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Tanaka, Machiko
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Aoki, Kenji
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THE CHARACTERIZATION OF PHENOTYPIC FEATURES OF CELLULOSE-FORMING ACETIC ACID BACTERIA

Machiko TANAKA,[1] Megumi YOSHIDA,[2] Syuichiro MURAKAMI,[3] Kenji AOKI[1] and Ryu SHINKE[1]

[1]*Laboratory of Bio-functional Chemistry, Division of Bioscience, The Graduate School of Science and Technology, Kobe University, 1 Rokkodaicho, Nada, Kobe 657, Japan*
[2]*Research & Development Department, Bunsen Co., Ltd., 387 Shingu, Shingu-cho, Ibo-gun, Hyogo 679-43, Japan*
[3]*Laboratory of Applied Microbiology, Department of Bio-functional Chemistry Faculty of Agriculture, Kobe University, 1 Rokkodaicho, Nada, Kobe 657, Japan*

Abstract

Twenty-eight strains of cellulose-forming acetic acid bacteria including twenty-one new isolates were examined for their phenotypic features. All the cellulose-forming strains were gram-negative, non-motile and non-flagellated rods, ranging from 0.4 × 1.0 to 0.9 × 3.0 μm in size and showing growth at pH 3.5 and 30℃. Most of them formed thick cellulosic pellicles except for strain IFO[1] 3144 which formed a little pellicles. All of these twenty-eight strains had the Q-10 system without any exception. They all formed acid from D-glucose without any exception. None of them formed acid from D-mannitol, dulcitol, L-arabitol and maltose. All the cellulose-forming strains including the new isolates were phenotypically situated at an identical taxonomic position with *A. xylinum* IFO 15237[T]. All the other strains except for *Acetobacter xylinum* IFO 15237[T] formed acid from ethanol and oxidized both acetate and lactate. In addition, all the other strains except for *A. xylinum* IFO 15237[T] and the isolate sukon-F oxidized glycerol to dihydroxyacetone. In this respect, the type strain of *A. xylinum* was unusual. Neither did they form acid from ribitol nor xylitol except for the isolate 2319. Furthermore, the isolate 2319 was unusual, as it formed very thick cellulosic pellicles from various kinds of sugar alcohols. They divided into two groups on both acid formation from sucrose and assimilation of sucrose.

Keywords: Acetobacter xylinum; *Acetobacter hansenii*; cellulose-forming acetic acid bacteria; taxonomy; identification

Introduction

Recently, people have become interested in foods containing dietary fiber. However, few foods containing bacterial cellulose have appeared on the consumer market except for "nata de coco." First of all, isolated cellulose-forming strains were examined taxonomically for organizing the base of bacterial cellulose food.

A cellulose-forming species, *Acetobacter xylinum* (ex Brown) Yamada was described with NCIB 11664[T](= IFO 15237[T]) as the designation of the type strain (Yamada, 1983; Yamada &

[1] Abbreviations: IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (Riken), Wako-shi, Saitama, Japan; BPR, Bio-Polymer Research Co., Ltd., Kawasaki-shi, Kanagawa, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland, U.K.; Q-n or Q_n, ubiquinone homologues with a specified number of isoprene units in a side chain, *e.g.*, Q-10 or Q_{10}

Kondo, 1984). This species was once classified as *Acetobacter aceti* subsp. *xylinum* De Ley and Frateur (De Ley & Frateur, 1974). However, the type strain of this subspecies, NCIB 4112 was found to have neither oxidation of acetate, nor formation of cellulose, nor ketogenic activity on D-mannitol (Gillis *et al.*, 1983). The type strain NCIB 4112 was reidentified as *Gluconobacter oxydans* De Ley (Gillis *et al.*, 1983), because the electrophoretic protein pattern of this strain was very similar to that of the type strain of *G. oxydans* (NCIB 9013^T = IFO 14819^T) which is the type species of the genus *Gluconobacter* Asai (De Ley, 1961). Therefore, Gillis *et al.* (1983) newly designated strain NCIB 11664 (Bertrand's sorbose bacterium) as the type strain of *Acetobacter aceti* subsp. *xylinum*.

