CHAPTER IV

RESULTS AND DISCUSSION

1. Isolation of acetic acid bacteria

The acetic acid bacteria were isolated from 89 samples in Thailand, using the enrichment medium. One hundred and eighty-one isolates produced acid and showed clearance zone around their colonies on GEY-$CaCO_3$ medium were isolated. The source of isolation and isolate number were listed in Table 4.1.

Table 4.1 Source and date of sample that isolated

Sample	Source	Date of Isolation	Isolate no.
Peach	Bangkok	2/5/2006	PH32-2
Musk-melon	Bangkok	2/5/2006	AK33-1, AK33-2
Allamanda	Bangkok	16/5/2006	TE37-1, TE37-2
Fetid passion flower	Bangkok	21/5/2006	SIS32-1, SIS32-2, SIS32-3
Radermachera	Bangkok	21/5/2006	MHM10-1
Cleome spinosa	Bangkok	21/5/2006	MR40-1, LM12-1,
Heliconia sp.	Bangkok	21/5/2006	LR4-1 , AM13-2
Adenium obsum	Bangkok	28/5/2006	PP42-1, PP42-2
Ixora chinensis	Bangkok	28/5/2006	KT43-1, KT43-2
Mango	Bangkok	28/5/2006	MG71-1, MG71-2
Fire bell	Bangkok	28/5/2006	KS72-1, KS72-2
Bauhinia purpurea Linn.	Bangkok	28/5/2006	SP73-1,SP73-2
Longan fruit	Changmai	30/6/2006	LG57-1, LG57-2
Tomato	Changmai	30/6/2006	TM58-1, TM58-2, PA3-3
Kaffir lime	Changmai	30/6/2006	BN1-1, BN1-2
Langsat	Chantaburi	6/7/2006	LS60-1, LS60-2
Star fruit	Chantaburi	6/7/2006	SF18-1, SF61-1, SF61-2
Tamarind	Chantaburi	6/7/2006	AM35, AM41-1, CD21-1
Sapodilla	Chantaburi	6/7/2006	SM63-1, SM63-2,CT 8-1

Sample	Source	Date of Isolation	Isolate no.
Sugar apple	Chantaburi	12/8/2005	CA76-2, CA127-1, CA127-2
Pineapple	Chantaburi	12/8/2005	PA3-2, PA3-3
Chrismas flower	Chonburi	8/6/2006	CM50-1, CM50-2
Plumeria flower	Chonburi	8/6/2006	LD51-1,LD51-2
Cordia sebstena	Chonburi	8/6/2006	DM52-1, DM52-2
Petunia	Chonburi	8/6/2006	PN53-1
Samanea saman	Chonburi	8/6/2006	JA54-1, AN1-1
Guava	Kanchanaburi	15/5/2006	GA8-1, GA8-2, HN9-1, HN9-2
Murraya paniculata Jack	Khampangphet	15/5/2006	DT4-2
Orange	Khon Kaen	13/9/2006	OR55-1, OR55-2, OR56-2, OR7-1, OR95-1
Dragon fruit	Khon Kaen	13/8/2006	DA3-1
Khao-mak	Khon Kaen	12/9/2005	EN6-3, BL13-1
Cassia fistula	Khon Kaen	20/6/2006	RP55-1, RP55-2
Rumbutan	Khon Kaen	20/6/2006	RB1-1, RB3-1
Look-pang	Khon Kaen	20/6/2006	LP92-1, LP92-2
Thaivermicelli	Khon Kaen	18/8/2006	TV83-1, TV83-2
Custard apple	Khon Kaen	18/8/2006	CR84-1, CR84-2
Grape	Roie	12/7/2006	GR64-1, GR64-2
Cananga	Roie	25/7/2006	KD66-1, KD66-2
Banana	Roie	25/7/2006	BB91-1, BBM91-1
Elaeocarpus	Nongkhai	12/8/2006	LBM3-1, LBM3-2
Antidesma sp.	Nongkhai	12/8/2006	AD8-1, AD8-2, AD8-3
Hog Plum	Nongkhai	14/9/2005	HP27-1, HP27-2
Jackfruit	Nongkhai	20/6/2006	JF81-1, PN19-1
Palm juice	Nongkhai	21/6/2006	PJ82-2
Chayote	Nontaburi	2/6/2006	FE68-1, FE68-2
Mango	Nontaburi	2/6/2006	JR70-1, JR70-2
Avocado	Phetchabun	5/9/2006	AV28-1
Papaya	Phuket	28/5/2006	MK44-1
Unkown flower	Nontaburi	4/6/2006	PS49-1, PS49-2

Sample	Source	Date of Isolation	Isolate no.
Honey	Phuket	28/5/2006	HG45-1, HG45-2
			AP60-1, AP94-1, AP94-2,
Apple	Nontaburi	2/6/2006	API-1, AP1-2
Apple	Rayong	4/11/2005	LR41-1
Pagoda flower	Rayong	4/11/2005	AM26, AM28, AM46
Cordia flower	Rayong	4/11/2005	CS15-2,CS15-4
Baccaurea ramiflora Lour	Rayong	4/11/2005	AM48, AM68, AM14-1
Cordia flower	Rayong	3/9/2006	AM10-1, AM10-3
Little Yellow Star	Rayong	3/9/2006	AM29, AM 47, AM24
Caesalpinia pulcherrima	Rayong	3/9/2006	FG13-1
Frangipani	Rayong	3/9/2006	MP11-1
Ixoria / Ixora	Rayong	3/9/2006	HM12-1, HM12-2
Seed Ixora	Rayong	3/9/2006	SI15-1, SI15-2
Caricature Plant	Rayong	3/9/2006	CR16-1, CR16-2
Night Jasmine	Rayong	3/9/2006	NJ17-3
Quassia	Rayong	3/9/2006	CM3-1
Periwinkle	Rayong	3/9/2006	PW19-2
Chaba	Rayong	3/9/2006	SB20-2
Unknown Flower	Rayong	3/9/2006	AG21-1, AG21-2
Zinnia	Rayong	3/9/2006	ZN22-1, ZN22-2
Red Grape	Rayong	12/9/2005	AR02, AR03
Long gong	Rayong	12/9/2005	LM26-1
Salas	Rayong	15/4/2006	BA28-1, BA28-2
Fermented starch	Saraburi	4/10/2005	FC4-3, FCL4-5, FBY4-3, FBM4-3
Night Jasmine	Saraburi	16/5/2006	AN34-1, AN34-2
Zinnia	Saraburi	16/5/2006	TP35-1, TP35-2
Caricature Plant	Saraburi	16/5/2006	CK36-1, CK36-2
Kaffir lime	Saraburi	5/7/2006	KLM13-1
Cantaloup	Saraburi	5/7/2006	CT85-1, CT85-2
Musk-melon	Saraburi	5/7/2006	MM86-1, MM86-2
Jujube	Trad	15/8/2006	JJ87-1, JJ87-2

Sample	Source	Date of Isolation	Isolate no.
Mangosteen	Trad	15/8/2006	MT78-1, MT78-2
Strawberry	Trad	15/8/2006	ST107-1, ST79-1
Long-gong	Trad	15/8/2006	LK88-1, LK88-2
Sapodilla	Trad	15/8/2006	SL89-1, SL89-2
Rose apple	Ubon	2/5/2006	RA103-1, RA30-1, RA30-2
Pum melo	Ubon	2/5/2006	PM169-2
Guava	Ubon	2/5/2006	GV74-1
Watermelon	Trad	15/8/2006	WM86-1, WM77-1
Unkown flower	Nontaburi	4/6/2006	FP47-1, FP47-2
Unkown flower	Khon Kaen	20/6/2006	PK48-1

2. Identification of isolates

2.1 Cell morphology and cultural characteristics

A total of 181 isolates were divided into 11 different Groups based on their morphological, cultural, physiological and biochemical characteristics including 16S-23S restriction pattern analyses and 16S rDNA sequence analysis. All were Gram-negative, strictly aerobic rod shaped bacteria. Ninety-seven strains (Group 1, 2 and 3) formed cream, shiny, circular and non-pigmented colonies. More than a half of tested isolates of these Group were motile with peritrichous flagella. Forty-five isolates (Group 4, 5, 6 and 7) formed white, shiny, raised circular and non-pigmented colonies. More than a half of tested isolates of these Group were non-motile. Twenty-one strains (Group 8) formed pink, shiny, raised circular and non-pigmented colonies. All the isolates were motile with peritrichous flagella. Fourteen strains (Group 9) formed orange, round, mucous, smooth and convex colonies. Colonies of Group 10 (2 isolates) were pink, smooth, circular and raised. All the isolates were non-motile. Group 11, 2 isolates formed pink, shiny, circular and raised colonies. All were non-motile and they produced levan-like mucous substance.

2.2 Physiological and biochemical characteristics

All of the isolates were strictly aerobic and positive for catalase, utilized glucose oxidatively. All Groups except Groups 8, 9 and 10 did not produce pigment. They grew at 30°C. Group 1, 2 and 3 oxidized acetate and lactate. Group 4, 5, 6 and 7 did not oxidize acetate and lactate. Group 8 grew in the presence of 30% D-glucose but grew in the presence of 0.35% acetic acid. Group 9 grew on D-mannitol and glutamate agar. Group 10 grew in the presence of 0.35% acetic acid. Acids were produced from L-arabinose, D-glucose, glycerol and D-mannose. Group 11 could produce levan-like mucous substance (Tables 4.2 and 4.3).

Table 4.2 Physiological and biochemical characteristic of 181 isolates

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
PA3-3	3+	-	-	-	+	+/-	+	+	-	-	1+	2+	3+	3+	3+	-
BBM91-1	3+	3+	-	1+	+	+/-	+	+	-	-	3+	3+	3+	3+	3+	-
CD21-1	3+	2+	-	1+	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	-
KLM13-1	3+	3+	-	-	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	-
MHM10-1	3+	3+	-	-	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	-
FBM4-3	3+	3+	-	-	+	+/-	+	+	-	-	2+	2+	3+	3+	3+	-
AK33-1	3+	3+	+	3+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
AM13-2	3+	2+	-	3+	+	+/-	+	+	+	3+	3+	3+	3+	3+	3+	+
AP60-1	3+	2+	1+	1+	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
AP94-1	3+	3+	-	-	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
AP94-2	3+	2+	-	-	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
AV28-1	2+	2+	-	2+	+	+/-	+	+	-	-	2+	3+	3+	3+	3+	+
BA28-2	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-
BB91-1	3+	-	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	+
CA127-1	3+	3+	-	1+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
CA127-2	3+	2+	-	1+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
CA76-2	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
CM50-1	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
CM50-2	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-
CR16-2	3+	2+	-	2+	+	+/-	+	+	+	2+	2+	2+	+	+	+	-
CR84-2	2+	2+	-	2+	+	+/-	+	+	-	-	2+	3+	3+	3+	3+	+
CS15-2	2+	2+	1	2+	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
CS15-4	2+	2+	-	2+	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	+
CT85-1	3+	2+	-	2+	+	+/-	+	+	2+	2+	2+	2+	2+	2+	2+	-
CT85-2	3+	3+	+	3+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
DM52-1	3+	3+	-	3+	+	+/-	+	+	3+	3+	2+	2+	2+	3+	3+	-
FBY4-3	3+	-	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
FC4-3	3+	3+	-	-	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	+
FCL4-5	2+	3+	-	2+	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	+
FP47-2	3+	3+	-	3+	+	+/-	+	+	2+	2+	2+	3+	3+	3+	3+	-
GR64-1	-	+	+	-	+	+/-	+	+	2+	2+	2+	3+	3+	3+	3+	+
GR64-2	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
GV74-1	3+	3+	-	-	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	2+
HG45-2	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
HP27-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
JA54-1	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
JF81-1	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-
JJ87-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-
KD66-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
LG57-2	-	+	+	-	+	+/-	+	+	2+	2+	2+	3+	3+	3+	3+	+
LK88-1	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
LM26-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
BB1-1	3+	3+	-	1+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
CM3-1	3+	2+	-	1+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
LM12-1	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
HM12-1	3+	2+	-	1+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
HM12-2	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
BN1-2	3+	3+	-	1+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
LR41-1	3+	3+	-	3+	+	+/-	+	+	2+	2+	2+	3+	3+	3+	3+	-
LS60-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
LS60-2	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
MM86-1	3+	3+	-	1+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
MT78-1	3+	2+	-	1+	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
MT78-2	3+	3+	-	-	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
NJ17-3	3+	3+	-	-	+	+/-	+	+	-	1+	2+	3+	3+	3+	3+	+
OR55-1	3+	3+	-	-	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	-
OR55-2	3+	3+	+	3+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
LBM3-1	3+	3+	-	1+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
OR56-2	3+	2+	-	3+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	-
OR95-1	3+	-	-	-	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	+
PK48-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
PM169-2	2+	2+	-	-	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
PN19-1	3+	2+	1+	1+	+	+/-	+	+	-	1+	2+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
PN53-1	3+	-	3+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
GA8-1	3+	2+	-	1+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
GA8-2	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
HN9-1	3+	2+	-	1+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
HN9-2	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
PS49-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-
PW19-2	3+	-	-	-	+	+/-	+	+	3+	2+	2+	2+	2+	2+	2+	2+
RA103-1	3+	3+	-	1+	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
RA30-1	3+	2+	-	1+	+	+/-	+	+	2+	2+	2+	3+	3+	3+	3+	-

