, 0.5% yeast extract (Oxoid), and 0.1% KNO₃. Indole formation was tested in a medium containing 1% tryptone (Oxoid), 0.2% yeast extract (Oxoid), and 1% D-glucose; H₂S formation was detected by lead acetate paper strips in a medium with 5% D-glucose, 0.5% peptone (Oxoid), 0.5% yeast extract (Oxoid), 0.1% L-cysteine, and 0.05% Na₂SO₄ (pH 7). Acetate and lactate oxidation and ketogenesis from glycerol, mannitol, and sorbitol were examined by the method of Frateur (14). For growth factor requirements, the procedure of Ameyama and

Kondô (1) was followed. Asai and Shoda's (5) ferric chloride reaction was utilized. The oxidase reaction was tested on filter paper soaked in 1% dimethyl-p-phenylenediamine. To test for catalase activity, young colonies (3 days old) were suspended in 10% (vol/vol) H₂O₂. The medium for the formation of ketogluconic acid consisted of 0.3% yeast extract (Oxoid) and 3% D-glucose. The 2-keto-, 5-keto-, and 2,5-diketogluconic acids were separated by thin-layer chromatography on microcrystalline (Merck) cellulose with ethyl acetate-water-acetic acid-formic acid (18:4:3:1, vol/vol) (6). The chromatograms were developed by use of the o-phenylenediamine spray reagent (9, 18).

Polyacrylamide gel electrophoresis of soluble proteins and numerical analysis of the electropherograms. Cells were grown for 40 h at 28°C in Roux bottles on the glucose-yeast extract-CaCO₃-agar medium. Cell-free extracts were prepared as described by Kersters and De Ley (16). The soluble protein fraction of each strain was dialyzed against 1 liter of 6.4 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 9.2). Polyacrylamide gel electrophoresis, densitometry, and photography of the stained gels, normalization of the densitometric tracings, computer-assisted calculations of the Pearson-product moment correlation coefficient *r*, and clustering were performed as described previously (16). Each protein extract was investigated in at least three different electrophoretic experiments.

DNA:rRNA hybridizations. The following strains were used: IFO 3246_t, 3247, 3248, 3249, 13330, and 13333. Cell growth, DNA preparation, fixation of DNA on filters, chemical determination of the amount of DNA on filter, the preparation of [¹⁴C]rRNA, and the hybridizations between fixed, single-stranded, high-molecular-weight DNA and 16S or 23S [¹⁴C]rRNA were carried out as described by De Ley and De Smedt (10). Reference [¹⁴C]rRNA was prepared from the type strains (22) of *Acetobacter aceti* subsp. *aceti* (NCIB 8621) (colony type t₁), *Gluconobacter oxydans* subsp. *oxydans* NCIB 9013 (= ATCC 19357), and *Pseudomonas fluorescens* ATCC 13525 and from *Xanthomonas campestris* NCPPB 528. From each hybrid the thermostability, expressed as $T_{m(e)}$ (the temperature at which half of the DNA:rRNA hybrid was denatured in 1.5 × SSC [SSC, 0.15 M NaCl–0.015 M sodium citrate, pH 7.0]–20% formamide), and the percent rRNA binding (the amount of [¹⁴C]rRNA, in micrograms, hybridized with 100 μg of single-stranded, high-molecular-weight DNA) were measured.

RESULTS

Phenotypic features. The properties of strains IFO 3245, 3247, 3249, 13328, 13330, 13331, and 13333 will be described first; strains IFO 3246_t and 3248 will be treated separately. Most of the features of these strains are shown in Table 2. We shall only clarify a few additional points here. Not all of the strains were motile. The actively motile strain 13331 had very thin polar flagella when stained by Rhodes' (19) flagella-staining technique. This confirmed the observations by the Japanese authors (2, 4, 25). Good growth was obtained on glucose-yeast extract–CaCO₃ agar. Strains 3249 and 13333 did not form a water-soluble, yellow-brown pigment. All strains tested grew easily without any growth factor. Some strains grew in yeast extract, in peptone broth, or in Hoyer media. Ammonium was utilized in a Hoyer medium containing mannitol as the sole carbon source. All strains grew very well on Casamino Acids, in contrast with other acetic acid bacteria, which do not. This phenomenon could also be deduced from Table 3 of Ameyama and Kondô (2). The oxidation of lactate to CO₂ was demonstrated by oxidograms. CaCO₃ precipitated when the strains were grown on a Ca-lactate-yeast extract-agar medium. In a liquid medium, every strain formed acid from ethanol, the final pH sometimes dropping down to 3.8. It was difficult, however, to get an unequivocal answer as to whether all strains formed acetic acid on ethanol-yeast extract–CaCO₃ plates. The oxidation of acetate was reported by Ameyama and Kondô (2) and Yamada et al. (25), but in the original description (17) it was clearly stated that the organisms did not oxidize acetic acid. From the respirometric data of Ameyama and Kondô (2), one can conclude