On the other hand, these cellulose-forming acetic acid bacteria were classified in two groups or two species, according to the numerical analysis of phenotypic features by Gosselé *et al.* (1983). One was the subphenon C or *Acetobacter hansenii* Gosselé *et al*, and the other was the subphenon E with a phenotypically broad spectrum of strains or *Acetobacter pasteurianus* (Hansen) Beijerinck emend. Gosselé *et al.* The type strain of *A. xylinum*, NCIB 11664^T was actually included in the subphenon E or *A. pasteurianus*. *Acetobacter xylinum* subsp. *sucrofermentans* Toyosaki *et al.* was recently described as the strain of JCM 9730 (= BPR 2001) (Toyosaki *et al.*, 1995).

This paper deals with the characterization of phenotypic features of the cellulose-forming acetic acid bacteria from the taxonomic point of view.

Materials and Methods

Bacterial strains Twenty-eight strains of cellulose-forming acetic acid bacteria including twenty-one new isolates were used in this experiment (Table 1). Some of the new isolates were from "sukonnyaku" formed on surfaces of fermenting vinegars in the breweries in Japan. The other strains were isolated from various kinds of fruit and from sticky materials on stems of a plant. The type strains of *Acetobacter aceti, A. pasteurianus, A. liquefaciens, A. hansenii* and *G. oxydans* were used as reference strains. The phenotypic features of these strains were examined after Asai *et al.* (1964) and Yamada *et al.* (1976a).

Form and size The organisms were grown on agar slants containing 3.0% D-glucose, 1.0% glycerol, 1.0% ethanol, 0.3% peptone, 0.2% yeast extract and 1.0% calcium carbonate at 30℃ for 2 days. Microscopic observation was made after staining with carbol fuchsin.

Motility The motility of the organisms grown on the agar slants at 20℃ for 24 hours, was judged by microscopic observation.

Effect of pH on growth The bacterial growth was measured at pH 3.0, 3.5, 4.0 and 6.8 in the liquid media composed of 2.0% D-glucose, 1.0% glycerol, 1.0% ethanol, 0.3% peptone and 0.2% yeast extract. For ajusting pH, hydrochloric acid or sodium hydroxide was added to media. The growth was judged by pellicle formation or turbidity after incubating at 30℃ for 7 days on slant.

Effect of temperature on growth The organisms were incubated at 30℃ and 37℃ for 7 days on the slant. The growth was judged by pellicle formation or turbidity.

Pigmentation The organisms were incubated at 30°C for 7 days on the agar slants mentioned above supplemented with 0.7% calcium carbonate. The pigmentation was judged by the formation of brown pigment in the agar slants.

Oxidation of acetate and lactate According to the method of Leifson (1954), the medium (pH 6.4) containing 0.2% sodium acetate or 0.2% sodium lactate, 0.3% peptone and 0.2% yeast extract was used. Bromothymol blue (0.002%) was added to the medium. The organisms were incubated at 30℃ for 7 days on slant. The oxidation of acetate and lactate was judged by the change of color from yellow to blue.

Formation of acetic acid from ethanol-calcium carbonate medium The organisms were incubated at 30℃ for 7 days on the agar plates containing 2.0% ethanol, 1.0% yeast extract, 2.0% calcium carbonate and 2.0% agar. The formation of acetic acid from ethanol was detected by clear zone around the colonies (Shimwell *et al.*, 1960).

Growth on glutamate agar According to the method of Iizuka and Komagata (1963), the organisms were incubated at 30℃ for 10 days on the agar slants (pH 7.2) containing 1.0% D-glucose, 0.5% sodium

glutamate, 0.1% potassium dihydrogen phosphate, 0.02% magnesium sulfate and 0.01% potassium chloride. Growth was judged whether there was the colony on the slant or not after incubation.

Growth on mannitol agar The organisms were incubated at 30℃ for 10 days on the agar slants (pH 6.0) containing 2.5% D-mannitol, 0.2% peptone and 0.2% yeast extract. Growth was judged whether there was the colony on the slant or not after incubation.