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
RB1-1	3+	3+	-	-	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
RB3-1	3+	3+	-	-	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
RP55-1	3+	2+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
SF18-1	3+	-	-	-	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
DA3-1	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
LBM3-2	3+	3+	-	1+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
SF61-2	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	-
SL89-1	3+	2+	1+	1+	+	+/-	+	+	-	2+	3+	3+	3+	3+	3+	+
SL89-2	3+	3+	-	-	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
SM63-1	3+	3+	+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
SM63-2	3+	3+	+	3+	+	+/-	+	+	3+	2+	2+	2+	2+	2+	2+	2+
ST107-1	3+	-	-	-	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
TE37-2	3+	2+	-	3+	+	+/-	+	+	-	-	+	+	+	+	+	2+
TM58-1	-	+	+	-	+	+/-	+	+	+	+	+	+	+	+	+	+
DT4-2	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
EN6-3	3+	3+	-	1+	+	+/-	+	+	-	3+	3+	3+	3+	3+	3+	-
TM58-2	-	+	+	-	+	+/-	+	+	-	+	+	+	+	+	+	+
TV83-2	3+	3+	+	3+	+	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
WM77-1	-	-	-	+	+	+/-	+	+	+	+	+	+	+	+	+	+
WM86-1	3+	3+	-	-	+	+/-	+	+	1+	2+	3+	3+	3+	3+	3+	+
ZN22-1	3+	3+	-	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+
AM35	3+	2+	-	2+	+	+/-	+	+	2+	2+	2+	2+	2+	+	3+	-
AM41	3+	2+	-	3+	+	+/-	+	+	2+	2+	2+	2+	2+	2+	2+	-
AD8-1	1+	2+	2+	2+	+	+/-	w	w	2+	3+	3+	3+	3+	3+	3+	2+
AD8-2	1+	2+	2+	2+	+	+/-	w	w	2+	3+	3+	3+	3+	3+	3+	+
AN34-1	+	3+	3+	+	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
AP1-2	-	3+	3+	-	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	3+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
AR02	+	3+	3+	+	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	3+
CK36-1	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
FE68-1	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
FE68-2	+	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
FG13-1	2+	3+	-	2+	+	+/-	w	w	-	1+	2+	2+	2+	2+	2+	2+
KD66-2	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	3+
KS72-2	-	2+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
KT43-1	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
MG71-1	+	3+	3+	+	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	3+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
PA3-2	3+	-	-	-	+	+/-	w	w	-	-	3+	3+	3+	3+	3+	2+
PJ82-2	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	2+	2+
PP42-2	-	3+	3+	-	+	+/-	w	w	2+	2+	3+	3+	3+	3+	3+	3+
SP73-1	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
SP73-2	+	3+	3+	+	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
TE37-1	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
TP35-1	+	3+	3+	+	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	2+
TP35-2	-	3+	3+	-	+	+/-	w	w	3+	3+	3+	3+	3+	3+	3+	3+
AD8-3	2+	3+	2+	3+	+	+/-	+	+	3+	3+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
MP11-1	-	+	-	-	+	+/-	+	+	+	+	+	+	+	+	+	+
AP1-1	-	2+	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+
AR03	3+	3+	-	3+	+	+/-	w	+	3+	3+	3+	3+	3+	3+	3+	+
FP47-1	3+	3+	-	3+	+	+/-	+	w	3+	3+	3+	3+	3+	3+	3+	2+
KS72-1	-	+	-	-	+	+/-	+	+	+	+	+	+	+	+	+	+
LG57-1	-	+	+	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	+
OR7-1	+	+	+	+	-	+/-	+	+	2+	3+	3+	3+	3+	3+	3+	+
RA30-2	3+	3+	-	3+	+	+/-	+	w	3+	3+	3+	3+	3+	3+	3+	2+
TV83-1	3+	2+	-	3+	+	+/-	+	w	+	2+	3+	3+	3+	3+	3+	-

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
BL13-1	3+	2+	-	-	+	+/-	+	+	2+	2+	3+	3+	3+	3+	3+	-
AG21-1	+	2+	3+	+	+	+/-	-	-	+	+	2+	2+	2+	2+	2+	3+
AG21-2	+	2+	3+	+	+	+/-	-	-	+	+	+	+	+	+	+	3+
AK33-2	+	+	3+	-	+	+/-	-	-	+	3+	3+	3+	3+	3+	3+	2+
AM10-1	+	+	3+	-	+	+/-	-	-	+	3+	3+	3+	3+	3+	3+	2+
AM10-3	2+	3+	2+	+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	+
AM14-1	-	+	+	-	+	+/-	-	-	2+	2+	2+	2+	2+	2+	2+	2+
AN34-2	-	-	-	-	+	+/-	-	-	+	+	+	+	+	+	+	2+
BA28-1	+	2+	3+	+	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	2+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
CK36-2	+	+	3+	-	+	+/-	-	-	+	+	+	2+	+	2+	+	+
CR16-1	+	2+	3+	+	+	+/-	-	-	+	+	2+	2+	2+	2+	2+	3+
CR84-1	-	+	3+	-	+	+/-	-	-	+	+	+	+	+	+	+	2+
DM52-2	+	+	3+	-	+	+/-	-	-	2+	2+	2+	2+	3+	3+	3+	2+
HG45-1	+	+	2+	+	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	-
HP27-2	-	+	3+	-	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	3+
JJ87-2	-	+	3+	-	+	+/-	-	-	2+	2+	2+	2+	2+	3+	3+	3+
JR70-1	-	+	+	-	+	+/-	-	-	+	+	+	+	+	+	+	+
JR70-2	+	2+	3+	+	+	+/-	-	-	+	2+	2+	+	+	+	+	2+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
KT43-2	+	+	+	-	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	+
LD51-1	+	2+	+	-	+	+/-	-	-	2+	2+	3+	3+	3+	3+	3+	+
LD51-2	+	+	3+	-	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	+
LK88-2	+	+	3+	-	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	+
LP92-1	-	+	3+	-	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	2+
LP92-2	-	+	3+	-	+	+/-	-	-	2+	2+	3+	3+	3+	3+	3+	2+
LR4-1	+	2+	3+	+	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	3+
MG71-2	-	+	2+	-	+	+/-	-	-	2+	2+	3+	3+	3+	3+	3+	+
MK44-1	+	2+	3+	+	+	+/-	-	-	+	2+	2+	2+	2+	2+	2+	2+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
MM86-2	+	+	3+	-	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	+
MR40-1	+	2+	3+	+	+	+/-	-	-	+	2+	2+	2+	2+	2+	2+	2+
PH32-2	-	+	2+	-	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	+
PP42-1	+	2+	3+	+	+	+/-	-	-	+	+	2+	2+	2+	2+	2+	3+
PS49-2	-	+	3+	-	+	+/-	-	-	3+	3+	3+	3+	3+	3+	3+	2+
RP55-2	+	+	3+	-	+	+/-	-	-	2+	2+	2+	3+	3+	3+	3+	+
SB20-2	+	3+	3+	+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	2+
SF61-1	-	+	3+	-	+	+/-	-	-	2+	2+	2+	2+	2+	3+	3+	3+
SIS32-1	3+	3+	2+	3+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	+

Table 4.2 Physiological and biochemical characteristic of 181 isolates (Continued)

Isolate no.	Growth on Glucose+0.3% acetic acid	Growth on Glucose w/o acetic acid	Growth on Sorbitol	Growth on Sucrose+0.3% acetic acid	Catalase test	O/F test	Oxidation of Acetate	Oxidation of Lactate	Growth at pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	Growth at 30% Glucose
SIS32-2	3+	3+	2+	3+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	+
SIS32-3	2+	2+	2+	2+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	+
ZN22-2	+	+	3+	+	+	+/-	-	-	2+	2+	3+	3+	3+	3+	3+	2+
AM24	+	2+	3+	+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	2+
AM26	+	2+	3+	+	+	+/-	-	-	+	+	2+	2+	2+	2+	2+	+
AM28	+	2+	3+	+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	3+
AN34-1	1+	2+	2+	2+	+	+/-	w	w	2+	3+	3+	3+	3+	3+	3+	2+
AM29	2+	3+	3+	+	+	+/-	-	-	2+	3+	3+	3+	3+	3+	3+	2+

+, positive; w, weak positive; -, negative

Table 4.3 Acid from carbohydrates of 181 isolates

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
AD8-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
AD8-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
AD8-3	+	+	+	+	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-
AG21-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
TP35-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
TE37-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
TP35-1	+	+	+	+	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-
AN34-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
DM52-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AG21-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
AK33-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
AK33-2	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM10-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM10-3	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM13-2	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+		-	+	-
AM14-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AN1-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
AN34-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
MP11-1	+	+	+	+	-	+	-	-		-	-	+	+	+	+	-	-	+	-
AN34-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
AK33-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
AK33-2	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM10-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM10-3	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM13-2	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+		-	+	-
AM14-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AN1-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
AN34-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
MP11-1	+	+	+	+	-	+	-	-		-	-	+	+	+	+	-	-	+	-
AN34-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
AP60-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
AP94-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
AP94-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
AP1-1	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	-	+	-
AP1-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
AR02	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
AM35	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
AM41	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
AR03	+	+	+	+	-	+	-	-		-	-	+	+	+	+	+	-	+	-
AV28-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
BA28-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
BA28-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
BN1-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
BN1-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
BB91-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
BBM91-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
CA127-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
CA127-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
CA76-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
CD21-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
CD21-2	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	-
CK36-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-		+	-	-	-
CK36-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
CM50-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
CM50-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
CR16-1	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-
CR16-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
CR84-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
CR84-2	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
CS15-2	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
CS15-4	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
CT85-1	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
CM3-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
CT85-2	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
DM52-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
DM52-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LM12-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
HM12-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
HM12-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
GA8-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
HN9-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
GA8-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
HN9-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
DA3-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
FBM4-3	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
FBW4-3	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	+	-
FBY4-3	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-
FC4-2	+	+	+	+	-	-	-	-	-	+	-	+	+	-	-	-	-	+	-
FC4-3	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
FCL4-5	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
FCS4-5	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-		-	+	-
FE68-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
FE68-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
FG13-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
FP47-1	+	+	+	+	+	+	-	-		-	-	+	-	-	+	-	-	+	-
FP47-2	+	+	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-
GR64-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
GR64-2	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-
GV74-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
HG45-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
HG45-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
HP27-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
HP27-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
IR14-1	+	+	+	+	-	-	-	-	-	+	-	+	+	-	-	-	-	+	-
JA54-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-
EN6-3	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
JF81-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
JJ87-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-
JJ87-2	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	+	+	+	-
JR70-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
JR70-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
KD66-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
KD66-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
BL13-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
ST79-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM26	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM28	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
KLM13-1	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-
KS72-1	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-
KS72-2	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
KT43-1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	+	-
KT43-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LD51-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LD51-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LG57-1	+	+	+	+	+	-	-	-		-	-	+	+	+	-	-	-	+	-
LG57-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
LK88-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
ZN22-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
LK88-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LM26-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
LP92-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LP92-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LR4-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
LR41-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
LS60-1	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-
LS60-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
LBM3-1	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-
LBM3-2	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-
AM46	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
MG71-1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
MG71-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
MHM10-1	+	+	+	-	-	-	-	-	-	+	-	+	-	-	+	+	-	+	-
MK44-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
MM86-1	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
MM86-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
MR40-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
MT78-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
MT78-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
AM48	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
AM68	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
NJ17-3	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
OR55-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
OR55-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
OR56-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
OR7-1	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-
OR95-1	+	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-
PA3-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
ZN22-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-
AM24	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-
AM29	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
AM10-3	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
PA3-3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
AM44	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-
AM47	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-
PH32-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
PJ82-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-
PK48-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
PM169-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
PN19-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
PN53-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
PP42-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
PP42-2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-
PS49-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
PS49-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
PW19-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
QS18-1	+	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-
RA103-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
RA30-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
RA30-2	+	+	+	+	+	-	-	-		-	-	+	-	-	+	-	-	+	-
RB1-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
RB3-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
TE37-2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
TV83-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
RP55-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
RP55-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
SB20-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	-
SF18-1	+	+	+	-	-	-	-	-	-	-	-	-				-	-	+	-
SF61-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
TM58-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
TM58-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-

