Microbiol. Cult. Coll. Dec. 2003. p. 81－90 Vol. 19, No. 2

Identification of *Frateuria aurantia* Strains Isolated from Indonesian Sources

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In the course of the isolation of acetic acid bacteria from tropical countries, 16 water-soluble brown pigment-producing bacteria were isolated from fruits and flowers in Indonesia. Two representative isolates were clustered in the *γ-Proteobacteria* together with the genus *Frateuria* by 16S rRNA gene sequence analysis. All isolates were identified as *Frateuria aurantia* by DNA-DNA similarity. The strains were aerobic, Gram-negative rods and motile with a single polar flagellum. They grew at pH 3.5, produced acetic acid from ethanol and brown water-soluble pigment, oxidized lactate but not acetate, did not grow in the presence of 0.35 % acetic acid, and did not produce H_2S. Further, they produced D-gluconate, 2-keto-D-gluconate, and 2, 5-diketo-D-gluconate from D-glucose but not 5-keto-D-gluconate. All isolates tested had Q-8 as the major ubiquinone and *iso*-branched acid of $C_{15:0}$ as the major cellular fatty acid. The DNA base composition of the isolates ranged from 62 to 63 mol %. This study is the fourth case of the isolation of *F. aurantia* strains so far, and showed the distribution of this species not only in Japan but also in tropical regions.

Key words: *Frateuria aurantia*, acetic acid bacteria

INTRODUCTION

The genus *Frateuria* was validly established by Swings et al. in 1980 (14, 15) for bacteria that are polarly flagellated, produce brown pigment, have branched cellular fatty acids , and are Q-8 equipped Gram-negative, incorporating "*Acetobacter aurantius*" (*Acetobacter aurantium* sic) described by Kondo and Ameyama (9, 10). *Frateuria aurantia* is the type species and only a species in this genus. The genus *Frateuria* is recognized as pseudo-acetic acid bacteria because the strains showed intermediate characteristics to acetic acid bacteria (1, 2, 20).

Up to now, three cases of the isolation of *F. aurantia* have been reported and were all conducted in Japan. First, Kondo and Ameyama (9, 10) reported the isolation of five strains from lily flowers (*Lilium aurantum*). The second case was reported by Ameyama and Kondo (1) with three strains isolated from rose (*Rose hybrida*), saffron (*Crocus sativus*), and three-lady bell (*Adenophora triphyla*), respectively. Thirdly, Yamada et al. (20) isolated six strains from raspberry (*Rubus parvifolius*). However, of the 14 above-mentioned isolates, only nine strains are preserved as *F. aurantia* in culture collections at present. Three strains (IFO 3245, 3247, and 3249) were those isolated by Kondo and Ameyama (9, 10) and six strains (IFO 13328 to 13333) by Yamada et al. (20).

In the course of isolation of acetic acid bacteria from sources of tropical countries, 16 water-soluble brown pigment-producing bacteria were isolated from flowers and fruits in Indonesia.

This study aims to identify the isolates compared with the strains of the genera in the family *Acetobacteraceae*.

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MATERIALS AND METHODS

Isolation and cultivation of acetic acid bacteria

One hundred and forty-nine sources including fermented foods, fruits, and flowers were collected in 1999 at Yogyakarta and Bogor in Indonesia. On-site isolation was conducted and three enrichment media at pH 3.5 (EM II, EM IV, and EM V) were employed for the isolation. EM II was composed of 2.0 % D-sorbitol, 0.5 % peptone, 0.3 % yeast extract, and 100 ppm cycloheximide. The compositions of EM IV and EM V were basically the same as that of EM II but 2.0 % D-sorbitol was replaced with 2.0 % dulcitol for EM IV, and 0.15 % D-glucose and 1.0 % methanol for EM V. When microbial growth occurred in enrichment media, bacterial strains were isolated by the method described by Yamada et al. (23). The isolation medium contained 2.0 % D-glucose, 0.8 % yeast extract, 0.5 % peptone, 0.5 % ethanol, 0.3 % $CaCO_3$, and 1.5 % agar. Isolates were maintained on agar slants of AG medium consisting of 0.1 % D-glucose, 1.5 % glycerol, 0.5 % peptone, 0.5 % yeast extract, 0.2 % malt extract, 0.7 % $CaCO_3$, and 1.5 % agar as described by Katsura et al. (7).