Ketogenic activity for glycerol The organisms were incubated at 30℃ for 7 days and 14 days in the liquid slants (pH 6.0) containing 3.0% glycerol, 0.1% peptone and 0.5% yeast extract. The production of dihydroxyacetone was judged by the appearance of orange color, when Fehling's solution was added to the cultures after incubation.

Assimilation of ammoniacal nitrogen The assimilation of ammoniacal nitrogen was examined by incubating the organisms at 30℃ for 7 days in the liquid slants containing 5.0% D-glucose, 0.3% ammonium sulfate, 0.3% potassium dihydrogen phosphate and 0.2% magnesium sulfate. Assimilation was judged whether there was the colony on the slant or not after incubation.

Ubiquinone homologues determination The organisms were incubated at 30℃ for 3 days on the fourteen agar plates (9 cm in diameter) per strain. The media contained 3.0% D-glucose, 1.0% glycerol, 1.0% ethanol, 0.3% peptone, 0.2% yeast extract and 0.2% calcium carbonate. The bacterial cells were collected from the surfaces of cultures. The ubiquinone homologues were extracted from the bacterial cells with ether-ethanol (3:1 by volume) and partially purified by thin-layer chromatography on a silica-gel plate (silica gel $60F_{254}$, Art 5715, 20 × 20 cm, 0.25 mm in thickness, Merck, Darmstadt, Germany) with pure benzene as developer.

The major ubiquinone systems of the strains as showing Table 2, were routinely estimated by reversed phase paper chromatography with two solvent systems composed of ethanol-ethyl acetate-water (5:3:1 by volume) and N,N-dimethylformamide-water (97:3 by volume) (Yamada *et al.*, 1969a).

The bacterial quinone systems as showing Table 3, were quantitatively analyzed by high performance liquid chromatography on a Shimadzu model HIC-6A liquid chromatograph equipped with a Novapak C_{18} column (3.9 mm × 15 cm, Nippon Waters, Ltd., Tokyo, Japan). The samples of ubiquinone homoloques extracted from the bacterial cells were developed with a solvent system of methanol-isopropanol (2:1 by volume) at a flow rate of 1.0 ml/min (Yamada & Kuraishi, 1983).

Acid formation from carbon sources The organisms were incubated at 30℃ for 7 days in the liquid slants (pH 6.8) containing 1.0% carbon source and 0.5% yeast extract, to which 0.002% bromocresol purple was added. Acid formation was judged by change of color from purple to yellow. At the same time, the bacterial growth was measured by the degree of pellicle formation.

Results and Discussion

All the cellulose-forming twenty-eight strains were found to be gram-negative, non-motile and non-flagellated rods (Table 1). They ranged from 0.4 × 1.0 to 0.9 × 3.0 μm in size. All of them formed much cellulose except for IFO 3144 which formed a little amount of cellulose.

All the twenty-eight strains grew well at pH 3.5, but some of them, did not grow well at pH 3.0. They grew well at 30℃, but about half of them did not grow at 37℃ (Table 2).

All of them except for the isolates 209 and the sukon-F grew on mannitol agar, and many strains grew on glutamate agar. Most of them assimilated ammoniacal nitrogen as the sole nitrogen source, when D-glucose was used as the sole carbon source.

The data of HPLC showed that the major ubiquinone-10 of the cellulose-forming strains was ranged from 85% to 96%, and the minor Q-9 of these strains was ranged from 4% to 15% (Table 3). The Q-8 homologue was not detected. The ubiquinone homologue compositions coincided with that of the type strain of *G. oxydans* (IFO 14819^T), but differed from those of the type strains of *A. aceti* (IFO 14815^T) and *A. pasteurianus* (JCM 7640^T) (Yamada *et al.*, 1969a; Urakami *et al.*, 1989).