Table 4.3 Acid from carbohydrates of 181 isolates (Continued)

Isolate no.	Acid production from D-Glucose	D-Mannose	D-Galactose	D-Fructose	L-Sorbose	D-Xylose	D-Arabinose	L-Rhamnose	D-Mannitol	D-Sorbitol	Dulcitol	Glycerol	Maltose	Lactose	Melibiose	Sucrose	Raffinose	Ethanol	Methanol
WM71-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
WM86-1	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
SF61-2	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
SI15-1	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-	-	+	-
SI15-2	+	+	+	+	+	-	+	+	-	+	-	+	+	-	-	-	-	+	-
SIS32-1	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
SIS32-2	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
SIS32-3	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-
SL89-2	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-

+, positive; - negative

2.3 Chemotaxonomic characteristics

2.3.1 Ubiquinone analysis

The representative strains in Group 1 (PA3-3, OR95-1, LS60-2 and LK88-1); Group 2 (MHM10-1, FBM4-3 and KLM13-1); Group 3 (LBM3-1 and LBM3-2) contained ubiquinone-9 as major quinine while Group 4 (JJ87-2, RP55-2 and LD51-1); Group 5 (PS49-2, AM10-3 and MK44-1); Group 6 (LD51-1 and JR70-2); Group 7 (MG71-2, ZN22-2); Group 8 (PJ82-2, FG13-1 and AP1-2); Group 9 (AP1-1 and OR7-1); Group 10 (SI15-1); Group 11 (CT8-1) contained ubiquinone-10 as major quinine (Fig. 4.1).

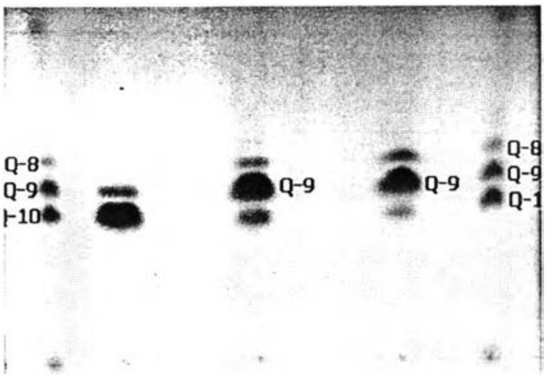


Fig 4.1 Ubiquinone of representative strains

2.3.2 DNA base composition

The DNA G+C contents of the representative strains in *Acetobacter*, PA3-3 and OR95-1 in Group 1 were 52.4 and 52 mol%; MHM10-1 and FBM 4-3 (Group 2) were 53.8 and 54.2 mol%; LBM 3-1 (Group 3) was 58.4 mol%, respectively. *Gluconobacter* strains, Group 4 (LD51-1) was 61.2 mol%; Group 5 (MK44-1); Group 6 (LD51-1); and Group 7 (ZN22-2) were 55.9, 51.3 and 55.4 mol%, respectively. The other strains, PJ82-2 (Group 8) was 60.4 mol%; Group 9 (AP1-1) was 58.5 mol%; Group 10 (SI15-1); Group 11 (CT8-1) were 60.7 and 58.2 mol%, respectively.

2.4 Molecular characteristics

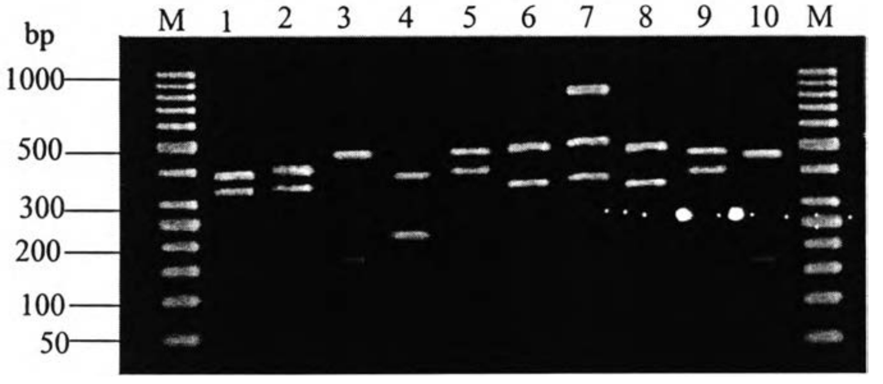
2.4.1 The 16S-23S rDNA internal transcribed spacer region restriction fragments length polymorphism (RFLP) analyses.

The 97 isolates that oxidized acetate and lactate, were divided into 3 groups, and designated as Group 1, Group 2 and Group 3, on the basis of the combination of the restriction patterns obtained by digestion with the two restriction endonuclease, *Hpa*II and *Hae*III (Table 4.4, Fig. 4.2).

Table 4.4 Restriction pattern digested by restriction endonucleases of isolates

Group/Isolate no.	Restriction pattern digested with	
	***Hpa*II**	***Hae*III**
Group 1 : PA3-3, AP94-1, BA28-2, CR16-2, CR84-2, CT85-1, CT85-2, FCL4-5, FP 47-2, GR64-1, GR64-2, GV74-1, HG45-2 ,HP27-1, KD66-1, LG57-2, LK88-1, LM26-1, LS60-1, LS60-2, OR55-1, OR55-2, OR56-2, OR95-1, PK48-1, PM169-2, PS49-1, PW19-2, RA103-1, RA30-1, RB1-1, RB3-1, SF61-2, SL89-1, SL89-2, SM63-1, SM 63-2, ST107-1, TM58-1, TM58-2, WM77-1, WM86-1, ZN22-1, AK33-1, AM13-2, AP 60-1, AP94-2, AV28-1, BB91-1, CS15-2, CS15-4, FBY4-3, FC4-3 (53 isolates)	*f*	*h*
Group 2 : MHM10-1, CA76-2, CM50-1, CM50-2, DM52-1, JA54-1, JF81-1, JJ87-1, LR41-1, MM86-1, MT78-1, MT78-2, NJ17-3, PN19-1, PN53-1, RP55-1, SF18-1, TE 37-2, TV83-2, AM35, AM41, FBM4-3, BBM91-1, CD21-1, KLM13-1, FBM4-3, BN 1-1, BN1-2, CM3-1, LM12-1, HM12-1, HM12-2, GA8-1, GA8-2, HN9-1, HN9-2, DA 3-1, DT4-2, EN6-3, BL13-12,CA127-1, CA127-2 (42 isolates)	*e*	*i*
Group 3 : LBM3-1, LBM3-2 (2 isolates)	*c*	*j*

(a)



(b)

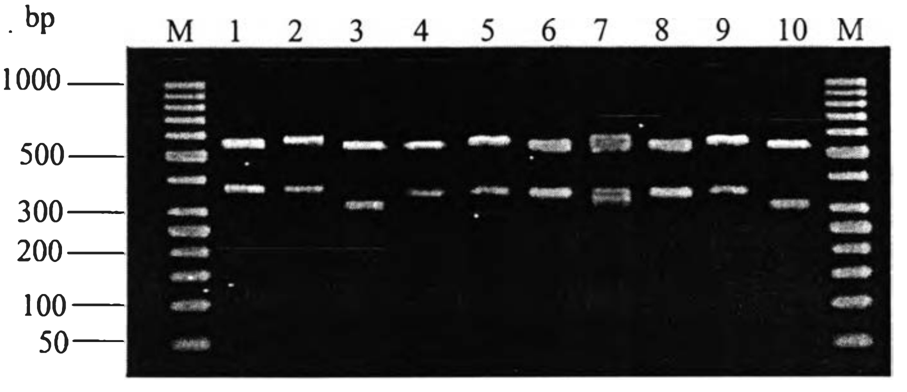


Fig. 4.2 Restriction patterns of 16S-23S rDNA ITS region PCR products of type strains of *Acetobacter* species by digestion with *Hpa*II (a) and *Hae*III (b) restriction endonuceases.

For estimation of digestion fragments produced from 16S-23S rDNA ITS region PCR products, 50 bp DNA markers were used in the agarose gel electrophoresis. Abbreviation: 1, *A. indonesiensis* NBRC 16471^T; 2, *A. cibinongensis* NBRC 16605^T; 3, *A. lovaniensis* NBRC 13753^T; 4 , *A. tropicalis* NBRC 16470^T; 5, *A. orientalis* NBRC 16606^T; 6, *A. pasteurianus* TISTR 1056^T; 7, *A. aceti* NBRC 14818^T; 8, Group 1; 9, Group 2; 10, Group 3; M, 50 bp DNA markers

Group 1 composed of 53 strains such as PA3-3, AP94-1 and so on (Table 10). It revealed the same restriction pattern f and h forms as *A. pasteurianus* TISTR 1056^T, when digested with *Hpa*II and *Hae*III restriction endonucleases, respectively (Table 4.4, Fig. 4.2).

Group 2 included 42 strains, MHM10-1, CA76-2, CM50-1 and so on (Table 10.). These strains showed the restriction pattern, e when digested with *Hpa*II and i when digested with *Hae*III restriction endonucleases. The pattern forms of Group 2 not so differenced from Group A (Table 4.4, Fig. 4.2). However, they can separate as two different groups.

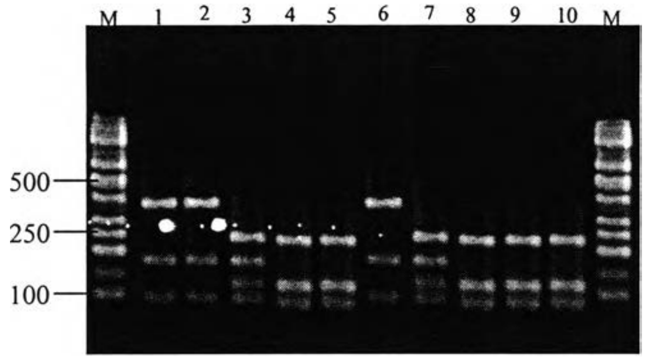
Group 3 contained 2 strains, LBM3-1 and LBM3-2. The DNA pattern of *Hpa*II digestion showed c form and j form presented in *Hae*III digestion (Table 4.4, Fig. 4.2).

The 45 isolates that did not oxidize acetate and lactate, were divided into 4 groups, designated as Group 4 to Group 7, on the basis of the combination of the restriction patterns obtained by digestion with the two restriction endonuclease, *Bsp*1286I, *Mbo*II and *Ava*II (Table 4.5 and Fig. 4.3).