Reference strains used

F. aurantia IFO 3245^T and IFO 13328, *Acetobacter aceti* IFO 14818^T, *Acidomonas methanolica* NRIC 0498^T, *Asaia bogorensis* NRIC 0311^T, *Gluconacetobacter liquefaciens* IFO 12238^T, *Gluconobacter oxydans* IFO 14819^T, *Kozakia baliensis* NRIC 0488^T, *Escherichia coli* NRIC 1999, and *Pseudomonas aeruginosa* NRIC 0201^T were used as reference strains.

16S rRNA gene sequencing and phylogenetic analysis

The amplification, sequencing, and analytical method of 16S rRNA genes were those described by Lisdiyanti et al. (12).

Determination of DNA base composition and DNA-DNA hybridization

DNAs were extracted by the method of Saito and Miura (13). DNA base composition was determined as described by Tamaoka and Komagata (17). DNA-DNA hybridization was carried out as described by Ezaki et al. (5).

Analysis of ubiquinones and cellular fatty acids

Cells cultivated on AG medium were used for the analysis of ubiquinones and cellular fatty acids. Ubiquinones were extracted as described by Yamada et al. (19) and analyzed as described by Tamaoka et al. (18). Methyl esters of cellular fatty acids were prepared by the direct transmethylation method with methanolic hydrochloride and were analyzed by gas chromatography as described by Komagata and Suzuki (8).

Phenotypic characterization

Phenotypic characterizations used in this study were mostly those described by Lisdiyanti et al. (12). Oxidation of acetate and lactate was tested by the method of Leifson (11). Ketogenesis from glycerol and mannitol were examined by the method of Shimwell et al. (16) and Asai et al. (2). Production of H_2S was detected by lead acetate paper strips on a medium containing 5.0 % D-glucose, 0.5 % peptone, 0.5 % yeast extract, 0.1 % L-cysteine, and 0.05 % Na_2SO_4 (pH 7.0) as described by Swings et al. (14) and on Triple Sugar Iron agar medium (TSI) (Eiken, Tokyo, Japan). Growth on methanol was examined by using yeast extract omitted medium C as described by Yamashita et al. (24).

RESULTS

Isolation of acetic acid bacteria and identification at the genus level

Of 149 samples collected in 1999 in Indonesia, only 24 samples showed bacterial growth on enrichment media. As a result, 62 isolates of acetic acid bacteria were selected for subsequent study because they were all Gram-negative, rod-shaped bacteria, produced clear zones on the isolation medium, and grew on AG medium at pH 3.5. Of the 62 isolates, 16 were regarded as *Frateuria* strains because they produced water-soluble brown pigment, oxidized ethanol, did not grow in the presence of 0.35 % acetic acid, and had Q-8 as the major ubiquinone. The remaining 11 isolates were presumed to be *Gluconobacter* and 35 isolates to be *Asaia*. The 16 *Frateuria* strains were subjected to the subsequent study. The designation of isolates is shown in Table 1.

Table 1. List of isolates and their sources

Isolate	Time of isolation		Sources	EM used	Place of isolation
B71D-1, B71D-3, B71D-4	February, 1999	fruit	*Mangifera foetida*	EM IV	Bogor
B72D-1, B72M-1, B72M-2, B72M-3, B72M-4, B72M-5	February, 1999	fruit	*Baccaurea racemosa*	EM IV and V	Bogor
B73M-1, B73M-3A, B73M-3B	February, 1999	fruit	*Artocarpus champeden*	EM V	Bogor
B79M-1	February, 1999	fruit	kemaris	EM V	Bogor
Y3S-1A, Y3S-1B, Y3S-3	February, 1999	flower	coconut flower	EM II	Yogyakarta

Abbreviation: EM., Enrichment medium.