All the twenty-eight strains formed acid from D-glucose (Table 4). Many of them produced acids from D-mannose, D-galactose, D-xylose, *meso*-erythritol and treharose. None of the twenty-eight strains formed acid from D-mannitol, maltose although the type strain of *G. oxydans* did. None of them formed acids from dulcitol, neither did the type strain of *G. oxydans*. Except

Table 1. Morphological characteristics of the cellulose-forming strains of acetic acid bacteria[a]

Species and strain	Source	Form	Size (μ m)
Acetobacter xylinum subsp.xylinum			
IFO 15237[T]	= NCIB 11664[T]	large rod	0.7-0.8x2.4-2.8
Acetobacter xylinum subsp.sucrofermentans			
JCM 9730[T]	= BPR 2001	rod	0.6-0.8x1.0-1.2
Strain			
IFO 3288	K.Kondo, 51, from vinegar	rod	0.7-0.8x1.2-1.4
IFO 3144	K.Kondo, 59	large rod	0.7-0.8x1.4-2.0
IFO 13693	= JCM 7664, M.Kozaki, 1-B	rod	0.7-0.8x1.2-1.4
IFO 13772	M.Ameyama, 5-7, film in fermentor of vinegar	large rod	0.7-0.8x2.0-2.4
IFO 13773	M.Ameyama, 5-8, film in fermentor of vinegar	rod	0.6-0.7x1.2-1.5
Isolate			
92A	Sticky material on stalk of garden catchfly (Silene armeria)	rod	0.6-0.8x1.6-1.8
105B	Chinese bush fruit (Prunus tomentosa)	rod	0.6-0.8x1.6-1.8
209	Fruit of gumi (Elaeagnus pungens)	rod	0.6-0.8x1.0-1.2
1083	Chinese jujube (Zizyphus jujuba)	rod	0.8-0.9x1.6-1.8
1414	Chinese jujube (Zizyphus jujuba)	rod	0.8-0.9x1.6-1.8
2256	Grape (Vitis 'malvasia')	rod	0.5-0.6x1.2-1.5
2319	Grape (Vitis 'merlot')	rod	0.6-0.8x1.0-1.2
2418	Grape (Vitis 'malvasia')	rod	0.4-0.5x1.0-1.2
2420	Grape (Vitis 'malvasia')	rod	0.6-0.7x0.6-1.5
2426	Grape (Vitis 'pinot noir')	rod	0.6-0.7x1.2-1.5
2443	Grape (Vitis 'zalagyöngye')	rod	0.8-0.9x1.7-1.8
2448	Grape (Vitis 'St-Èmilion')	rod	0.7-0.8x1.0-1.2
2641	Apple (Malus 'fuji')	rod	0.8-0.9x1.2-1.6
2835	Dried fig (Ficus carica)	rod	0.7-0.8x1.2-1.5
2898	Grape (Vitis 'R-13')	rod	0.7-0.9x1.5-1.8
Sukon-A	Sukonnyaku at vinegar brewery, Shizuoka	large rod	0.6-0.7x2.0-2.4
Sukon-E1	Sukonnyaku at vinegar brewery, Hiroshima	large rod	0.7-0.8x1.9-2.0
Sukon-E2	Sukonnyaku (apple vinegar) at vinegar brewery, Hiroshima	rod	0.6-0.7x1.2-1.4
Sukon-F	Sukonnyaku at vinegar brewery, Osaka	rod	0.8-0.9x1.5-1.8
Sukon-G	Sukonnyaku at vinegar brewery, Wakayama	large rod	0.6-0.7x1.4-2.0
Sukon-H	Sukonnyaku at vinegar brewery, Wakayama	large rod	0.6-0.7x2.4-3.0
Acetobacter aceti			
IFO 14818[T]	= NCIB 8621[T]	large rod	0.8-0.9x1.2-2.0
Acetobacter pasteurianus			
JCM 7640[T]	= NCIB 12228[T], LMD 22.1	rod	0.7-0.9x1.5-1.8
Acetobacter liquefaciens			
IFO 12388[T]	= IAM 1834[T]	rod	0.7-0.8x1.0-1.2
Acetobacter hansenii			
IFO 14820[T]	= NCIB 8746[T]	large rod	0.7-0.8x1.8-2.0
Gluconobacter oxydans			
IFO 14819[T]	= NCIB 9013[T]	rod	0.6-0.8x1.0-1.2

[a]All the cellulose-forming strains examined were gram-negative, non-motile and non-flagellated, and formed thick cellulosic pellicles. However, the strain IFO 3144 formed a little amount of cellulosic substance.