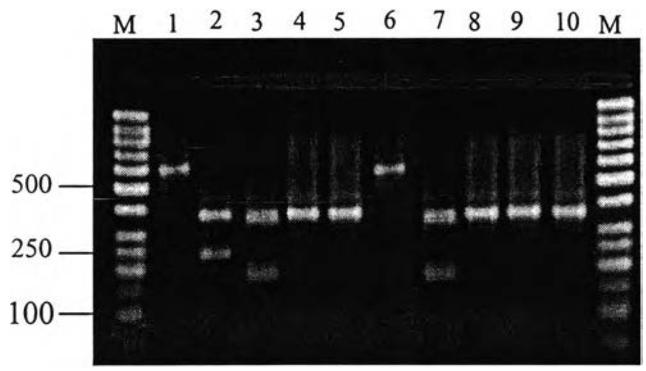
Table 4.5 Restriction pattern digested by restriction endonucleases of isolates

Group/Number of Isolates	Restriction pattern by digestion with		
	***Bsp*1286I**	***Mbo*II**	***Ava*II**
Group 4: AG21-1, BA28-1, HG45-1, JJ87-2, JR70-1, LD51-2, LK88-2, LP92-1, RP55-2, SB20-2, SF61-1, ST79-1, AM26, AM28, AM46,AM48, AM68 (17 isolates)	*Go*	*Go*	-
Group 5: DM52-2, MK44-1, MM86-2, AG21-2, AK33-2, AM10-3, AM14-1, AN34-2, MR40-1, PH32-2, PP42-1, PS 49-2 (12 isolates)	*Gc*	*Gc*	-
Group 6: AM10-1, CR16-1, CR84-1, HP27-2, JR70-2, KT 43-2, LD51-1, LP92-2, LR4-1 (9 isolates)	*Gf*	*Gf*	*Gf*
Group 7: MG71-2, ZN22-2, AM24, AM29, AM47, CK36-2 (7 isolates)	*Gt*	*Gt*	*Gt*
AN1-1 (in Group 7)	*Gt*	*Gt*	*Gs*

(a)



(b)



(c)

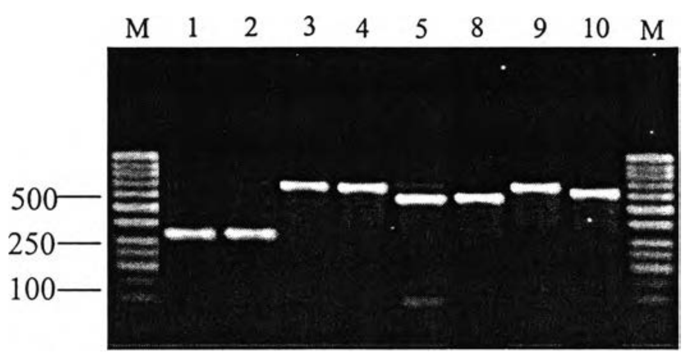


Fig. 4.3 Restriction patterns of 16S-23S rDNA ITS region PCR products of type strains of *Gluconobacter* species by digestion with *Bsp*1286I (a) *Mbo*II (b) and *Ava*II (c) endonuclease.

For estimation of digestion fragments produced from 16S-23S rDNA ITS region PCR products, 50bp DNA markers were used in the agarose gel electrophoresis. Abbreviation: 1, *G. oxydans* NBRC 14819^T; 2, *G. albidus* NBRC 3250^T; 3, *G. cerinus* NBRC 3267^T; 4, *G. thailandicus* NBRC 100600^T; 5, *G. frateurii* NBRC 3264^T; M ; 6, Group 4; 7, Group 5; 8, Group 6; 9, Group 7; 10, AN1-1; M,50bp DNA marker

Group 4 contained 17 isolates, AG21-1, BA28-1 and so on as in Table 11. They showed the same restriction patterns as *G. oxydans* NBRC 14819^T when digested by *Bsp*1286I and *Mbo*II restriction endonucleases, *G. oxydans*/ *G. oxydans* pattern in Yukphan *et al.*, 2004 (Fig. 4.3).

Group 5 contained 12 isolates, DM52-2, MK44-1 and so on (Table 11), gave the same restriction patterns as *G. cerinus* NBRC 3267^T, *G. cerinus*/*G. cerinus* pattern when digested by *Bsp*1286I and *Mbo*II restriction endonucleases (Fig. 4.3) (Yukphan *et al.*, 2004).

Group 6 contained 9 isolates, AM10-1, CR16-1, CR84-1, HP27-2, JR70-2, KT43-2, LD 51-1, LP92-2 and LR4-1 presented as *G. frateurii*/*G. frateurii* pattern, as described by Yukphan *et al.*, 2004 (Table 4.5 and Fig. 4.3). In the previous paper, *G. thailandicus* and *G. frateurii* complex were showed closely relationship together by the same patterns based on *Bsp*1286I and *Mbo*II restriction analyses. However, *Ava*II restriction analyses could be differentiated *G. frateurii* from *G. thailandicus* strains (Tanasupawat *et al.*, 2004., Malimas *et al.*, 2006).

Group 7 contained 7 isolates, MG71-2, ZN22-2, AM24, AM29, AM47 and CK36-2 showed the same restriction patterns with *G. thailandicus* NBRC 100600^T when analyzed with *Ava*II endonuclease. They were belonged to *G. frateurii*/ *G. frateurii*/ *G. thailandicus* patterns (Table 4.5 and Fig. 4.3). (Yukphan *et al.*, 2004, Tanasupawat *et al.*, 2004., Malimas *et al.*, 2006).

Only one strain, AN1-1 in group 7 presented restriction pattern different from other group when digested by *Ava*II endonuclease (Table 4.5), as a cutting position and higher band of 610 bp but lower 714 bp of *G. frateurii* and *G. thailandicus* type strains (Fig. 4.3) , respectively.

2.4.2 16S rDNA sequence and phylogenetic analysis

The PCR products of 10 selected strains were determined for their 16S rDNA nucleotide sequences. Their nucleotide sequences were illustrated in Appendix D. The completes 16S rDNA sequence consisting of about 1300-1500 nucleotides were determined for some type strains of *A. pasteurianus, A. orientalis, A. lovaniensis, G. oxydans, G. cerinus, G. frateurii, G. thailandicus Sw. salitolerans K. baliensis*. Based on 16S rDNA s equences from the selected strains, the phylogenetic tree were constructed from evolutionary distances by using neighbor-joining method in the MEGA program version 2.1.

The representative isolates of Group 1, PA3-3 was 100% related to each other and shared 16S rDNA nucleotide similarity within 99.8% with *A. pasteurianus* TISTR 1056^{T}. Strains KLM13-1, MHM10-1, FBM4-3 and BBM91-1 (Group 2) showed 99.5%, 99.4%, 99.7 and 99.6% sequence similarities, respectively. with *A. orientalis* NRIC 0481^{T}. LBM3-1 (Group 3) showed 99.8% similarity with *A. lovaniensis* IFO 13753^{T}. JR70-1 (Group 4) showed 99.8% similarity with *G. oxydans* NBRC 14819^{T}. AK33-2 (Group 5) showed 99.7% similarity with *G. cerinus* NBRC 3267^{T}. LD51-1(Group 6) showed 99.6% similarity with *G. frateurii* NBRC 3264^{T}. MG 71-2 (Group 7) showed 99.8% similarity with *G. thailandicus* NBRC 100600^{T} while AN1-1 showed 98.7% similarity with *G. frateurii* NBRC 3264^{T}. MG71-1 (Group 8) showed 99.7% similarity with *As. bogorensis* NBRC 12264^{T}. SIS32-2 (Group 9) showed 97.8% similarity with *Ga. liquefacien*s IFO 12388^{T}. SI15-1 (Group 10) showed 97.7% similarity with *Sw. salitolerans* PA51^{T}. Group 11, CT8-1 showed 96.4% similarity with *K. baliensis* NRIC 0488^{T}.

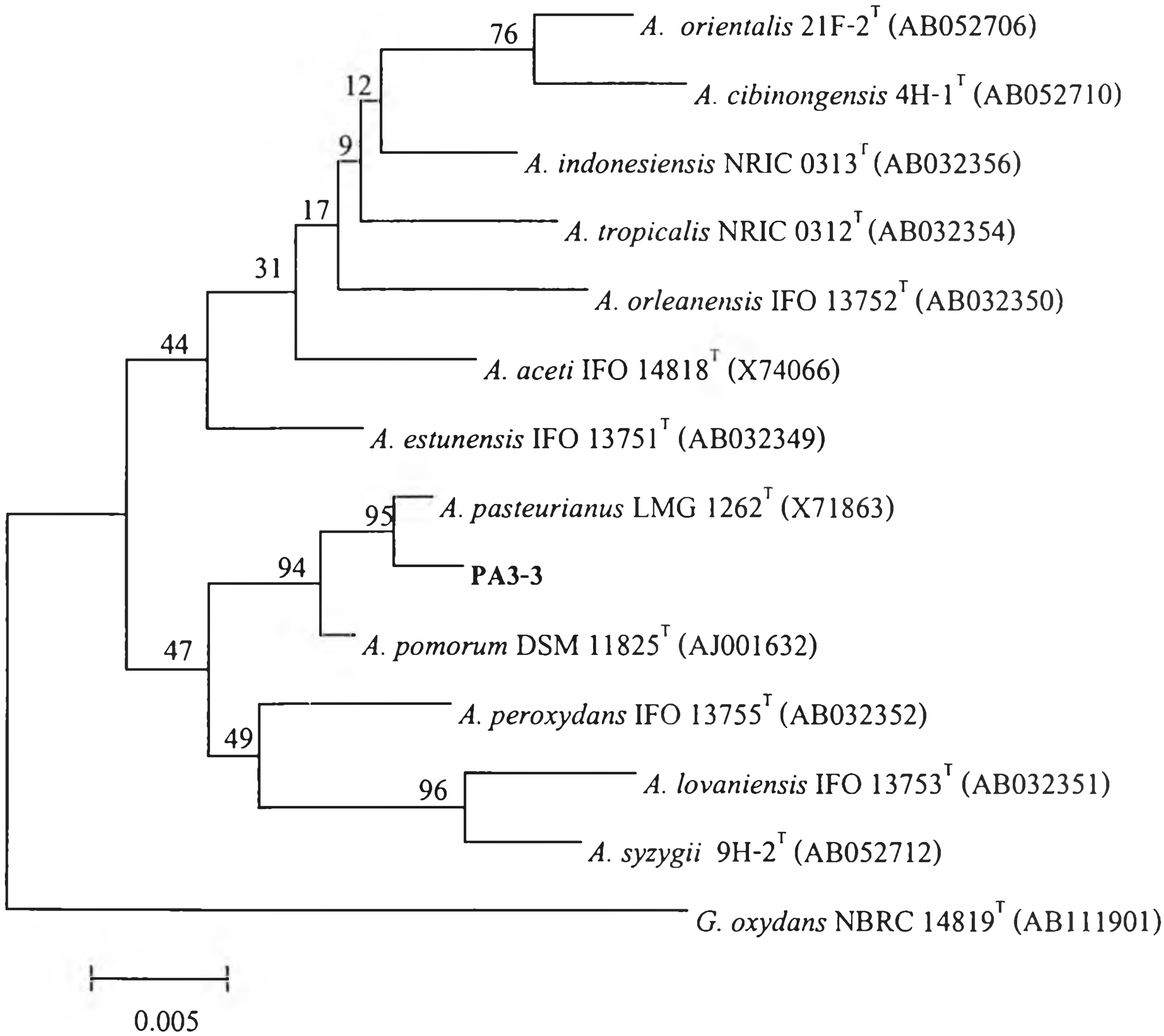


Fig. 4.4 Neighbour-joining-tree showing the phylogenetic position of strain PA3-3 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

