Facultative Psychrophilic Bacteria Isolated from the Tropical Districts in Indonesia

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Abstract: In this study the bacteria investigated were 34 strains (P-1~P-34) isolated at 4~5°C by enrichment culture in Indonesia. These strains were divided into 7 subgroups (Group I~Group VII) from the results of cultural, morphological, temperature dependency of growth, biophysical investigations and cellular fatty acid composition. Group I includes 5 strains, of which one strain was mesophile and four strains were facultative psychrophiles. Group II includes 4 strains, which were concluded to be in the genus *Sporosarcina* and to have high salt concentration dependency of growth. Group III includes 2 strains, which showed rhizoid rough colony formation and were considered to be in the genus *Alcaligenes* or *Flavobacterium*. The relation with salt was considered to be interesting as a growth factor in this Group. Group IV includes 10 strains, of which only one strain was mesophile and remaining 9 strains were facultative psychrophiles. These strains were considered to be in the genus *Alcaligenes* or *Pseudomonas*. Their sources were distributed over very wide range.

Group V includes 7 strains, and Group VI includes 3 strains, all of which were considered as facultative psychrophile. Their optimum growth temperature were low (4~27°C). Group VII includes 9 strains, all of which were considered to be in the genus *Pseudomonas*. In conclusion, there were no obligate psychrophile and 23 strains were facultative psychrophile. In the case of Group VI and VII, the growth investigation and analyses of fatty acid composition by gas chromatography were carried out. As the results of these precise examinations, Group VI was considered to be very near to the genus *Moraxella* or *Acinetobacter*, and Group VII near to the genus *Chromobacterium* or *Pseudomonas*. On the level of species determination, it was concluded that P-26, P-27 and P-33 were the same species near to *Pseudomonas caryophylli*, and P-6, P-31 and P-29 were considered as *Moraxella bovis* or *Moraxella*-Like-species.

Key words: Psychrophilic bacteria, Indonesia, taxonomy

Introduction

For the first time, from the standpoint of the global ecology of microorganisms, and for production, processing and storage of food materials in Indonesia, biologists in Indonesia and Japan cooperated in the isolation works of many bacteria existing in the middle and eastern districts of Java Island, Madura Island and Bali Island etc. (Suhadi *et al.*, 1977, 1979, 1979; Kaneko *et al.*, 1979; Okasawa *et al.*, 1980). These bacteria included halophilic, thermophilic, psychrophilic and radio-resistant groups. In this study, only psychrophilic bacteria were investigated. The bacteria were

enrichment cultured in Tryptocase Soy Broth (B. B.L.), and a single colony was isolated three times on the same agar plate medium. The taxonomical characteristics and the temperature dependency of growth were investigated.

Materials and Methods

Bacterial strains

Bacteria isolated in Indonesia with enrichment-culture at 5°C in Nutrient broth (pH 7.2) were suspended in sterilized water three times and a single colony was reisolated. The sources and 34 strains obtained are shown in Table 1.

Table 1. Strains isolated and their sources

Strain	Location and type of samples
P-1	Sand, Pari Island
P-2	Rice-field water, Genting village
P-3	"
P-4	Wet soil, Tapaksiring, Bali Island
P-5	"
P-6	Fresh fish, Tuban (Banjar)
P-7	"
P-8	"
P-9	Sea water, Pari Island
P-10	Mud, small river, Sapikerep village
P-11	"
P-12	"
P-13	Water, Kodalam village
P-14	Algae, Lasam salt-field area
P-15	"
P-16	"
P-17	Salted fish, Muncar (Sotong)
P-18	Shell fish, Kenjeran coastal area
P-19	Rice field mud, mountain area (+700 m)
P-20	Mud, village, mountain area (+1000 m)
P-21	Fresh water fish, Gibanas (Nilem)
P-22	Fresh fish, Kragan (Pungkur)
P-23	Shell fish, Kongsu Island
P-24	"
P-25	Pindang fish, Bancar (Layang)
P-26	Algae, Banyupah coastal area
P-27	Running water, salt field, Gresik
P-28	Surface mud, salt field, Gresik
P-29	Salt, salt field, Batangan
P-30	"
P-31	Emas fish, fresh fish, Cipanas
P-32	Sisik, salted fish, Ngadisare
P-33	Algae, rice field, Kejajar
P-34	"

Methods for approximate classification

We investigated taxonomical characteristics following Jitsukenho Nogeikagaku vol. 2 (Asakura Shoten 1978) and Identification of Medical Bacteria 2nd Ed. (Cowan *et al.*, 1974). Bacteria were precultured on Nutrient agar slant at 25°C for about 20 hrs. Cultural characteristics were observed

in Nutrient agar and liquid media by plate, slant, static liquid and stab cultures. Morphological characteristics were investigated with strains Nutrient slant cultured at 25°C for 20 hrs and negative stained by nigrosine solution. Motility characteristics were studied by hanging drops preparation methods. Flagella stain was done by Nishizawa and Sugawara method. Gram stain was done by the modified method of Hucker. Spore formation was examined by Scheffer-Fulton method with Gram-positive strains cultured on potato agar slant medium. The temperature dependency of growth was studied with strains which were inoculated in Nutrient liquid medium by one loopfull amount of slant cultured sample above mentioned and reciprocally cultured in experimental tube at 2~3°C, 40°C and 45°C respectively. The degree of growth was measured by the decrease of transmittance at 660 nm.