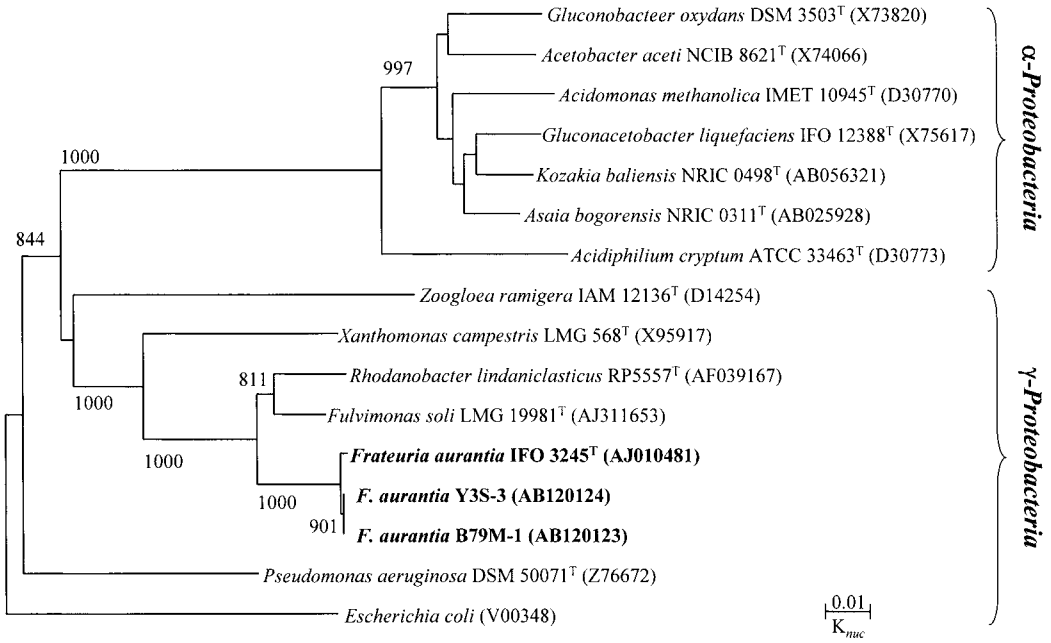

Fig. 1. Phylogenetic relationship of *Frateuria* strains deduced from 16S rRNA gene sequences
The scale bar represents 1 nucleotide substitution per 100 nucleotides. Numerals indicate the bootstrap value derived from 1000 replications.

Phylogenetic analysis

Two isolates (B79M-1 and Y3S-3) were determined for their 16S rRNA gene sequences. The phylogenetic tree of the isolates is shown in Fig. 1. The two representative isolates were located in the γ-Proteobacteria together with *F. aurantia* IFO 3245^T (AJ010481) and were closely related to the genera *Fulvimonas* and *Rhodanobacter*. Sequence similarity of the isolates was 99.8 % to *F. aurantia* IFO 3245^T (AJ010481). The accession number of 16S rRNA gene sequences in DDBJ was AB120123 for B79M-1 and AB120124 for Y3S-3.

DNA base composition and DNA-DNA similarity

All isolates had the DNA base composition ranging from 62 to 63 mol %. They showed high values (76 to 100 %) of DNA-DNA similarity to the type strain of *F. aurantia* IFO 3245^T and *F. aurantia* IFO 13328. They showed low values (10 to 36 %) of DNA-DNA similarity to other reference strains. Thus, all strains studied were identified as *F. aurantia*. Table 2 shows DNA base compositions and values of DNA-DNA similarity of the 16 isolates and the reference strains.

Ubiquinone systems and fatty acid compositions

The 16 isolates had Q-8 as the major ubiquinone that accounted for 97.5 to 100 %, and *iso*-branched acid of $C_{15:0}$ as the major cellular fatty acid that accounted for 40.6 to 51.0 % of the total acids (Table 3).