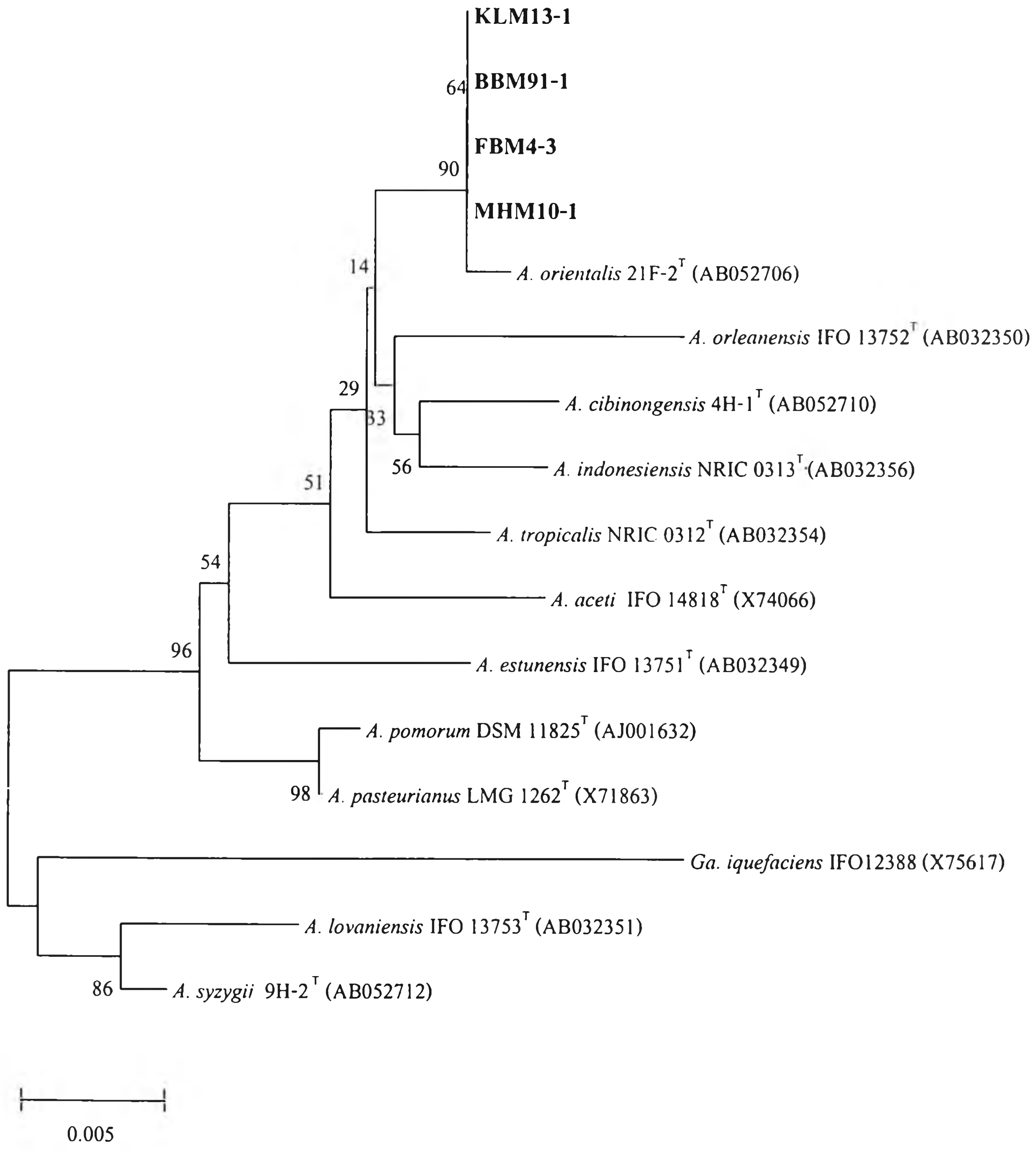


Fig. 4.5 Neighbour-joining-tree showing the phylogenetic position of strains KLM13-1 BBM91-1, FBM4-3 and MHM10-1 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

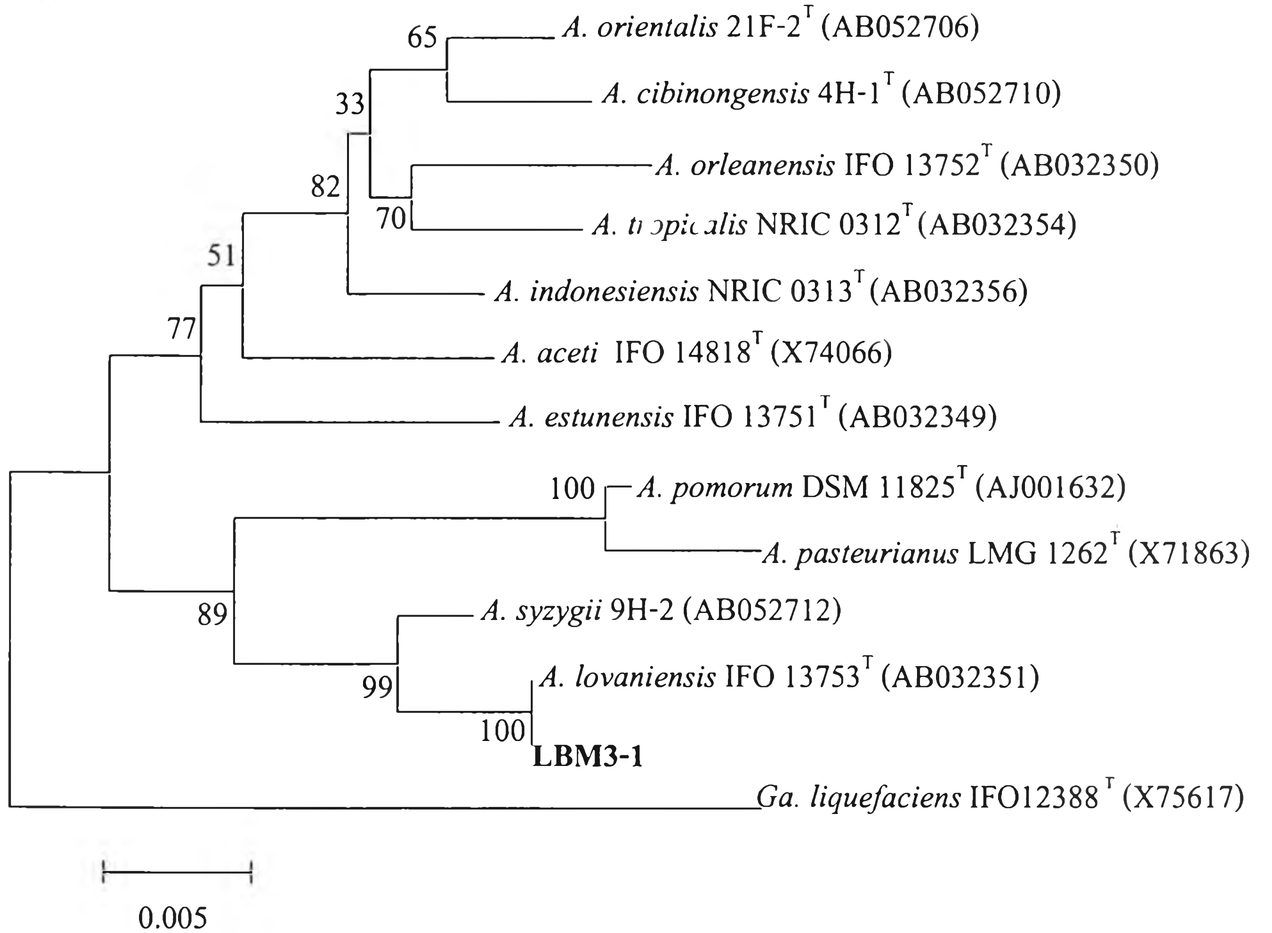


Fig. 4.6 Neighbour-joining-tree showing the phylogenetic position of strain LBM3-1 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

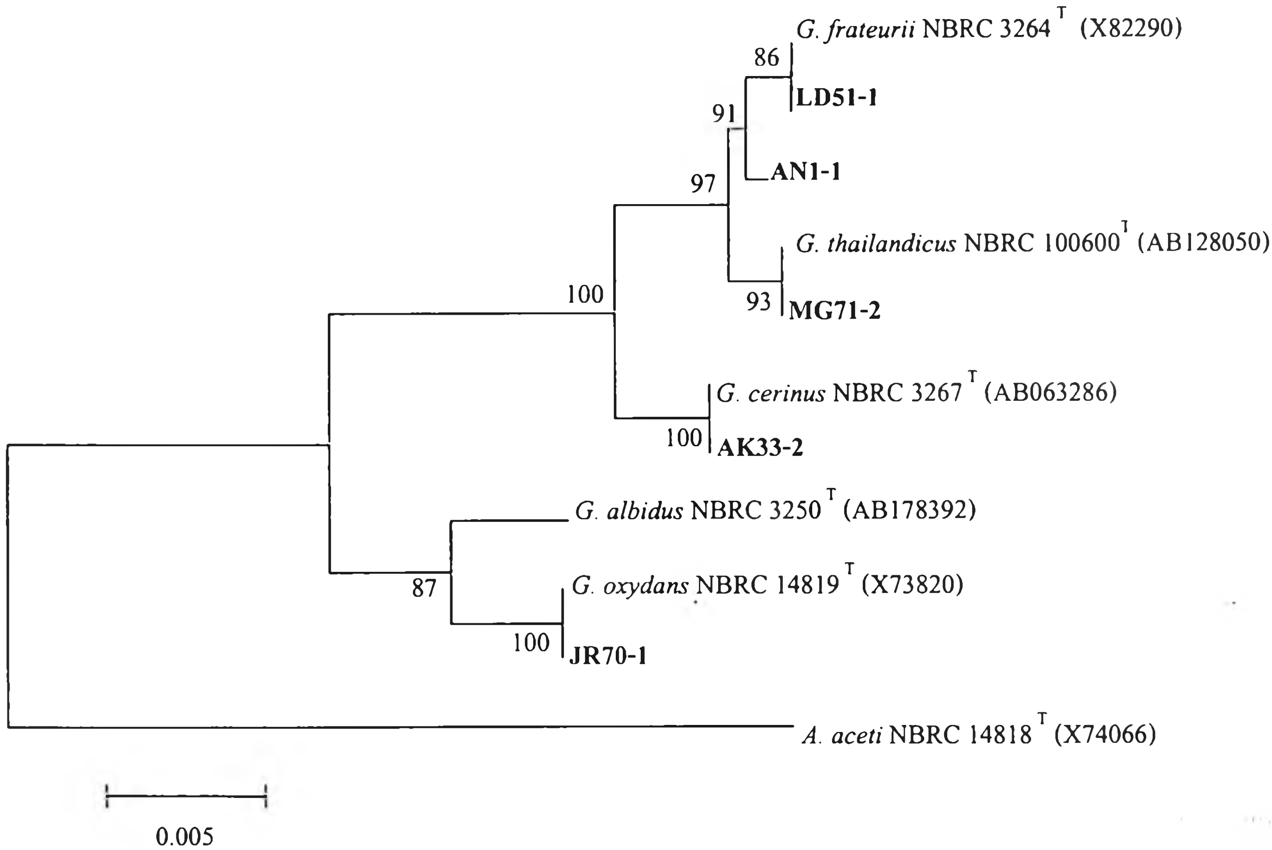


Fig. 4.7 Neighbour-joining-tree showing the phylogenetic position of strains JR70-1, AK33-2, LD51-1, MG71-2 and AN1-1 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

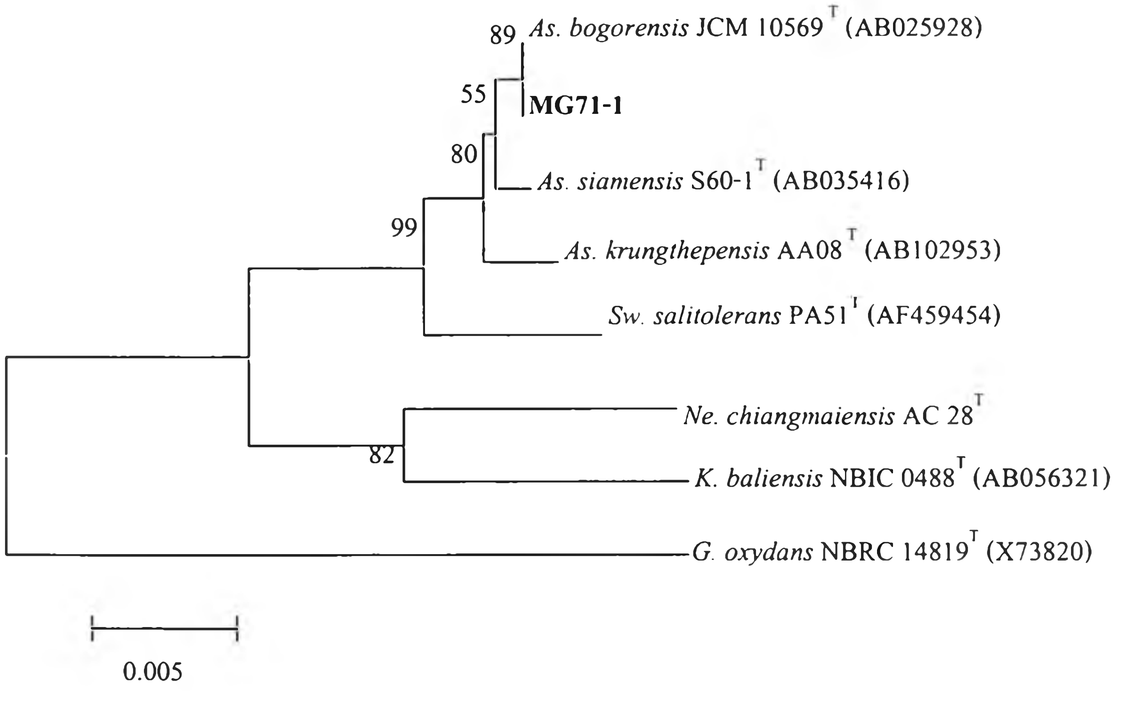


Fig. 4.8 Neighbour-joining-tree showing the phylogenetic position of strains MG71-1 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