that these strains possess a distinct but very weak ability to oxidize acetate. On Frateur's acetate oxidograms or in the overoxidation test on ethanol-yeast extract–CaCO₃ medium, we found all strains to be negative. These contradictory results suggest that the eventual oxidation of acetate is very weak and that its detection depends on the sensitivity of the method. The ketogenic activity is another example of a weak or doubtful phenomenon. Ameyama and Kondô (2) found no ketogenic activity with strains 3245, 3246, 3247, α, and β. Yamada et al. (25) retested these strains and found no reaction. Asai et al. (4) had also noted a negative result with strains 3245 and 3246, but Asai (3) reported a positive reaction. In our hands a positive, but weak, ketogenic reaction was found with glycerol, mannitol, and sorbitol for all the strains tested. The test conditions were probably different, so that a slightly positive reaction might appear to be negative to some. We incubated the cells for 2 h at 30°C, covered the plates with Benedict reagent, and left them for 3 h at 30°C (for glycerol) or at 45°C (for sorbitol and mannitol). The Japanese authors attached great importance to the ferric chloride reaction. According to Yamada et al. (25), all of the "*A. aurantius*" strains gave a positive ferric chloride reaction, but we found no reaction for strains IFO 13328, 13330, 13331, and 13333. The ferric chloride reaction has some inconveniences: it is rather unspecific, because it reacts with phenols, enols, oximes and hydroxamic acids (20), and the different shades of red, violet, and brown are difficult to interpret as a definite positive or negative reaction; this is particularly true for the "*A. aurantius*" strains which produce water-soluble brown pigments. Kondô and Ameyama (17) stated that gluconic acid and a strong reducing acid, but not 5-ketogluconic acid, were formed from glucose. Subsequently, the formation of ketogluconic acids has not been mentioned. We established the production of both 2-keto- and 2,5-diketogluconic acids but not 5-ketogluconic acid by the seven strains tested. This combination of keto acids also occurs—although very rarely—with *Gluconobacter* and *Acetobacter* (15a). We detected acid formation from ethanol, glycerol, D-arabinose, D-xylose, D-ribose, D-fructose, D-glucose, D-galactose, and D-mannose, in agreement with previous observations (4, 17, 25). The production of acid from D-glucose and D-xylose was particularly manifest, since the pH always dropped below 4. We found the "*A. aurantius*" strains to grow at low pH values (pH 3.6), as was previously observed by Asai et al. (4) and Yamada et al. (25). These authors also grew them at 37°C, but we could not confirm this for all of the strains. At 34°C, however, the seven strains

[illegible]