Table 2. DNA base compositions and values of DNA-DNA similarity

Strain or isolate	G+C content (mol%)	DNA-DNA similarity (%) with strain: IFO 3245^T	IFO 13328	B79M-1	Y3S-3
F. aurantia IFO 3245^T	63	100	82	83	76
IFO 13328	62	100	100	100	92
B71D-1	62	100	93	100	90
B71D-3	63	100	87	95	84
B71D-4	62	100	92	93	91
B72D-1	62	88	nd	nd	nd
B72M-1	62	86	nd	nd	nd
B72M-2	63	100	93	99	92
B72M-3	62	89	76	79	69
B72M-4	62	95	78	78	71
B72M-5	62	89	75	77	76
B73M-1	62	92	nd	nd	nd
B73M-3A	62	89	nd	nd	nd
B73M-3B	62	91	78	74	73
B79M-1	62	94	79	100	73
Y3S-1A	62	100	100	100	100
Y3S-1B	63	100	98	97	97
Y3S-3	63	100	95	100	100
Ga. liquefaciens IFO 12388^T	64	20	nd	23	nd
G. oxydans IFO 14819^T	62	36	nd	25	nd
As. bogorensis NRIC 0311^T	60	21	nd	26	nd
K. baliensis NRIC 0488^T	57	32	nd	nd	nd
A. aceti IFO 14818^T	57	12	nd	nd	nd
Ac. methanolica NRIC 0458^T	62 *	15	nd	nd	nd

Abbreviations: *A., Acetobacter*; *Ac., Acidomonas*; *As., Asaia*; *F., Frateuria*; *Ga., Gluconacetobacter*; *G., Gluconobacter*; *K., Kozakia*; ^{T}, type strain; nd, not determined.
* cited from reference (24).

Phenotypic characteristics of 16 *Frateuria* strains

All isolates were aerobic, Gram-negative rods measuring from 0.4 to 0.8 μm by 0.8 to 2.0 μm, motile with a single polar flagellum, and occurring singly and in pairs. They produced water-soluble brown pigment on glucose-yeast extract-$CaCO_3$ agar medium as did *Gluconacetobacter liquefaciens* strains and their colonies were orange on mannitol-yeast extract-peptone agar medium. The strains produced catalase but not oxidase. They produced acetic acid from ethanol, and oxidized lactate but not acetate. The ketogenesis from glycerol was found on 10 isolates and from mannitol on 7 isolates. The isolates grew at pH 3.5 but did not grow in the presence of 0.35% acetic acid, as did *Asaia* strains. All isolates produced D-gluconate, 2-keto-D-gluconate, and 2, 5-diketo-D-gluconate from D-glucose but not 5-keto-D-gluconate. They utilized ammoniac nitrogen with mannitol as a sole source of carbon, but did not utilize it with glucose or ethanol. They also grew on D-mannitol agar and glutamate agar. The isolates and *F. aurantia* IFO 3245^T and IFO 13328 slightly blackened the tip of lead acetate paper strips, and *P. aeruginosa* NRIC 0201^T and *E. coli* NRIC 1999 did as well. The production of H_2S was not detected along the line of the stab inoculation of TSI agar medium on the isolates, *F. aurantia* strains, acetic acid bacteria, *P. aeruginosa* NRIC 0201^T, and *Escherichia coli* NRIC 1999.

The isolates did not utilize methanol as a sole source of carbon on yeast extract omitted medium C. The produc-