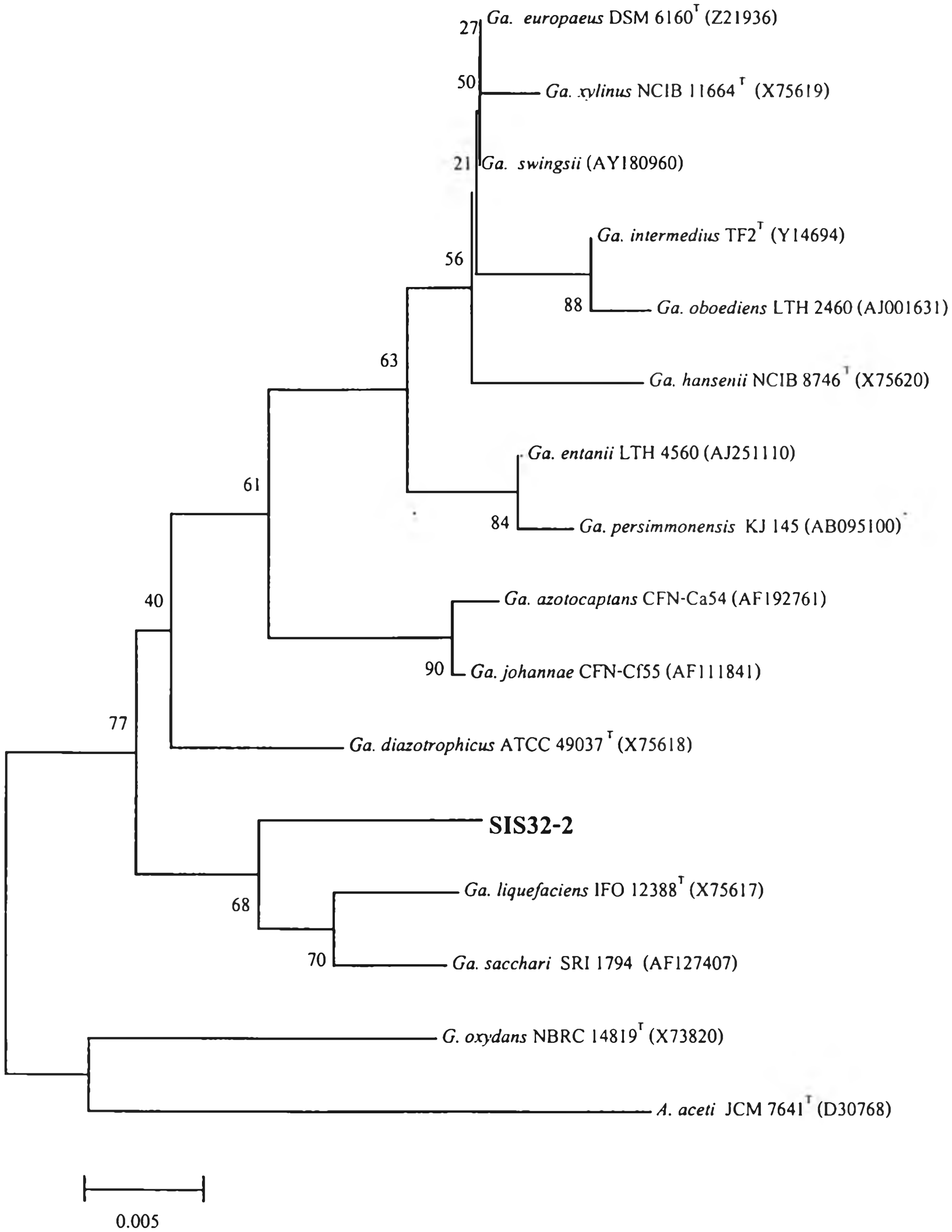


Fig. 4.9 Neighbour-joining-tree shoeing the phylogenetic position of strain SIS32-2 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

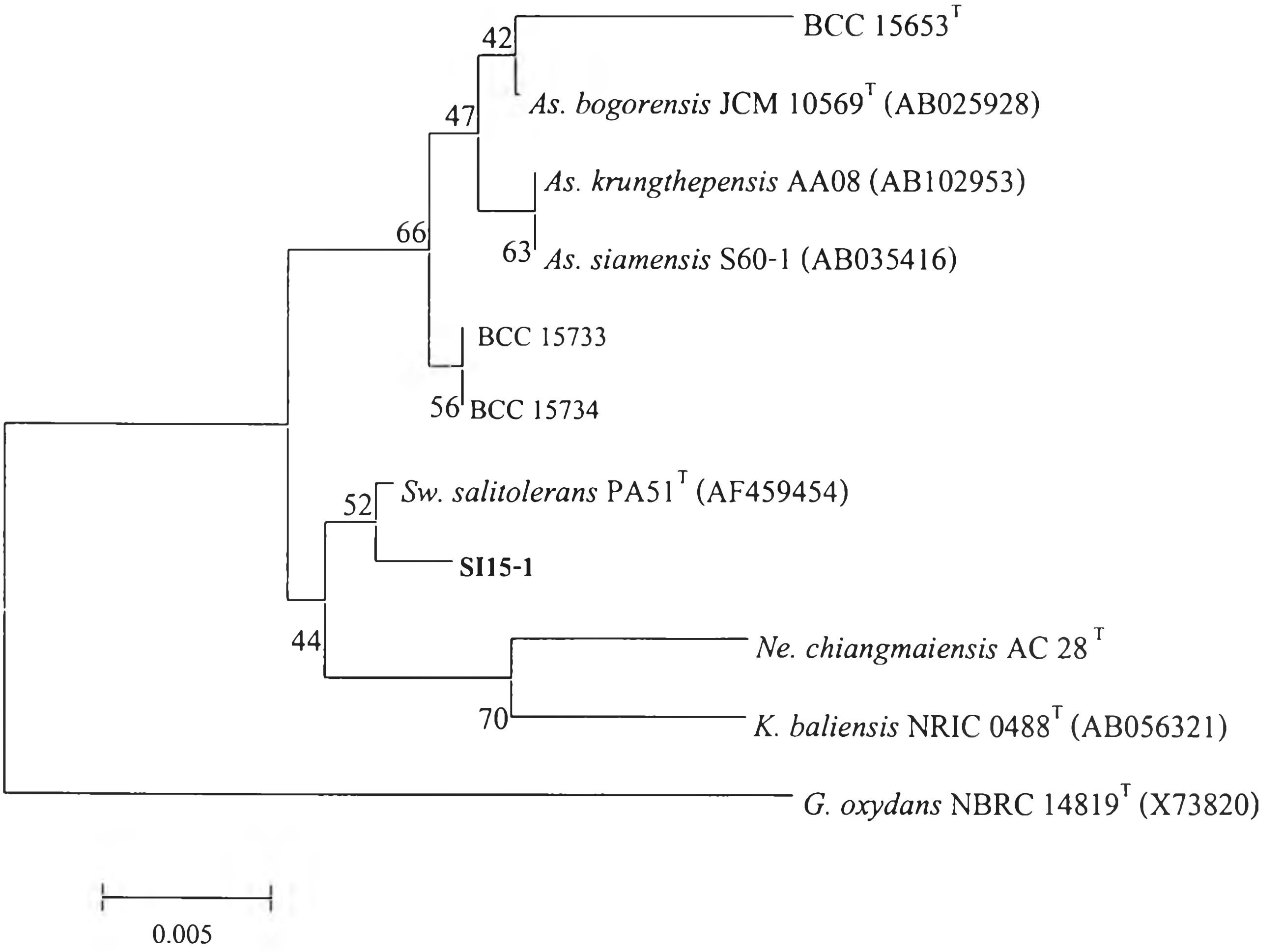


Fig. 4.10 Neighbour-joining-tree shoeing the phylogenetic position of strain SI15-1 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

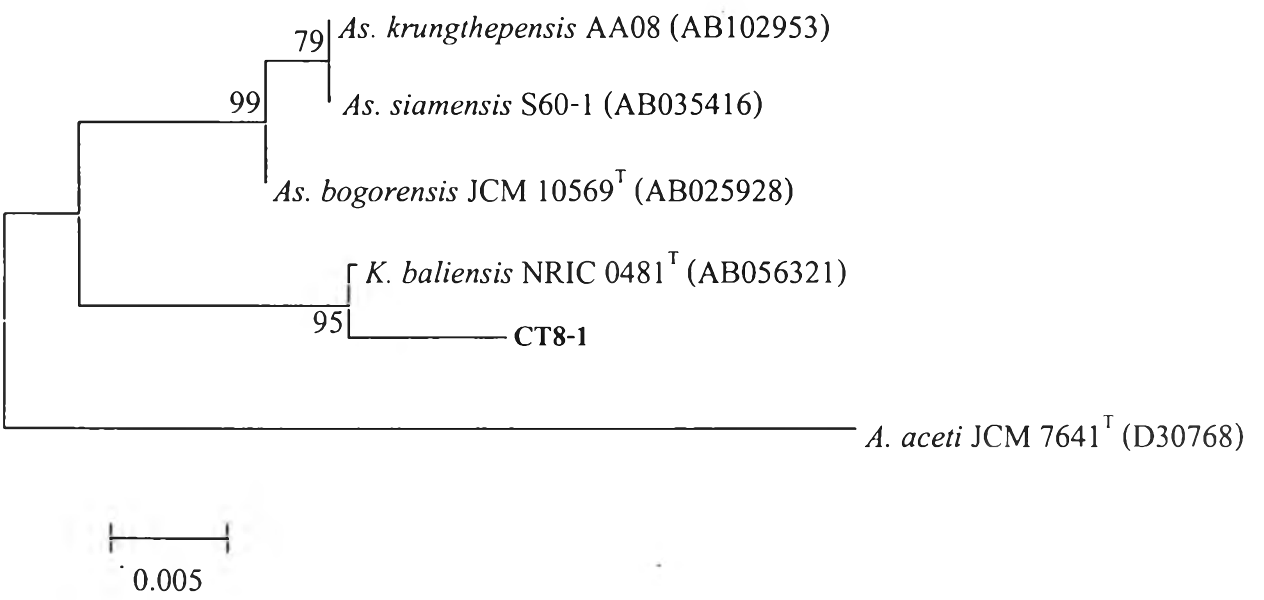


Fig. 4.11 Neighbour-joining-tree shoeing the phylogenetic position of strain CT8-1 based on 16S rDNA sequences. Bar, 0.005 substitution per nucleotide position. Bootstrap values expressed as 1,000 replications.

Chracterization of *Acetobacter and Gluconobacter* strains

Group 1 contained 53 isolates, as shown in Table 4.4

The representative strain, PA3-3 was located in the cluster of *A. pasteurianus* LMG 1262^T of the phylogenetic analyses based on the 16S rDNA gene sequences and had 99.8% sequence similarity to the type strain of this species. The isolates produced acetylmethyl carbinol from lactate and grew at 37-40°C. 2-Ketogluconate was not produced in all the isolates. The isolates were characterized by Q-9 as a major ubiquinone in the coexistence of a considerable quantity of Q-8, the data of which coincided with those of Yamada *et al.* (1968). DNA G+C contents of tested strains PA3-3 and OR95-1 were 52.4 and 52 mol%, respectively were identified as *A. pasteurianus* (Lisdiyanti *et al.*, 2002). On the basis of restriction pattern and 16S rDNA sequence analysis were closed to *A. pasteurianus*. The 53 isolates were therefore identified as *A. pasteurianus* (Swings, 1992; Yamada *et al.*, 1997; Wayne *et al.*, 1987). These isolates were found in Rumbutan, Starfruit, Hog plum, Watermelon, Zinnia, Salas, Caricature Plant, Cantaloup, Custard apple, Strawbeery, Pum melo, Orange, Tomato, Apple, Unkown flower, Musk-melon, Honey, Grape, Long gong, Heliconia, Periwinkle, Langsat, Avocado, Banana, Cordia flower, Rose apple, Periwinkle and Fermented starch.