[T],type strain.

Table 2. Physiological and biochemical characteristics of the cellulose-forming strains of acetic acid bacteria

Species and strain	Growth at pH[a] 3.0	3.5	Growth at 30	37°C	Reaction in acetate	lactate	Production of acetic acid on EtOH-$CaCO_3$	Pigmentation	Growth on glutamate agar	mannitol agar	DHA[b] from glycerol	Assimilation of ammoniacal nitrogen	Ubiquinone system[c]
Acetobacter xylinum subsp.xylinum													
IFO 15237[T]	±	+	+	−	acid	acid	−	−	+	+	−	+	Q-10
Acetobacter xylinum subsp.sucrofermentans													
JCM 9730[T]	+	+	+	+	alk	alk	+	−	+	+	+	+	Q-10
Strain													
IFO 3288	+	+	+	−	alk	alk	+	−	+	+	+	−	Q-10
IFO 3144	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
IFO 13693	+	+	+	+	alk	alk	+	−	−	+	+	+	Q-10
IFO 13772	±	+	+	+	alk	alk	+	−	−	+	+	+	Q-10
IFO 13773	+	+	+	+	alk	alk	+	−	+	+	+	+	Q-10 olate
Isolate													
92A	±	+	+	−	alk[w]	alk[w]	+	−	+	+	+	±	Q-10
105B	±	+	+	−	alk	alk	+	−	−	+	+	±	Q-10
209	+	+	+	±	alk	alk	+	−	+	−	+	+	Q-10
1083	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
1414	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
2256	±	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
2319	+	+	+	±	alk	alk[w]	+	−	−	+	+	+	Q-10
2418	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
2420	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
2426	+	+	+	−	alk	alk[w]	+	−	−	+	+	−	Q-10
2443	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
2448	+	+	+	+	alk	alk	+	−	+	+	+	+	Q-10
2641	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
2835	±	+	+	−	alk	alk	+	−	−	+	+	±	Q-10
2898	+	+	+	−	alk	alk	+	−	−	+	+	±	Q-10
Sukon-A	+	+	+	+	alk	alk	+	−	+	+	+	+	Q-10
Sukon-E1	+	+	+	+	alk	alk	+	−	−	+	+	±	Q-10
Sukon-E2	+	+	+	+	alk	alk	+	−	−	+	+	+	Q-10
Sukon-F	+	+	+	+	alk	alk	+	−	−	−	−	+	Q-10
Sukon-G	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
Sukon-H	+	+	+	+	alk	alk	+	−	+	+	+	±	Q-10
Acetobacter aceti													
IFO 14818[T]	+	+	+	−	alk	alk	+	−	+	−	+	+	Q-9
Acetobacter pasteurianus													
JCM 7640[T]	+	+	+	+	alk	alk	+	−	+	−	−	−	Q-9
Acetobacter liquefaciens													
IFO 12388[T]	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
Acetobacter hansenii													
IFO 14820[T]	+	+	+	−	alk	alk	+	−	+	+	+	+	Q-10
Gluconobacter oxydans													
IFO 14819[T]	±	+	+	−	acid	acid	+	−	−	+	+	−	Q-10

[a]In addition, all the cellulose-forming strains were grown at pH 4.0, 4.5 and 6.8.
[b]Dihydroxyacetone.
[c]All the cellulose-forming strains and the type strain of *A. liquefaciens* gave a single spot corresponding to Q-10 on reversed-phase paper chromatographies. In the data obtained here on *A. liquefaciens*, a small spot of Q-9 was not found, differing from those of Yamada *et al.* (1969a).
+, positive; −, negative; ±, very weak; [w], weak; [T], type strain.

for the isolate 2319, none of the twenty-eight strains formed acids from ribitol and xylitol, neither did the type strain of *G. oxydans*. Moreover, the isolate 2319 formed much cellulose from various kinds of sugar alcohols. In these respects, the isolate 2319 was unusual.