Table 3. Ubiquinone systems and fatty acid compositions of the isolates

Strain No.	Ubiquinone composition (%)			Fatty acid compisition (%)*						
				Saturated		Unsaturated				
	Q-8	Q-9	Q-10	$C_{14:0}$	$C_{16:0}$	2-OH $C_{12:0}$	3-OH $C_{12:0}$	*i*-$C_{15:0}$	*i*-$C_{17:1}$w9c	*i*-$C_{17:0}$
B71D-1	100.0	0.0	0.0	2.8	9.1	2.8	7.0	46.0	2.3	5.8
B71D-3	99.1	0.6	0.3	nd	nd	nd	nd	nd	nd	nd
B71D-4	99.8	0.2	0.0	2.4	5.1	2.0	6.4	51.0	3.5	5.3
B72D-1	99.2	0.4	0.4	3.1	8.2	2.5	8.5	45.4	2.3	5.1
B72M-1	99.3	0.4	0.3	3.4	10.9	2.7	10.5	49.4	1.8	5.0
B72M-2	98.8	0.5	0.6	4.1	11.9	2.8	8.2	40.6	1.8	5.6
B72M-3	99.1	0.4	0.4	3.1	8.9	2.2	7.1	46.7	2.4	5.6
B72M-4	98.8	0.3	0.9	2.5	5.9	1.9	6.9	49.7	2.5	4.1
B72M-5	99.2	0.5	0.4	2.4	7.2	2.0	10.7	42.8	2.5	5.5
B73M-1	98.7	0.5	0.8	2.8	8.2	2.3	7.7	46.5	2.4	5.7
B73M-3A	99.0	0.5	0.6	2.5	5.7	2.1	7.2	49.8	2.4	4.0
B73M-3B	100.0	0.0	0.0	nd	nd	nd	nd	nd	nd	nd
B79M-1	97.6	0.5	1.8	2.3	5.9	2.4	7.0	49.7	2.6	5.1
Y3S-1A	98.4	0.6	1.0	2.8	5.6	2.6	7.8	45.2	2.4	5.2
Y3S-1B	100.0	0.0	0.0	nd	nd	nd	nd	nd	nd	nd
Y3S-3	97.5	0.4	2.1	2.9	8.3	2.3	7.5	46.7	2.5	5.1

Abbreviations: nd, not determined.

* Values are percentage of total fatty acids; values less than 1 % are not shown.

tion of γ-pyrones from glucose and fructose was difficult to determine because the isolates produced brown pigment in the medium.

The 16 isolates produced acid from L-arabinose, D-arabinose, D-ribose, D-xylose, L-rhamnose, D-glucose, D-galactose, D-mannose, D-fructose, glycerol, and ethanol but not from L-sorbose, sucrose, D-mannitol, and D-sorbose. Tables 4 and 5 present the differential phenotypic characteristics of the isolates and reference strains used in this study.

DISCUSSION

Since the last isolation of *Frateuria* strains reported by Yamada et al. (20) in 1976, we have succeeded after 23 years in isolating 16 *F. aurantia* strains from fruits and flowers in Indonesia. The isolates were properly identified by phenotypic characteristics, ubiquinone systems, cellular fatty acid compositions, DNA base compositions, levels of DNA-DNA similarity, and 16S rRNA gene sequences. In this study, we revealed that *Frateuria* strains were distributed not only in Japan but also in tropical regions. The same as the previous isolation, *F. aurantia* inhabited fruits and flowers.

The oxidation of acetate is problematic in taxonomic interpretation for the genus *Frateuria*. Kondo and Ameyama (9, 10) stated acetate oxidation-negative for "*Acetobacter aurantium*" based on the data by respirometry in the original description. Later, Ameyama and Kondo (1) reported the growth of "*Acetobacter aurantium*" at the expense of acetate and lactate and oxygen uptake for these substances. Meanwhile, Asai et al. (2) and Yamada et al. (20) tested the oxidation of acetate and lactate by Leifson's method (11), and stated a positive reaction with "*A. aurantium*" strains for acetate and lactate. In contrast, Swings et al. (14) examined the oxidation of acetate and lactate by the method of Frateur (6), and described negative for acetate and positive for lactate with *F. aurantia*. In this study, the 16 isolates and two *F. aurantia* strains showed weak positive for acetate and positive for lactate when tested by Leifson's method (11). However, a weak positive reaction was found also for a control. Thus, we concluded that *F. aurantia* strains showed a negative reaction for acetate compared with the control. These contradictory

Table 4. Phenotypic characteristics of *F. aurantia* strains compared with acetic acid bacteria