Group 2 contained 42 isolates, as shown in Table 4.4

The representative strains (isolates KLM13-1, MHM10-1, FBM4-3 and BBM91-1) were phylogenetically located in the cluster of *A. orientalis* and had 99.5%, 99.4%, 99.7 and 99.6% sequence similarities, respectively to the type strain of this species. The isolates produced 2-keto-D-gluconic acid. The 40 isolates were therefore identified as *A. orientalis* (Lisdiyanti *et al.*, 2000). They were differentiated from the strains in Group 1 by the restriction pattern. DNA G+C contents of tested strains MHM10-1 and FBM4-3 contained 53.8 and 54.2 mol%, respectively identified as *A. orientalis* (Lisdiyanti *et al.*, 2002). These isolates were found in Radermachera, Sugar apple, Chrismas flower, *Cordia sebstena*, Apple, Starfruit, Dragon fruit, Allamanda, *Murraya paniculata* Jack, Musk-melon, Khao-mak, Tamarind, Thaivermicelli, Night jasmine, Caricature Plant, Petunia, Sugar apple, Mangosteen, *Samanea saman*, Jackfruit, Jujube, Banana, Quassia, Ixora, Kaffir lime, Guava and Fermented flour.

Group 3 contained 2 isolates, as shown in Table 4.4

The representative strains (isolates LBM3-1) was phylogenetically located in the cluster of *A. lovaniensis* and had 99.5% sequence similarities respectively to the type strain of this species. The isolates had Q-9 as found in the isolates of Group 1. The 2 isolates were therefore identified as *A. lovaniensis* (Lisdiyanti *et al.*, 2000). They were differentiated from the isolates of Group A by restriction pattern. DNA G+C contents of tested strains LBM3-1 was 58.4 mol% identified as *A. lovaniensis* (Lisdiyanti *et al.*, 2002). These isolates were found in flowers of Elaeocarpus.

Table 4.6 Differential characteristics of *Acetobacter* strains

Characteristics	Group 1 53 isolates	*A. pasteurianus* NBRC 1056^T	Group 2 42 isolates	*A. orientalis* NBRC 16606^T	Group 3 2 isolates	*A. lovaniensis* NBRC 13753^T
Acetate oxidation	+	+	+	+	+	+
Lactate oxidation	+	+	+	+	+	+
Ketogenesis	-	-	-	-	-	-
Acetylmethyl carbinol	+	+	w	w	w	w
2-Keto-D-gluconic acid	-	-	+	+	-	-
Growth in the present of						
10% ethanol	+	+	-	-	-	-
Nitrate reduction	+	+	-	-	+	+
Ubiquinone	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9
Growth at 40°C	+	+	-	-	-	-
Growth on						
L-Arabitol	-	-	-	-	w	w
Acid production from						
Cellobiose	+	+	- (w6)	-	w	w
D-Xylose	-(w8)	-	+	+	+	+
Melibiose	-	-	+	+	-	-

+, positive; w, weak positive; -, negative

Group 4 contained 17 isolates, as shown in Table 4.5

The isolates produced acetylmethyl carbinol from lactate and grew at 30°C. 2-ketogluconate and 5-ketogluconate were produced in all the isolates but 2,5-ketogluconate were not produced in all the isolates. The 17 isolates were therefore identified as *G. oxydans*. On the basis of restriction pattern and 16S rDNA sequence analysis were identified as *G. oxydans*, these 17 strains gave the same restriction patterns as the type strains of *G. oxydans* NBRC 14819^T when digested by *Bsp*1286I and *Mbo*II restriction endonucleases, *G. oxydans/G. oxydans* pattern in Yukphan *et al.*, 2004. These isolates were found in Honey, Salas, Unkown flower, Plumeria flower, Jujube, Mangosteen, Long-gong, Chaba, Cassia, Strawberry, Pagoda flower, Look-pang, Star fruit and *Baccaurea ramiflora Lour.*

Group 5 contained 12 isolates, as shown in Table 4.5

The isolates were growth at 30°C but were not growth at 37°C and growth on D-Arabitol. Acid production from *myo*-Inositol. D-Mannose and D-Sorbitol. They were identified as *G. cerinus*. On the basis of restriction patterns, 12 strains gave the same restriction patterns as the type strains of *G. cerinus* NBRC 3267^T, *G. cerinus/G. cerinus* pattern forms when digested by *Bsp*1286I and *Mbo*II restriction endonucleases (Yukphan *et al.*, 2004). DNA G+C contents of tested strains LD51-1 was 61.2 mol% identified as *G. cerinus* (Lisdiyanti *et al.*, 2002). These isolates were found in Long-gong, Rose apple, Kaffir lime, Red apple, Guava, Banana, Unkown flower, Petunia, Papaya, *Baccaurea ramiflora Lour*, *Cleome spinosa*, Night Jasmine, Cordia flower, Peach, *Adenium obsum*, Unkown flower and Musk-melon.

Group 6 contained 9 isolates, as shown in Table 4.5

The isolates were growth at 30°C but were not growth at 37°C and growth on D-Arabitol. Acid production from *myo*-inositol. D-mannose and melibiose and sucrose. They were identified as *G. cerinus*. On the basis of restriction pattern, the isolates presented as *G. frateurii/ G. frateurii* pattern, described with Yukphan *et al.*, 2004. In the previous paper *G. thailandicus* and *G. frateurii* complex were described and showed the closely relationship between *G. frateurii* and *G. thailandicus* by the same pattern based on *Bsp*1286I and *Mbo*II restriction analysis., However, *Ava*II restriction analysis presented differentiation of *G. frateurii* and *G. thailandicus* (Tanasupawat *et al.*, 2004., Malimas *et al.*, 2006). DNA G+C contents of tested strains AN1-1 51.3 mol%. These isolates were found in *Cordia sebstena* Caricature Plant, Custard apple, Mango, *Ixora chinensis*, Plumeria flower, *Heliconia*, and Look-pang.

Group 7 contained 9 isolates, as shown in Table 4.5

The isolates were growth at 37°C and growth on L-arabitol and *meso*-ribitol. No acid production from *myo*-inositol. D-mannose and melibiose. They were identified as *G. thailandicus* (Tanasupawat *et al*., 2004). On the basis of restriction pattern showed the same restriction patterns forms the type strains of *G. thailandicus* NBRC 100600^T, were identified as *G. thailandicus*. DNA G+C contents of tested strains AN1-1 51.3 mol% identified as *G. thailandicus* (Tanasupawat *et al*., 2004). These isolates were found in Caricature Plant, Little Yellow Star, Zinnia and Mango.

In the phylogenetic analyses based on 16S rDNA sequences, strain AN1-1 of Group IV was located in the sublineage of *G. frateurii* and *G. thailandicus*, however, the calculated sequence similarities of the strain were low but not so high, namely, 98.7% to *G. frateurii* NBRC 3264^T and 98.6% to *G. thailandicus* NBRC 100600^T. AN1-1 was found in *Samanea saman*.

Table 4.7 Differential characteristics of *Gluconobacter* strains

Characteristics	Group 4 17 isolates	*G. oxydans* NBRC 14819[T]	Group 5 12 isolates	*G. cerinus* NBRC 3267[T]	Group 6 9 isolates	*G. frateurii* NBRC 3264[T]	Group 7 7 isolates	*G. thailandicus* NBRC 100600[T]	AN 1-1 1 isolates
Acetate oxidation	-	-	-	-	-	-	-	-	-
Lactate oxidation	-	-	-	-	-	-	-	-	-
Ubiquinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10
Growth at 37°C	+	+	-	-	-	-	w	w	-
Growth at pH 3.0	+	+	-	-	+	+	+	+	+
Growth on									
D-Arabitol	-	-	+	+	+	+	+ (-1)	+	-
L-Arabitol	-	-	-	-	w	w	w (+1)	w	+
meso-Ribitol	-	-	-	-	w	w	w (+1)	w	+
Acid production from									
D-Mannose	w	w	+	+	+	+	- (+1)	-	+
D-Sorbitol	-	-	+	+	-	-	-	-	-
myo-Inositol	w	w	+	+	+	+	-	-	-
Maltose	+	+	-	-	-	-	- (+1)	-	+
Melibiose	w	w	+	+	+	+	- (+1)	-	+
Sucrose	-	-	-	-	+	+	- (+1)	-	+

+, positive; w, weak positive; -, negative

Chracterization of *Asaia, Gluconacetobacter, Swaminathania,* and *Kozakia* strains

Group 8 contained 8 isolates, TP35-2, TP35-1, AD8-1, AD8-2, AN34-1, AP1-2, AR02, CK36-1, FE68-1, FE68-2, FG13-1, KD66-2, KS72-2, KT43-1, MG71-1, PA3-2, PJ82-2, PP42-2, SP73-1, SP73-2 and TE37-1. There were Gram-negative, obligate aerobes, motile and non motile rods. Colonies are pink, shiny, smooth and raised. They were oxidize acetate and lactate. All the isolates grew in the presence of 30% D-glucose. They grew on glutamate agar. Acid was produced from L-arabinose, D-xylose, D-mannose, L-sorbose and D-galactose. The major quinone of the representative (PJ82-2, FG13-1 and AP1-2) as Q-10. DNA G+C contents of tested strains PJ82-2 was 60.4 mol% were identified as *Asaia* (Yamada *et al.*, 2000). There were found in Zinnia, Apple, Night Jasmine, *Antidesma*, Caricature Plant, Red Grape, Fire bell, *Ixora chinensis*, *Caesalpinia pulcherrima*, Cananga, Mango, Plumeria Allamanda, *Adenium obsum*, *Bauhinia purpurea* Linn, Pineapple, Palm juice and Chayot.

Group 9 contained 14 isolates, LD51-2, RA30-2, TV83-1, AD8-3, MP11-1, AP1-1, AR03, FP 47-1, KS72-1, LG57-1, OR7-1, SIS32-1, SIS32-2 and SIS32-3. There were Gram-negative, obligate aerobes, motile and non motile rods. Formed orange, round, mucous, smooth and convex colonies. They were oxidize acetate and lactate. All the isolates grew in the presence of 0.35% acetic acid. They grew on Glutamate agar. Acid was produced from Glycerol L-arabinone, D-glucose, glycerol, D-mannose and sorbitol *meso*-erythritol but not on dcitol. Acid was produced from L-arabinose, D-fructose, D-galactose, D-glucose, glycerol, D-mannitol (variable and weak), D-ribose, L-sorbose, D-xylose. The major quinone of the representative AP1-1 and OR7-1 as Q-10. DNA G+C contents of tested strains AP1-1 was 58.5 mol%. In a phylogenetic analyses SIS32-2 showed 97.8% similarity with *Ga. liquafacien*. They were identified as *Gluconactcetobacter* (Yamada and Condo., 1984). There were found in Plumeria flower, Rose apple, Thaivermicelli, Frangipani, Apple, Red Grape, Unkown flower, Fire bell, Long gong and Fetid passion flower.

Group 10 contained 2 isolates, SI15-1 and SI15-2. There were Gram-negative, obligate aerobes and non motile rods. Colonies were pink, smooth and raised. They were oxidize acetate and lactate. Able to produced water soluble brown pigments. Grows well in the presence of 0.35% acetic acid. Produces acid from Glycerol, L-arabinose and D-mannose. The major quinone of the representative SI15-1 as Q-10. DNA G+C contents of tested strains SI15-1 was 60.7 were identified as *Sw. salitolerans* (Loganathan P. and Sudha N., 2004). In a phylogenetic analyses SI15-1 showed 97.7% similarity with *Sw. salitolerans* PA51^{T}. They were identified as *Sw. salitolerans* (Loganathan P. and Sudha N., 2004) There were found in Seed Ixora.