Table 3. Ubiquinone homologue composition in the cellulose-forming strains of acetic acid bacteria

Species and strain	Concentration (%) of		
	Q-8	Q-9	Q-10
Acetobacter xylinum subsp.*xylinum* IFO 15237^T	–	15	85
Acetobacter xylinum subsp.*sucrofermentans* JCM 9730^T	–	8	92
Strain			
IFO 3288	–	7	93
IFO 3144	–	6	94
IFO 13772	–	11	89
Isolate			
92A	–	6	94
105B	–	5	95
2256	–	5	95
2835	–	5	95
Sukon-F	–	4	96
Sukon-G	–	12	88
Acetobacter liquefaciens IFO 12388^T	–	10	90
Acetobacter hansenii IFO 14820^T	–	11	89
Acetobacter aceti IFO 14818^T	13	79	8
Acetobacter pasteurianus JCM 7640T	13	75	12

–, not detected or negligible (below 1%); ^{T},type strain.

Most of the twenty-eight strains formed dihydroxyacetone from glycerol as *G. oxydans* IFO 14819^T did, but *A. xylinum* IFO 15237^T and the isolate sukon-F did not. Furthermore, the type strain of *A. xylinum* was different from other cellulose forming strains and from the type strain of *G. oxydans*, because *A. xylinum* IFO 15237^T did not form acid from ethanol, nor did it grow on an ethanol-calcium carbonate medium. The type strain of *A. xylinum* was unusual, because it did not oxidize acetate and lactate to carbon dioxide and water, and did not form dihydroxyacetone from glycerol.

As for the cellulose-forming strains, some spontaneous mutants which lost cellulose-forming ability were reported (Schramm & Hestrin, 1954; Steel & Walker, 1957; Shimwell & Carr, 1958). Shimwell and Carr (1958) described that such mutants as lost cellulose-forming ability could not be distinguished phenotypically from the non cellulose-forming strains which were classified in *A. aceti*. De Ley and Frateur (1974) once distinguished the cellulose-forming strains from non cellulose-forming ones at the subspecific level and classified the former in *A. aceti* subsp. *xylinum*.

Yamada *et al.* (1969b, 1976b) pointed out that in the ubiquinone system, the cellulose-forming strains having Q-10 can be distinguished chemotaxonomically from *A. aceti* having Q-9, whether it has cellulose forming ability or not.

Recently, the cellulose-forming strains were divided into two groups on acid formation from

Table 4. Acid formation from sugars and sugar alcohols in the cellulose-forming strains of acetic acid bacteriaa