Strain No.	Brown pigment Production	Oxidation of			Ketogenesis from		Production of				Growth on Hoyer-Frateur with mannitol as a carbon source	H_2S production
		ethantol	acetate	lactate	glycerol	mannitol	GA	2-KGA	5-KGA	2,5-diKGA		
B71D-1	+	+	−	+	−	−	+	+	−	+	+	−
B71D-3	+	+	−	+	+	−	+	+	−	+	+	−
B71D-4	+	+	−	+	+	+	+	+	−	+	+	−
B72D-1	+	+	−	+	+	+	+	+	−	+	+	−
B72M-1	+	+	−	+	−	−	+	+	−	+	+	−
B72M-2	+	+	−	+	−	−	+	+	−	+	+	−
B72M-3	+	+	−	+	−	−	+	+	−	+	+	−
B72M-4	+	+	−	+	−	−	+	+	−	+	+	−
B72M-5	+	+	−	+	−	−	+	+	−	+	+	−
B73M-1	+	+	−	+	+	−	+	+	−	+	+	−
B73M-3A	+	+	−	+	+	+	+	+	−	+	+	−
B73M-3B	+	+	−	+	+	+	+	+	−	+	+	−
B79M-1	+	+	−	+	+	−	+	+	−	+	+	−
Y3S-1B	+	+	−	+	+	+	+	+	−	+	+	−
Y3S-1B	+	+	−	+	+	+	+	+	−	+	+	−
Y3S-3	+	+	−	+	+	+	+	+	−	+	+	−
F. aurantia IFO 3245^T	+	+	−	+	+	+	+	+	−	+	+	−
F. aurantia IFO 13328	+	+	−	+	+	+	+	+	−	+	+	−
Ga. liquefaciens IFO 12228^T	+	+	+	+	+	−	+	+	+	+	−	−
G. oxydans IFO 14819^T	−	+	−	−	+	−	+	+	+	−	−	−
As. bogorensis NRIC 0311^T	−	−	w	w	+	−	+	+	+	−	+	−
K. baliensis NRIC 0488^T	−	+	w	w	+	−	+	+	+	−	−	−
A. aceti IFO 14818^T	−	+	+	+	+	−	+	+	+	−	−	−
Ac. methanolica NRIC 0498^T	−	+	+	−	−	−	+	+	−	−	−	−

Abbreviations: *A., Acetobacter*; *Ac., Acidomonas, As., Asaia*; *F., Frateuria*; *Ga., Gluconacetobacter*; *G., Gluconobacter*; *K., Kozakia*; ^{T}, type strain; GA., gluconate; KGA., keto-D-gluconate; +, positive; −, negative; w,weak.

results would be attributed to a weak reaction for acetate with *F. aurantia* strains.

Ketogenesis is also confusing for the identification of *F. aurantia*. Asai et al. (2) reported a negative reaction from glycerol with "*A. aurantium*" strains, and Ameyama and Kondo (1) described no production of reducing substances from glycerol and mannitol with the "intermediate strains" of acetic acid bacteria. In contrast, Yamada et al. (20) reported a positive reaction from glycerol with the "intermediate strains" of acetic acid bacteria. Meanwhile, Swings et al. (14) mentioned a positive but weak ketogenic reaction from glycerol, mannitol, and sorbitol with all strains tested, but their method was a little different from that of Shimwell et al. (16) which was employed by Asai et al. (2) and Yamada et al. (20). In this study, 10 of the 16 isolates and two *F. aurantia* strains showed yellow halos around streak cultures on yeast extract-glycerol agar plates, and 7 isolates and two *F. aurantia* strains on yeast extract-mannitol agar plates. The yellow halos of positive strains were not intense, unlike those of acetic acid bacteria. Thus, the ketogenic activity of the *Frateuria* strains was determined to be weak positive, as found by Swings et al. (14). However, Fehling reagent reacts not only with dihyroxyacetone but also with a variety of reducing compounds. Therefore, more selective reagents specific for

Table 5. Acid production from sugars, sugar alcohols, and alcohols by *Frateuria* strains and acetic acid bacteria