Table 4.8 Differential characteristics of SI15-1

Characteristics	**SI15-1**	***Sw. salitolerans* LMG 21291^{T}**
Production of water soluble brown pigments	-	+
Growth on		
Mannitol agar	w	+
Glutamate agar	+	+
Growth in the presence of		
3%NaCl	-	+
1%KNO_3	-	+
Acid production from		
L-Arabinose	-	+
D-Mannitol	+	-
Rhamnose	+	-
Sorbitol	-	+

+, positive; w, weak positive; -, negative

Group 11 contained 14 isolates, CT8-1 and CT 8-2. There were growth at pH 3.0 and 30°C. They were oxidize acetate and lactate (weak). Dose not grew on 30% D-Glucose. Dose not utilize methanol. Produce dihydroxyacetone from glycerol. Produce levan-like mucous substance Produces acid from glycerol L-arabinose, L-sorbose, D-mannitol. The major quinone of the representative CT8-1 as Q-10. DNA G+C contents of tested strains CT8-1 was 58.2 mol%. In a phylogenetic analyses CT8-1 showed 96.4% similarity with *K. baliensis*. They were identified as *Kozakia* (Lisdiyanti *et al.*, 2002). There were found in Sapodilla.

3. Screening of high acetic acid-producing thermotolerant strains

The alcohol dehydrogenase (ADH) activity of the tested isolates ranged from 4.7 to 8.2 unit/mg at 30°C and 4.06 to 7.49 unit/mg at 40°C. The strain PA 3-3 showed the highest ADH activity, 8.2 unit/mg at 30°C and 7.49 unit/mg at 40°C (Tables 4.9 and 4.10).

Table 4.9 Alcohol dehydrogenase (ADH) activity of isolates at 30°C

Isolate no.	Proteins content (mg)	ADH activity (Unit)	Specific activity (Unit/mg)
PA3-3	48.4	398	8.2
AP94-1	45.2	320	7.1
BA28-2	50.6	240	4.7
CR16-2	43.4	312	7.2
CR84-2	44.2	305	6.9
CT85-1	46.3	312	6.74
CT85-2	40.2	260	6.47
CL4-5	55.5	328	5.91
FP47-2	46.4	260	5.6
GR64-1	52.4	342	6.53
GR64-2	50.5	335	6.63
GV74-1	48.5	296	6.1
HG45-2	51.3	315	6.14
HP27-1	49.5	274	5.54
KD66-1	46.8	249	5.75
LG57-2	46.5	286	6.15
LK88-1	43.8	273	6.23
LM26-1	52.8	288	5.45
LS60-1	52.9	301	5.69
LS60-2	55.2	324	5.87
OR55-1	49.8	287	5.98
OR55-2	53.9	336	6.23
OR95-1	46.5	316	6.79
FC4-3	50.8	285	5.61
PK48-1	50.2	292	5.82

Table 4.9 Alcohol dehydrogenase (ADH) activity of isolates at 30°C (Continued)

Isolate no.	Proteins content (mg)	ADH activity (Unit)	Specific activity (Unit/mg)
PM169-2	55.8	356	6.38
PS49-1	54.3	362	6.67
PW19-2	48.8	316	6.48
RA103-1	46.9	309	6.59
RA30-1	47.3	324	6.85
RB1-1	52.8	367	6.95
RB3-1	54.3	374	6.89
SF61-2	50.1	316	6.31
SL89-1	52.6	294	5.59
SL89-2	49.8	315	6.33
SM63-1	53.6	285	5.32
SM63-2	48.7	306	6.28
ST107-1	56.9	374	6.57
TM58-1	58.7	330	5.62
TM58-2	55.2	354	6.41
WM77-1	49.8	298	5.98
WM86-1	52.8	320	6.06
ZN22-1	59.5	362	6.08
AK33-1	56.7	343	6.05
AM13-2	53.8	320	5.95
AP60-1	49.2	338	6.89
AP94-2	49.8	316	6.38
AV28-1	52.9	332	6.28
BB91-1	56.2	347	6.17
CA127-1	50.3	297	5.9
CA127-2	52.8	354	6.7
CS15-2	55.4	341	6.16
CS15-4	53.8	336	6.25
FBY4-3	52.1	276	5.29

Table 4.10 Alcohol dehydrogenase (ADH) activity of isolates at 40°C

Isolate no.	Proteins content (mg)	ADH activity (Unit)	Specific activity (Unit/mg)
PA3-3	44.2	331	7.49
AP94-1	43.1	268.5	6.23
BA28-2	-	-	-
CR16-2	42.1	276.8	6.56
CR84-2	-	-	-
CT85-1	40.6	241.2	5.94
CT85-2	-	-	-
CL4-5	-	-	-
FP47-2	-	-	-
GR64-1	44.3	245.4	5.54
GR64-2	43.2	243.2	5.63
GV74-1	40.2	209.4	5.21
HG45-2	-	-	-
HP27-1	-	-	-
KD66-1	-	-	-
LG57-2	42.4	226	5.33
LK88-1	39.8	215.7	5.42
LM26-1	36.4	160.5	4.41
LS60-1	42.4	179.4	4.23
LS60-2	41.8	182.2	4.36
OR55-1	40.5	132	3.26
OR55-2	-	-	-
OR95-1	42.3	239	5.65
PK48-1	41.3	175.5	4.25
PM169-2	39.7	212.8	5.36
PS49-1	-	-	-
PW19-2	-	-	-
RA103-1	39.9	232.2	5.82
RA30-1	40.2	237.2	5.9
RB1-1	39.4	238.4	6.05

Table 4.10 Alcohol dehydrogenase (ADH) activity of isolates at 40°C (Continued)

Isolate no.	Proteins content (mg)	ADH activity (Unit)	Specific activity (Unit/mg)
RB3-1	40.3	218.8	5.43
SF61-2	-	-	-
SL89-1	-	-	-
SL89-2	-	-	-
SM63-1	38.7	163	4.21
SM63-2	39.2	208.5	5.32
ST107-1	40.1	222.6	5.55
TM58-1	-	-	-
TM58-2	38.6	208.8	5.41
WM77-1	40.4	166	4.11
WM86-1	39.5	198.3	5.02
ZN22-1	-	-	-
AK33-1	-	-	-
AM13-2	-	-	-
AP60-1	41.2	219.2	5.32
AP94-2	40.6	208.7	5.14
AV28-1	38.8	194.8	5.02
BB91-1	42.1	208.4	4.95
CA127-1	39.3	190.6	4.85
CA127-2	40.2	209.4	5.21
CS15-2	-	-	-
CS15-4	-	-	-
FBY4-3	-	-	-
FC4-3	-	-	-

(-) : No growth at 40°C

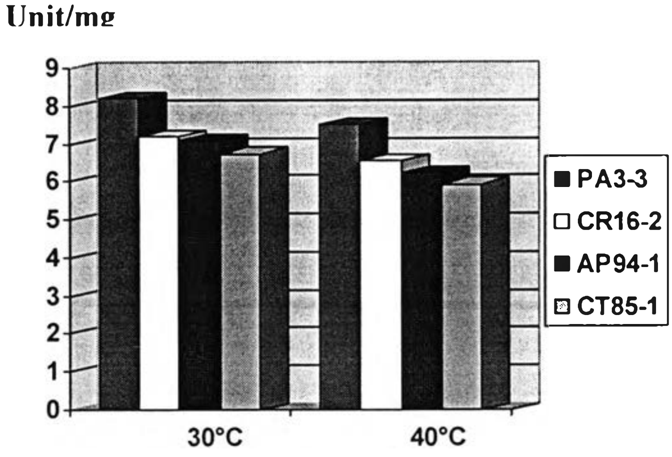


Fig. 4.12 Effect of temperature on ADH specific activity of the top 4 strains

4. Acetic acid production of PA3-3

The selected strain, PA3-3 (highest ADH activity) cultivated in GGYP medium showed a maximum growth (OD, 1.2631) after incubating for 4 days as shown in Fig. 4.13.

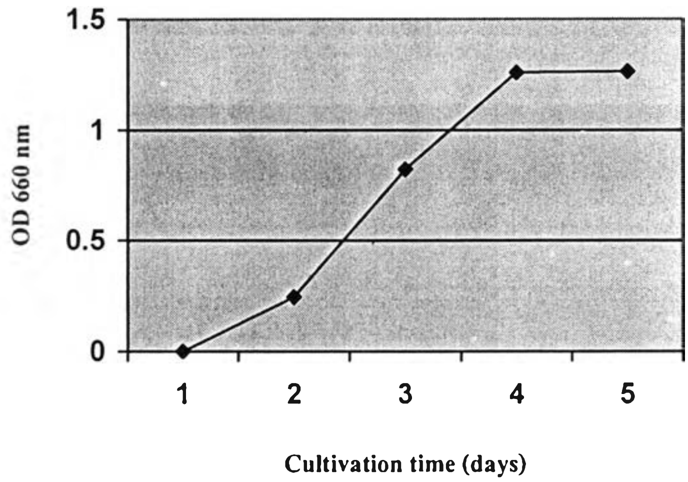


Fig. 4.13 Growth curve of PA3-3 at 30°C

PA3-3 was determined for vinegar fermentation using a medium containing 0.5%yeast extract, 1%acetic acid and 4%ethanol and cultivated various temperatures; 30, 37 and 40°C. PA3-3 produced high acetic acid at temperature at 30°C but it also could do at 37°C and 40°C almost the same as *A. pasteurianus* TISTR 1056^{T} (Table 4.13). This thermotolerant bacterium could produced 28.87 g/l, 25.63 g/l and 18.75 g/l of acetic acid after 3 days incubation at 30°C, 37°C and 40°C, respectively (Table 4.13).

4.1 Effects of ethanol and acetic acid concentration

Acetic acid production by PA3-3 reached the highest acetic acid accumulation level of 4% ethanol and 1% acetic acid (Tables 4.11 and 4.12).

Table 4.11 Effects of ethanol concentration on PA 3-3

%ethanol	Acetic acid production
2	0.998
4	1.659
6	1.118
8	1.016

Table 4.12 Effects of acetic acid concentration on PA 3-3

%acetic acid	Acetic acid production
0.5	1.512
1.0	2.047
1.5	1.262
2.0	1.016

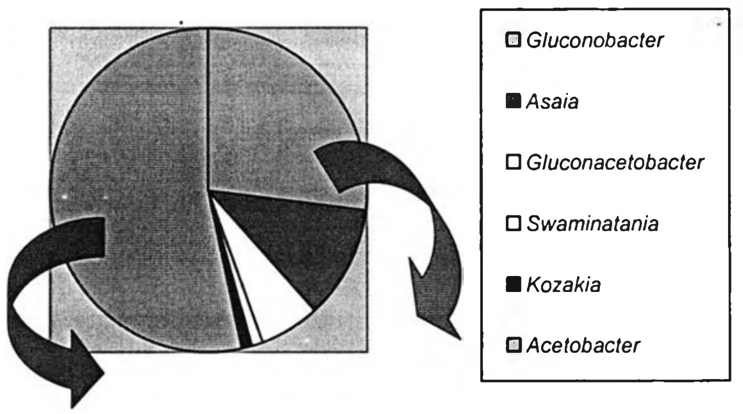
4.2 Effects of temperature

PA3-3 could produced 28.87 g/l, 25.63 g/l and 18.75 g/l of acetic acid after 3 days incubation at 30°C, 37°C and 40°C, respectively (Table 4.13).

Table 4.13 Acetic acid production on PA 3-3

Temperature (°C)	*A. pasteurianus* TISTR 1056^T	Acetic acid production
30	2.098	2.887
37	1.413	2.563
40	0.012	1.875

As mentioned above, the total of 181 isolates of acetic acid bacteria were isolated from 89 samples of fruits, flowers, and fermented starch in Thailand. They were identified as *Acetobacter* (97 strains; *A. pasteurinus, A. orentalis* and *A. lovaniensis*), *Gluconobacter* (45 strains; *G. oxydans, G. cerinus, G. frateurii, G. thailandicus, Gluconobacter* sp.), *Asaia* (21 strains), *Gluconacetobacter* (14 strains), *Swaminatania* (2 strains) and *Kozakia* (2 strains) as shown in Fig. 4.14. Strain PA3-3 identified as *A. pasteurinus* was selected to produce high acetic acid at temperature at 30°C and also could do at 37°C and 40°C better than *A. pasteurianus* TISTR 1056^{T}.



A. pasteurianus,53 strains
A. orentalis, 42 strains
A. lovaniensis, 2 strains

G. oxydans, 17 strains
G. cerinus, 12 strains
G. frateurii, 9 strains
G. thailandicus, 6 strains
Gluconobacter sp., 1 strain

Fig. 4.14 Species of acetic acid bacteria found in fruits, flowers and other materials