Species and strain	Acid formation from																								
	D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	D-Mannitol	Dulcitol	D-Sorbitol	Xylitol	L-Arabitol	D-Arabitol	Ribitol	*meso*-Erythritol	Glycerol	*myo*-Inositol	Maltose	Melibiose	Lactose	Trehalose	Sucrose	Ethanol
Acetobacter xylinum subsp.xylinum																									
IFO 15237^T	+	±	±	±	±	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−
Acetobacter xylinum subsp.sucrofermentans																									
JCM 9730^T	+	±	±	−	−	+	±	−	−	−	−	−	−	−	±	−	+	−	−	−	±	−	±	+	+
Strain																									
IFO 3288	+	+	+	−	−	+	+	−	−	−	−	−	−	−	±	−	+	±	−	−	+	−	+	+	+
IFO 3144	+	+	+	±	−	+	+	−	+	−	−	−	−	−	+	−	+	+	−	−	$+_s$	−	+	−	+
IFO 13693	+	+	±	−	−	+	−	−	−	−	−	−	−	−	$+_s$	−	+	±	+	−	−	−	$+_s$	$+_s$	+
IFO 13772	+	±	±	−	−	−	−	−	−	−	−	−	−	−	+	−	+	±	+	−	$±_s$	−	+	±	+
IFO 13773	+	±	±	−	−	−	−	−	−	−	−	−	−	−	$+_s$	−	$+_s$	±	+	−	$±_s$	−	−	±	+
Isolate																									
92A	+	+	±	−	−	+	−	−	−	−	−	−	−	−	−	−	$+_s$	−	−	−	−	−	±	+	$+_s$
105B	+	+	±	−	−	+	±	−	±	−	−	−	−	−	+	−	+	$±_s$	±	−	+	−	±	+	+
209	+	−	−	−	−	−	−	−	±	−	−	−	−	−	+	±	+	+	−	−	−	−	−	−	+
1083	+	+	+	−	−	+	+	−	+	−	−	−	−	−	$+_s$	−	+	+	−	−	+	−	+	−	+
1414	+	−	±	−	±	−	−	−	±	−	−	−	−	−	−	−	+	$±_s$	−	−	−	−	$+_s$	+	+
2256	+	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	+
2319	+	+	*	±	±	+	+	−	−	−	−	±	+	−	−	+	+	+	$+_s$	−	+	−	$+_s$	−	+
2418	+	±	±	−	−	−	−	−	−	−	−	−	−	−	±	−	+	±	−	−	−	−	+	+	+
2420	+	±	±	−	−	±	−	−	−	−	−	−	−	−	$+_s$	−	−	−	−	−	$+_s$	−	−	−	+
2426	+	±	±	±	−	+	±	±	−	−	−	−	−	−	−	−	$+_s$	−	−	−	±	−	±	+	+
2443	+	+	*	−	−	+	−	−	−	−	−	−	−	−	$+_s$	−	+	+	−	−	$+_s$	−	+	+	+
2448	+	±	±	−	−	+	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	+
2641	+	+	+	−	−	+	−	−	−	−	−	−	−	−	±	−	+	−	+	−	±	−	±	+	+
2835	+	+	±	−	−	+	+	−	−	−	−	±	−	−	−	−	±	−	−	−	±	−	$+_s$	+	+
2898	+	+	±	±	−	+	±	−	−	−	−	±	−	−	−	−	$+_s$	−	−	−	+	−	+	+	+
Sukon-A	+	+	±	−	−	+	+	−	−	−	−	−	−	−	$+_s$	−	±	−	−	−	±	−	+	−	+
Sukon-E1	+	+	+	−	−	+	+	−	−	−	−	−	−	−	+	−	−	±	+	−	±	−	$+_s$	+	+
Sukon-E2	+	+	+	−	−	+	+	−	−	−	−	−	−	−	$+_s$	−	−	±	+	−	+	−	$+_s$	+	+
Sukon-F	+	±	±	−	−	+	±	−	−	−	−	−	−	−	−	−	−	−	±	−	±	−	−	−	+
Sukon-G	+	±	±	−	−	+	±	−	−	−	−	−	−	−	$+_s$	−	$+_s$	−	−	−	±	−	+	−	+
Sukon-H	+	±	±	−	−	+	±	−	−	−	−	−	−	−	−	−	$+_s$	−	−	−	−	−	$+_s$	−	+
Acetobacter aceti																									
IFO 14818^T	+	+	+	−	−	+	+	±	+	−	−	−	−	−	−	−	+	+	−	−	$+_s$	−	$+_s$	−	+
Acetobacter pasteurianus																									
JCM 7640^T	+	±	±	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Acetobacter liquefaciens																									
IFO 12388^T	+	+	+	−	−	+	−	−	−	−	−	−	−	−	±	−	+	−	−	−	−	−	$+_s$	−	+
Acetobacter hansenii																									
IFO 14820^T	+	+	+	−	−	+	+	−	−	−	−	−	−	−	±	−	+	−	−	−	$+_s$	−	$+_s$	−	+
Gluconobacter oxydans																									
IFO 14819^T	+	+	+	+	+	+	+	+	+	+	−	+	±	±	+	−	+	+	−	−	+	−	−	+	+

+, positive; −, negative; $+_s$, positive but slow; ±, very weak; ^{T}, type strain.

sucrose. One is composed of the strains including *A. xylinum* subsp. *xylinum* IFO 15237^T, which did not form acid from sucrose. The other is composed of the strains including *A. xylinum* subsp. *sucrofermentans* JCM 9730^T (Toyosaki *et al.*, 1995), which formed acid from sucrose.

Gosselé *et al.*(1983) reclassified the cellulose-forming strains which were once accommodated in *A. aceti* subsp.*xylinum* De Ley and Frateur into two groups or two species. One is the group which they named the subphenon C or *A. hansenii* Gosselé et al. (Q-10) (Urakami *et al.*, 1989; Toyosaki *et al.*, 1995). The other is the group which they named the subphenon E (Q-9) with a phenotypically broad spectrum of strains or *A. pasteurianus* (Hansen) Beijerinck emend. Gosselé *et al.* The type strains of these two groups or species can be distinguished from each other by their ubiquinone systems.