Strain No.	Acid production from														
	L-arabinose	D-arabinose	D-ribose	D-xylose	L-rhamnose	D-glucose	D-galactose	D-mannose	D-fructose	L-sorbose	sucrose	D-mannitol	D-sorbitol	glycerol	ethanol
B71D-1	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B71D-3	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B71D-4	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B72D-1	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B72M-1	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B72M-2	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B72M-3	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B72M-4	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B72M-5	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B73M-1	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B73M-3A	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B73M-3B	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B79M-1	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
Y3S-1B	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
Y3S-1B	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
Y3S-3	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
F. aurantia IFO 3245^T	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
F. aurantia IFO 13328	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
Ga. liquefaciens IFO 12228^T	+	−	nd	+	−	+	+	+	+	−	−	+	−	+	+
G. oxydans IFO 14819^T	+	−	nd	+	−	+	+	+	+	+	+	+	+	+	+
As. bogorensis NRIC 0311^T	+	+	nd	+	+	+	+	+	+	+	+	+	+	+	−
K. baliensis NRIC 0488^T	+	−	nd	+	−	+	+	+	−	−	+	−	−	+	+
A. aceti IFO 14818^T	+	−	−	+	−	+	+	+	−	−	−	−	−	−	+
Ac. methanolica NRIC 0498^T	+	−	nd	+	−	+	+	+	−	−	−	−	−	+	+

Abbreviations: *A., Acetobacter*; *Ac., Acidomonas*; *As., Asaia*; *F., Frateuria*; *Ga., Gluconacetobacter*; *G., Gluconobacter*; *K., Kozakia*; ^{T}, type strain; nd, not determined.

dihydroxyacetone are needed.

The utilization of ammoniac nitrogen is another problem for the classification and identification of acetic acid bacteria. Asai et al. (2) tested the growth of the "*A. aurantium*" strains on a defined medium consisting of $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, and a carbon source without vitamins, and reported the growth of "*A. aurantium*" IFO 3245 on mannitol and glucose and no growth of "*A. aurantium*" IFO 3246. Yamada et al. (20) described the growth of the "intermediate strains" on glucose under the same conditions. Swings et al. (14) mentioned the growth of seven *F. aurantia* strains tested on Frateur modified Hoyer medium (with vitamins) (4), two of seven strains on glucose, and no strains on ethanol. In this study, the 16 isolates and two *F. aurantia* strains utilized ammoniac nitrogen on Hoyer-Frateur medium (without vitamins) (3) when mannitol was supplied as a sole source of carbon, but did not utilize it when ethanol or glucose was supplied. Further, the strains tested did not utilize ethanol or glucose on Frateur modified Hoyer medium (data not shown). However, the growth on mannitol was enhanced on Frateur modified Hoyer medium (data not shown). Lisdiyanti et al. (12) reported a false-positive growth of *Acetobacter* strains on ammoniac nitrogen, and the growth was indefinable compared with those of the genera *Gluconobacter, Gluconacetobacter*, and *Asaia*. Thus, utilization of ammoniac nitrogen by acetic acid bacteria should be test-

ed carefully, and the carry-over of nutrients and growth factors from precultures should be prevented.

Swings et al. (14, 15) reported the differentiation of *F. aurantia* from acetic acid bacteria by the production of H_2S that was detected by lead acetate paper strips. However, the isolates and *F. aurantia* strains slightly blackened the tip of lead acetate paper strips in this study, and *P. aeruginosa* and *E. coli* strains, which were used as negative controls, did as well. Further, the production of H_2S was not detected on the isolates, *F. aurantia* strains, *P. aeruginosa* and *E. coli* strains by using TSI agar. Lead acetate paper strips are much more sensitive for detecting H_2S compared with TSI agar, and will give false-positive reactions. Therefore, its interpretation should be done very carefully. Thus, *F. Frateuria* was concluded not to produce H_2S. The production of H_2S reported by Swings et al. (14, 15) is not useful for the differentiation of *F. aurantia* from acetic acid bacteria.