TABLE 2—continued

Feature	Strain no.								
	IFO 3245	IFO 3247	IFO 3249	IFO 13328	IFO 13330	IFO 13331	IFO 13333	IFO 3248	IFO 3246t ₁
Requirement for growth factors									
<i>p</i> -Aminobenzoic acid	—	—	—	—	—	—	—	—	—
Thiamine	—	—	—	—	—	—	—	—	—
Nicotinic acid	—	—	—	—	—	—	—	—	+
Pantothenic acid	—	—	—	—	—	—	—	—	+
Biochemical reactions:									
Oxidase	—	—	—	—	—	—	—	—	—
Catalase	+	+	+	+	+	+	+	+	+
Reduction of nitrates	—	—	—	—	—	—	—	—	—
Formation of indole	—	—	—	—	—	—	—	—	—
Formation of H ₂ S	+	+	+	+	+	+	+	—	—
Ferric chloride reaction	+	+	+	—	—	—	—	—	—
Ketogenesis with:									
Glycerol	+	+	+	+	+	+	+	—	+
D-Mannitol	+	+	+	+	+	+	+	—	+
D-Sorbitol	+	+	+	+	+	±	+	—	+
Oxidogram on:									
Acetate	—	—	—	—	—	—	—	—	—
Lactate	+	+	+	+	+	+	+	—	—
Gelatinase	—	—	—	—	—	—	—	—	—
Acid from ethanol (Frateur)	+	+	+	—	—	—	+	+	—
Overoxidation of ethanol (Frateur)	—	—	—	—	—	—	—	+	—
Formation of 5-ketogluconic acid	—	—	—	—	—	—	—	—	+
Formation of 2-ketogluconic acid	+	+	+	+	+	+	+	+	+
Formation of 2,5-diketogluconic acid	+	+	+	+	+	+	+	—	—
Acid from:									
Ethanol	+	+	+	+	+	+	+	+	—
Glycerol	—	+	+	+	+	+	+	—	—
<i>m</i> -Erythritol	—	—	—	—	—	+	—	—	+
D-Arabinose	+	+	+	+	+	+	+	—	+
D-Ribose	+	+	+	+	+	+	+	—	+
D-Xylose	+	+	+	+	+	+	+	+	+
L-Rhamnose	—	—	—	+	+	+	+	—	—
D-Fructose	+	+	+	+	+	+	+	—	+
D-Galactose	+	+	+	+	+	+	+	—	+
D-Glucose	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	—
L-Sorbose	—	—	—	—	—	—	—	—	+
Mannitol	—	—	—	—	—	—	—	—	+
D-Sorbitol	—	—	—	—	—	—	—	—	+
D-Cellobiose	—	—	—	—	—	+	+	—	—
D-Lactose	—	—	—	—	—	+	+	—	—
Maltose	—	—	—	—	—	—	—	+	+
Sucrose	+	+	+	—	—	—	—	—	+
Raffinose	—	—	—	—	—	—	—	—	+
Dextrin	—	—	—	—	—	—	—	—	+
Starch	—	—	—	—	—	—	—	—	—
Susceptibility to:									
Kanamycin (30 µg)	+	+	+	+	+	+	+	—	+
Streptomycin (10 µg)	+	+	+	+	+	+	+	—	+
Tetracycline (30 µg)	+	+	+	+	+	+	+	+	+
Novobiocin (30 µg)	+	+	+	+	+	+	+	+	+
Ampicillin (10 µg)	+	+	+	+	—	+	+	+	—
Nalidixic acid (30 µg)	+	+	+	—	+	+	—	+	+
Gentamicin (10 µg)	—	—	—	—	—	—	—	—	—
Chloramphenicol (30 µg)	—	—	—	—	—	—	—	—	—
Cephaloridine (25 µg)	+	—	+	+	+	—	—	—	—

TABLE 2—continued

Feature	Strain no.								
	IFO 3245	IFO 3247	IFO 3249	IFO 13328	IFO 13330	IFO 13331	IFO 13333	IFO 3248	IFO 3246t ₁
Bacitracin (10 U)	—	—	—	—	—	—	—	—	—
Penicillin G (10 U)	—	—	—	—	—	—	—	—	—
Erythromycin (10 µg)	—	—	—	—	—	—	—	—	—
Lincomycin (2 µg)	—	—	—	—	—	—	—	—	—
Colistin sulfate (10 µg)	—	—	—	—	—	—	—	—	—
Fusidic acid (10 µg)	—	—	—	—	—	—	—	—	—
Nitrofurantoin (200 µg)	—	—	—	—	—	—	—	—	—
Methicillin (10 µg)	—	—	—	—	—	—	—	—	—
Neomycin (30 µg)	—	—	—	—	—	—	—	—	—
Polymyxin B (300 U)	—	—	—	—	—	—	—	—	—
Sulfafurazole (100 µg)	—	—	—	—	—	—	—	—	—

^a These strains were received as "*Acetobacter aurantius*."

grew well. Kondô and Ameyama (17) characterized "*A. aurantius*" as a gelatin liquefier, but in a later publication (2) they reported no gelatin liquefaction for strains IFO 3245, 3246, 3247, 3248, α , β , and γ . We found no gelatin liquefaction. We also examined a series of features that was not tested by other authors. The sensitivity to 0.5 to 2% NaCl was determined, and strains 3245, 3247, and 3249 grew very well; the others did not. Ethanol concentrations above 5% were not tolerated. Five of the seven strains grew in 25 to 30% glucose concentrations. All of the "*A. aurantius*" strains produced catalase and H₂S; they do not possess oxidase, do not produce indole, and do not reduce nitrates. Acetic acid bacteria do not produce H₂S.