Yamada (1983) postulated the new species, *A. xylinum* (ex Brown) Yamada with the type strain of NCIB 11664 (= IFO 15237^T), which had been designated *A. aceti* subsp. *xylinum* by Gillis *et al.*(1983). Namely, *A. xylinum* (Q-10) was separated from the subphenon E or *A. pasteurianus* (Q-9)(Gosselé *et al.* 1983) because of the differences in the ubiquinone system. Toyosaki *et al.*(1995) also pointed out that by their DNA-DNA hybridization experiments both the type strains of *A. xylinum* subsp. *xylinum* and *A. xylinum* subsp. *sucrofermentans* were distinguishable from that of *A. pasteurianus* (homology indexes, 9.1% and 0.3%, respectively) and *A. hansenii* (homology indexes, 24.3% and 15.6%, respectively) at the specific level.

The type strain of *A. xylinum* did not have the oxidative abilities of lactate and acetate, as *G. oxydans* IFO 14819^T did not. However, it is obvious that it cannot be accommodated in the genus *Gluconobacter* because the type strain of *A. xylinum* subsp. *xylinum* showed a relatively high homology index (56.5%) when hybridized with *A. xylinum* subsp. *sucrofermentans* which oxidized acetate and lactate (Toyosaki *et al.* 1995). In addition, the type strain constituted a cluster along with those of *A. hansenii and A. liquefaciens* apart from that of *G. oxydans* in the 16s ribosomal RNA sequence analysis.

According to Gosselé *et al.*(1983), the strains classified in the subphenon E or *A. pateurianus* cannot assimilate sucrose as the sole carbon source. On the other hand, some strains classified in the subphenon C or *A. hansenii* can assimilate sucrose. The type strain of *A. xylinum* subsp. *sucrofermentans* actually grew well on sucrose, but that of *A. xylinum* did not grow on sucrose (Toyosaki *et al.*, 1995).

The results obtained here may suggest that the cellulose-forming twenty-eight strains, including the twenty-one new isolates,shoud be placed in an identical taxonomic position along with the type strain of *A. xylinum* , which has a wide range of DNA base composition (ranging 54.9-62.6 mol% G+C) (Yamada, 1983).

To solve the precise taxonomic positions of these cellulose-forming strains, the DNA-DNA homology experiments are necessary and will be reported in the future.

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セルロース生産性酢酸菌を特徴づける表現形質

田中満智子　吉田恵美*　村上周一郎**　青木健次　新家 龍
(神戸大学大学院自然科学研究科、*ブンセン株式会社開発部、**神戸大学農学部)

新たに分離したセルロース生産性酢酸菌21菌株に、基準株を含む *Acetobacter xylinum* の保存株7株（全てセルロース生産性菌株）を加えた28菌株の表現型を試験した。28菌株は全てが、グラム陰性、鞭毛を持たず、非運動性の桿菌、pH 3.5、30℃でよく生育、キノン系はQ－10であった。さらに28菌株全てはグルコースから酸を生成し、D-マンニトール、D-ダルシトール、L-アラビトール、マルトースからは酸を生成しなかった。IFO 3144はごく僅かではあったが、28菌株全てがセルロース様物質を生成した。以上により、新たに分離した21菌株は表現型を見る限り、*A. xylinum* と同じ分類学的位置にある。 *A. xylinum* の基準株と分離株 sukon F はグリセロールからジハイドロキシアセトンを生成しなかった。その上、*A. xylinum* の基準株のみはエタノールから酸を生成せず、乳酸も酢酸も酸化できなかった。その観点では *A. xylinum* の基準株は特異な存在である。なお、分離菌株2319のみはリビトールとキシリトールから酸を生成し、その上、殆どの糖アルコールから旺盛にセルロース様物質を生成した事で注目された。なお、28株のセルロース生産菌はスクロースからの酸生成の有無で二分された。