We assigned by using new isolates that the genus *Frateuria* is phylogenetically located in the *γ-Proteobacteria*. Members of *F. aurantia* strains are characterized by the following characteristics: Gram-negative, strictly aerobic, rod-shaped, polarly flagellated when motile, requiring no growth factors when mannitol was supplied as a sole source of carbon, producing a brown water-soluble pigment, growing at pH 3.5 and on Hoyer-Frateur mannitol medium, producing acid from ethanol, pentoses (L-arabinose, D-arabinose, D-ribose and D-xylose), and hexoses (D-glucose, D-galactose, D-mannose, and D-fructose), not producing H_2S, and producing gluconate, 2-keto- and 2, 5-diketo-D-gluconate from glucose but not 5-keto-D-gluconate. The major ubiquinone is Q-8 as described by Yamada et al. (19), and the major cellular fatty acid is *iso*-$C_{15:0}$ as reported by Yamada et al. (21). The DNA base composition ranges from 62 to 63 mol%, which is a little lower than those reported by Yamada et al. (22).

The genus *Frateuria* is similar to acetic acid bacteria from the viewpoint of biochemistry together with *Acetobacter, Acidomonas, Asaia, Gluconacetobacter, Gluconobacter,* and *Kozakia* rather than as fluorescent pseudomonads. This is because the members of the genus *Frateuria* are able to grow at pH 3.5, oxidize ethanol to acetic acid, and oxidize D-glucose to D-gluconate, 2-keto-D-gluconate and 2,5-diketo-D-gluconate. Further, their growth is inhibited by acetic acid.

The strains newly isolated in this study have been deposited in the Nodai Culture Collection Center (NRIC), Tokyo University of Agriculture and Biotechnology Culture Collection (BTCC), Indonesian Institute of Sciences with the following accession numbers: NRIC 0567 (= BTCC B-532 = B71D-1), NRIC 0568 (= BTCC B-533 = B71D-3), NRIC 0569 (= BTCC B-534 = B71D-4), NRIC 0570 (= BTCC B-535 = B72D-1), NRIC 0571 (= BTCC B-536 = B72M-1), NRIC 0572 (= BTCC B-537 = B72M-2), NRIC 0573 (= BTCC B-538 = B72M-3), NRIC 0574 (= BTCC B-539 = B72M-4), NRIC 0575 (= BTCC B-540 = B72M-5), NRIC 0576 (= BTCC B-541 = B73M-1), NRIC 0577 (= BTCC B-542 = B73M-3A), NRIC 0578 (= BTCC B-543 = B73M-3B), NRIC 0579 (= BTCC B-544 = B79M-1), NRIC 0580 (= BTCC B-545 = Y3S-1A), NRIC 0581 (= BTCC B-546 = Y3S-1B), and NRIC 0582 (= BTCC B-547 = Y3S-3).

ACKNOWLEDGEMENT

This work was supported in part by a research grant (2003) of Institute for Fermentation, Osaka, Japan.

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インドネシア試料より分離した*Frateuria aurantia*の同定について

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熱帯地域の酢酸菌分離の過程で，インドネシアの果物と花より水溶性褐色色素を生成する酢酸菌16株を分離した．代表株2株の16S rRNA遺伝子全塩基配列解析から，両株ともγ-*Proteobacteria*の*Frateuria*属に位置していた．全ての分離株は*Frateuria aurantia*の基準株（IFO3245）と高いDNA-DNA相同値を示し，*F.aurantia*と同定した．分離株は好気性，グラム陰性桿菌で，単一極鞭毛により運動した．また，pH3.5で生育し，エタノールから酢酸を生成し，水溶性褐色色素を生成し，乳酸を酸化したが酢酸は酸化しなかった．0.35％の酢酸の存在下では生育せず，硫化水素を生成しなかった．グルコースからグルコン酸，2-ケトグルコン酸，2，5-ジケトグルコン酸を生成した．ユビキノンQ-8を有し，主要菌体脂肪酸は*iso*-$C_{15:0}$であり，G+C含量は62-63mol％であった．本研究は*F.aurantia*の4回目の分離例であり，この種が日本のみならず熱帯地域にも生育していることを明らかにした．