Strain IFO 3246t₁ is very different from the other strains described here. It did not grow at or above 34°C, in 20% or more glucose, or in Hoyer's medium with mannitol. It did not produce brown pigment on glucose-yeast extract-CaCO₃ agar; it required nicotinic acid and pantothenate as growth factors; it was not susceptible to 10 µg of ampicillin; it produced 5-ketogluconic but not 2,5-diketogluconic acid; it did not produce H₂S; it did not produce CO₂ from lactate or detectable acid from ethanol, meso-erythritol, or mannose; and it produced acid from L-sorbose, mannitol, sorbitol, raffinose, and dextrin (Table 2). Phenotypically, IFO 3246t₁ is a *Gluconobacter*, but an atypical one because it forms no detectable acetic acid from ethanol.

Strain IFO 3248 is likewise very different from the above-mentioned "*A. aurantius*" strains (Table 2). It grew very poorly on glucose or on Hoyer-mannitol media and did not form a water-soluble brown pigment. From ethanol it produced acetic acid, which was further oxidized to CO₂ and water; it did not form H₂S and was not ketogenic. This strain is an atypical *Acetobacter*

because it does not oxidize lactate to CO₂ and water. Recently, this strain was examined at the IFO (I. Banno, personal communication): it grew weakly, produced no diffusible pigment on glucose medium, was peritrichous, was not ketogenic on glycerol, acidified ethanol, oxidized lactate very slowly but not acetate, and could not utilize NH₄⁺ as a sole source of nitrogen. The IFO confirmed our conclusion that it was different from the other "*A. aurantius*" strains studied.

Protein electropherograms. Forty-four normalized scans of protein electropherograms from nine "*A. aurantius*" strains were clustered by the unweighted average-pair group method. The lower limits of reproducibility were as follows: for strains 3245 (6 gels) and 3247 (6 gels), $r = 0.98$; for strain 3249 (3 gels), $r = 0.99$; and for strains 13328 to 13333 (29 gels), $r = 0.97$. The most typical electropherogram of each strain (23) was used for the final clustering represented in Fig. 1. The electrophoretic protein patterns of strains IFO 3249, 13328, 13329, 13330, 13331, 13332, and 13333 are almost indistinguishable from each other and cluster at $r \geq 0.97$. Their electropherograms are very similar to, but not identical with, those of strains 3245 and 3247. The protein electropherograms of strains IFO 3246t₁ and IFO 3248 are completely different from those of the nine strains mentioned above and from each other.

DNA:[¹⁴C]rRNA hybridizations. The results of the DNA:[¹⁴C]rRNA hybridizations are shown in Table 3. The hybridizations between labeled rRNA and the DNAs from the reference *Acetobacter*, *Gluconobacter*, *Pseudomonas*, and *Xanthomonas* strains are reported as controls. The DNA:rRNA hybrids of the "*A. aurantius*" strains IFO 3247, 3249, 13330, and 13333 have similar thermal stabilities versus each reference

FIG. 1. Quantitative correlations between normalized electropherograms of soluble proteins from nine *Frateuria aurantia* strains, *Gluconobacter* sp. IFO 3246_{t1}, and *Acetobacter* sp. IFO 3248. All strains were received as "*Acetobacter aurantius*."

TABLE 3. Properties of the DNA:[¹⁴C]rRNA hybrids between [¹⁴C]rRNA from several type and reference strains^a and DNA from four *Frateuria aurantia* strains^b

DNA from:	Source of [¹⁴ C]rRNA							
	<i>Acetobacter aceti</i> subsp. <i>aceti</i> NCIB 8621 _{t1}		<i>Gluconobacter oxydans</i> subsp. <i>oxydans</i> NCIB 9013		<i>Pseudomonas fluorescens</i> ATCC 13525		<i>Xanthomonas campestris</i> NCPPB 528	
	<i>T</i> _{m(e)} (°C)	% Binding	<i>T</i> _{m(e)} (°C)	% Binding	<i>T</i> _{m(e)} (°C)	% Binding	<i>T</i> _{m(e)} (°C)	% Binding
<i>Acetobacter aceti</i> subsp. <i>aceti</i> NCIB 8621 _{t1}	81.0	0.17	76.0	0.13	57.0	0.04	58.5	0.04
<i>Gluconobacter oxydans</i> subsp. <i>oxydans</i> NCIB 9013	77.5	0.22	81.5	0.27	ND	ND	ND	ND
<i>Pseudomonas fluorescens</i> ATCC 13525	ND	ND	58.0	0.15	81.0	0.14	69.0	0.08
<i>Xanthomonas campestris</i> NCPPB 528	ND	ND	ND	ND	69.0	0.07	81.0	0.09
<i>Frateuria aurantia</i> IFO 13330	59.8	0.11	57.8	0.10	65.1	0.13	72.5	0.14
<i>Frateuria aurantia</i> IFO 13333	60.0	0.08	60.0	0.10	ND	ND	71.0	0.13
<i>Frateuria aurantia</i> IFO 3249	59.3	0.10	61.8	0.12	66.0	0.10	72.0	0.11
<i>Frateuria aurantia</i> IFO 3247	60.5	0.09	60.5	0.15	66.5	0.12	71.5	0.15
<i>Acetobacter</i> sp. IFO 3248	78.0	0.18	77.3	0.19	57.5	0.10	61.0	0.08
<i>Gluconobacter</i> sp. IFO 3246 _{t1}	76.8	0.13	80.3	0.14	57.0	0.08	61.0	0.09

^a DNA from the latter strains was included for comparison. A mixture of 16S and fragmented 23S rRNA had to be used from the *Acetobacter* and *Gluconobacter* strains; 23S rRNA was used from the *Pseudomonas* and *Xanthomonas* strains. *T*_{m(e)} and percent rRNA binding have been defined in the text; ND, Not determined.

^b All IFO strains were received as "*Acetobacter aurantius*."

DNA:rRNA duplex. The rRNA cistrons of these four strains are more similar to those of *Xanthomonas* and *Pseudomonas* than to those of *Acetobacter* and *Gluconobacter*. The rRNA cistrons of "*A. aurantius*" IFO 3246_{t1} are indistinguishable from those of *Gluconobacter* (15). The rRNA cistrons of strain IFO 3248 are closer to those of *Acetobacter* than to those of *Gluconobacter*.

DISCUSSION

It may be useful first to consider briefly the taxonomic conclusions to be drawn from the methods of DNA:rRNA hybridizations and protein gel electropherograms. The former method, conceived and developed in this laboratory, is most useful for grouping and identifying organisms at the levels of genus, tribe, family, and

order. The method usually does not have enough resolution to distinguish taxa below the genus or subgenus level (11-13). It has been found that $T_{m(e)}$, the midpoint of the thermal denaturation curve of the DNA:rRNA hybrid and a measure of base differences between rRNA cistrons, is most meaningful in drawing taxonomic conclusions because it is empirically directly correlated with overall phenotypic similarities. The more the $T_{m(e)}$ value of the hybrid resembles the $T_{m(e)}$ value of the homologous duplex, the more similar are the rRNA cistrons and the more closely the organisms are related. The comparison of protein electropherograms (16; Kersters and De Ley, in press) has a different value. It has a very high resolution and is capable of detecting nearly identical strains. It is most useful in elucidating the internal taxonomic structures of genera and species.

From the three methods used (phenotypic features, protein gel electropherograms, DNA:rRNA hybridizations), we have determined unambiguously that the "*A. aurantius*" strains can be placed into three groups: (group 1) IFO 3246t₁, (group 2) IFO 3248, and (group 3) IFO 3245, 3247, 3249, and 13328 to 13333.

Strain IFO 3248. Hybrids between DNA from strain IFO 3248 and [¹⁴C]rRNA from the type strains of the type species of *Acetobacter* and *Gluconobacter* (Table 3) have features indicating that IFO 3248 belongs in *Acetobacter*; this conclusion is clear from our previous extensive study on the acetic acid bacteria (15). Our phenotypic analysis (Table 2) confirms that this

strain is an *Acetobacter*, although an atypical one.

Strain IFO 3246t₁. Hybrids between DNA from strain IFO 3246t₁ and [¹⁴C]rRNA from the type strains of the type species of *Gluconobacter* and *Acetobacter* (Table 3) show unambiguously that this strain is an authentic *Gluconobacter* (15). Our phenotypic analysis (Table 2) of these strains confirms this. The exact taxonomic position of both strains within the genera *Acetobacter* and *Gluconobacter* is being examined and will be reported later.

Strains IFO 3245, 3247, 3249, and 13328 to 13333. Of 99 phenotypic characters of strains IFO 3245, 3247, 3249, and 13328 to 13333, 77 are identical or nearly so (Table 2). The protein electropherograms of these nine strains are very nearly identical. Bacteria with similar protein electropherograms are genetically very similar (16; Kersters and De Ley, in press). We can thus conclude that the nine strains mentioned above constitute a genetically and phenotypically homogeneous group. A number of phenotypic features show that these bacteria are quite different from both *Gluconobacter* and *Acetobacter* (Table 4).

The fact that these nine strains are able to produce acetic acid from ethanol and to grow at pH 3.6 suggests superficially that taxonomically they are close relatives of *Gluconobacter* and *Acetobacter*. However, the similarity of a few phenotypic features is often an unreliable and fallacious guide in detecting relationships at the inter- and suprageneric levels. Our DNA:rRNA

TABLE 4. Features differentiating *Frateruia*, *Gluconobacter*, and *Acetobacter*^a

Feature	<i>Frateruia</i>	<i>Gluconobacter</i>	<i>Acetobacter</i>
Flagellar arrangement	Polar or none	Polar or none	Peritrichous or none
Oxidation of acetate to CO ₂	—	—	+
Oxidation of lactate to CO ₂	+	—	+
Ketogenesis	+	+	(—)
Vitamin requirements	—	+	(—)
Formation of a yellow-brown soluble pigment on glucose-yeast extract-CaCO ₃	(+)	(—)	(—)
Formation of H ₂ S	+	—	—
Formation from glucose of:			
5-Ketogluconic acid	—	+	D
2-Ketogluconic acid	+	+	(+)
2,5-Diketogluconic acid	+	(—)	(—)
Presence of ubiquinone	Q8	Q10	Q9-Q10
Growth on Frateur's Hoyer mannitol medium	+	(—)	(—)
Guanine plus cytosine content of DNA (mol %)	62-64	57-64	51-65

^a This table includes our own data, these from *Bergey's Manual* (7) from Yamada et al. (24, 25), Asai et al. (4), Ameyama and Kondô (1, 2), and Gillis and De Ley (15). Symbols: + or —: all the strains are positive or negative; (+) or (—): most strains are positive or negative; D: different reactions.

hybridization method is one of the few methods which so far have been able to detect these relationships.

Our results (Table 3) show that the rRNA cistrons from strains IFO 3247, 3249, 13330, and 13333 are not related to those from *Acetobacter* and *Gluconobacter*. With a $T_{m(e)}$ of about 60°C, they cannot belong to *Acetobacter* or *Gluconobacter* and are not even members of the rRNA superfamily (15) of which these genera constitute a separate branch. Our rRNA data (Table 3) show that these nine strains do not fit in any known genus and that they belong in a separate genus. We propose to name this new genus *Frateruria* after the late Joseph Frateur (1903–1974), eminent Belgian microbiologist, renowned for his important contributions to the taxonomy of the acetic acid bacteria.

DNA:rRNA hybrids between DNA from strains of *Frateruria* and 23S [¹⁴C]rRNA from *Pseudomonas fluorescens* ATCC 13525 and *Xanthomonas campestris* NCPPB 528 show $T_{m(e)}$'s of about 67 and 72°C, respectively. It should be stressed that the strains of *P. fluorescens* and *X. campestris* used here are type strains, each representative of a large cluster of related strains (De Vos and De Ley, manuscript in preparation). The rRNA cistrons of the *Frateruria* strains clearly have many base sequences in common with those from *P. fluorescens* ATCC 13525 and still more with those from *X. campestris* NCPPB 528. The genera *Xanthomonas*, *Azotobacter*, and *Azomonas*, the pseudomonads of Section I (7), and a few other organisms constitute a separate rRNA superfamily (12; De Vos and De Ley, manuscript in preparation). *Frateruria* also belongs in this rRNA superfamily. Thus, although it is taxonomically most closely related to the genus *Xanthomonas*, it does not belong in it. This is supported not only by phenotypic differences but also by the fact that no member of the genus *Xanthomonas* forms a DNA:rRNA hybrid with [¹⁴C]-rRNA from *X. campestris* with a $T_{m(e)}$ below 75°C.

Description of *Frateruria* gen. nov. Cell morphology. Gram-negative, straight rods, 0.5 to 0.7 by 0.7 to 3.5 μ m, which occur singly or in pairs, rarely in filaments or as irregular cells and never in chains. Motile by means of polar flagella.

Colonial morphology. Luxuriant growth occurs on glucose-yeast extract-CaCO₃ medium. After 6 days at 30°C, the brownish colonies measure 2 to 5 mm in diameter, have a dark brown center, and are glistening, flat, or raised and circular with a regular edge. Some strains show a typical browning of the medium—due to

a water-soluble pigment—after 3 days of incubation, others only after 6 to 10 days. The color tends to become dark coffee brown. After 4 weeks no visible crystals of Ca-gluconate are formed. Good growth is also obtained on mannitol-yeast extract-peptone agar. After 6 days at 30°C the colonies are yellow to orange, glistening or rough, regular or irregular, highly convex with undulate or regular edge, and 1 to 3 mm in diameter. They become pale brown and reach a diameter of up to 6 mm after 6 to 19 days.

Growth characteristics. Strictly aerobic. Growth occurs in beer wort, but in peptone broth, yeast extract broth, and Hoyer medium with mannitol, growth is faint. Growth factors are not required. Growth occurs at pH 3.6, in a medium containing 20% glucose, and at 30 and 34°C but not at 4°C. One percent ethanol is tolerated.

Susceptibility towards antibiotics. Susceptible to kanamycin (30 μ g), streptomycin (10 μ g), tetracycline (30 μ g), and novobiocin (30 μ g). Resistant to gentamicin (10 μ g), chloramphenicol (30 μ g), bacitracin (10 U), penicillin G (10 U), erythromycin (10 μ g), lincomycin (2 μ g), colistin sulfate (10 μ g), fucidin (10 μ g), nitrofurantoin (200 μ g), methicillin (10 μ g), neomycin (30 μ g), polymyxin B (300 U), and sulfafurazole (100 μ g).

Biochemical features. Acid is formed from ethanol, glycerol, D-arabinose, D-ribose, D-xylose, D-fructose, D-galactose, D-glucose, and D-mannose. Acid is not produced from L-sorbose, raffinose, dextrin, or starch. With D-glucose and D-xylose, the pH drops below 4. The strains are ketogenic on glycerol, mannitol, and sorbitol. From D-glucose, 2-keto- and 2,5-diketogluconic acids are formed, but not 5-ketogluconic acid. Lactate, but not acetate, is oxidized to CO₂ and water. Oxidase and gelatinase are not produced. Litmus milk is unchanged. Nitrates are not reduced. Indole is not produced. Catalase, a characteristic ubiquinone, Q8, and H₂S are produced.

The only species, and thus the type species, is *Frateruria aurantia* sp. nov. (au.rañ tia, M.L. adj. *aurantius* gold colored; derived from *aurare*, C.L. verb, to overlay with gold; refers to the gold-yellow color of the strains on mannitol-yeast extract peptone agar). The description of this species is the same as that given above for the genus. The type strain of *F. aurantia* is IFO 3245. A description of this strain is given in Table 2. Diagnostic features useful in differentiating *Frateruria* from *Gluconobacter* and *Acetobacter* can be found in Table 4.

For the genus *Frateruria* as well as for its only species, *Frateruria aurantia*, we propose as minimal standards: gram-negative, polarly flagellated if motile, strictly aerobic rods requiring no

growth factors, producing a brown water-soluble pigment on glucose-yeast extract- CaCO_3 agar, producing the ubiquinone Q8, able to grow at pH 3.6 and on Frateur's Hoyer mannitol medium, producing acid from ethanol, glucose, and xylose, and lacking oxidase, gelatinase, nitrate reduction, and starch hydrolysis; they produce H_2S . From glucose, 2-keto- and 2,5-diketogluconic acids are formed, but not 5-ketogluconic acid. The guanine plus cytosine content of the DNA ranges from 62 to 64 mol%.

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