The optimum growth temperature was determined by the maximum decrease of transmittance (at 660 nm). In this case, 0.2 ml suspended samples were inoculated in L-type glass tube and cultured at 2.5~41°C for 12~18 hrs in ToYo Kagaku Sangyo temperature gradient incubator Model TN-3. The biophysical characters were studied by the measurements of oxidase activity (Cytochromeoxidase test paper, Nissui), catalase activity (3% H₂O₂ solution), acid formation from glucose and O-F test.

Fig. 1. Temperature ranges for multiplication of psychrophilic, mesophilic, and thermophilic bacteria. Hatched portions indicate approximate optima. (From Doetsch and Cook, 1973)

Table 2. Cultural characteristics (1)

Strain	Nutrient agar colonies				Nutrient agar slant		
	Form	Color	Surface	Growth	Form	Luster	Pigment production
P-1	C	PB	S	W	F	G	End orange
P-2	P	W	S	W	F	G	
P-3	P	PW	S	W	F	G	
P-4	P	PW	R	G	F	D	
P-5	P	W	R	G	F	D	
P-6	P	MW	R	G	F	G	
P-7	P	PW	S	G	F	G	
P-8	C	PB	S	G	F	G	
P-9	C	O	S	G	F	G	
P-10	C	W	R	G	F	G	
P-11	C	PW	S	G	F	G	
P-12	C	MW	S	G	F	G	End orange End orange
P-13	C	MW	S	G	F	G	
P-14	P	PP	S	G	F	G	
P-15	P	O	S	G	F	G	
P-16	C	O	S	G	F	G	
P-17	R	PY	R	G	F	G	
P-18	C	PB	S	G	F	G	
P-19	R	PW	S	G	F	G	
P-20	C	W	R	G	F	D	
P-21	C	PP	S	G	F	G	
P-22	C	PB	S	G	F	G	Exo brown Exo brown
P-23	C	PP	S	G	F	G	
P-24	C	PB	S	G	F	G	
P-25	R	PY	R	G	F	G	
P-26	C	PB	S	G	F	G	
P-27	C	PB	S	G	F	G	
P-28	C	PB	S	G	F	G	
P-29	C	MW	S	G	F	G	
P-30	C	O	S	G	F	G	
P-31	C	MW	S	G	F	G	
P-32	C	MW	S	G	F	G	Exo brown
P-33	C	PB	S	G	F	G	
P-34	C	PB	S	G	F	G	

Nutrient agar colonies : 20°C, 2 days.

From : C, circular ; P, punctiform ; R, rhizoid.

Color : PB, pale brown ; W, white ; PW, pale white ; MW, milky white ; O, orange ; PP, pale pink ; PY, pale yellow.

Surface : S, smooth ; R, rough.

Growth : W, weak ; G, good.

Nutrient agar slant : 25°C, 24-48 hrs.

Form : F, filiform.

Luster : G, glistening ; D, dull.

Methods for precise classification

For the accurate classification Group VI and VII were selected. These Groups showed typical psychrophilic characteristics. In this paper the definition of psychrophile was according to Fig. 1 (Doetsch and Cook, 1973). In this case, growth curve, morphological experiments, observation of flagella, carbon sources for growth (Stanier *et al.* 1966) and many other biophysical characteristics were studied. The fatty acid compositions of the two Groups were analysed by gas chromatographical method, the precise process of which was reported by Nishimura *et al.* (1979).

Results

Cultural characteristics are summarized in Table 2 and 3. In Table 2, the results of Nutrient agar colonies and Nutrient agar slant are shown. In Table 3, the results of Nutrient broth and Nutrient stab culture are shown. Morphological characteristics are summarized in Table 4, the temperature dependency of growth and the optimum growth temperature are shown in Table 5. The degree of growth was divided into four transmittance range 0-4% (#), 40-70% (+), and 70-90% ((+)) and 90-100%(-).

Physiological properties are summarized in Table 6. From the above several experimental results and with the aid of the manual of Identification Medical Bacteria and Bergey's manual of Determinative Bacteriology 8th Ed. (Buchanan and Gibbons, 1974), 34 strains were divided into 7 groups as shown in Table 7, Table 8 and Table 9.

Among the 7 Groups, Group VI and Group VII were adopted as typical facultative psychrophilic bacteria and their more precise physiological characteristics are shown in Table 10. Group VI includes P-6, P-31 and P-29, and Group VII includes P-26, P-27 and P-33.

Table 3. Cultural characteristics (2)

Strain	Nutrient broth			Nutrient stab
	Surface	Turbidity	Odor	Form
P-1	S	+	-	V
P-2	S	(+)	-	F
P-3	S	(+)	-	F
P-4	P	(+)	-	P
P-5	P	(+)	-	P
P-6	S	(+)	+	P
P-7	S	(+)	+	P
P-8	S	(+)	-	V
P-9	S	(+)	-	V
P-10	P	(+)	-	V
P-11	S	(+)	-	P
P-12	S	(+)	-	V
P-13	S	+	-	F
P-14	R	+	+	F
P-15	S	(+)	-	P
P-16	S	(+)	-	P
P-17	S	(+)	+	F
P-18	P	+	+	P
P-19	R	+	+	V
P-20	P	(+)	-	V
P-21	R	+	+	P
P-22	S	+	+	F
P-23	M	+	+	F
P-24	S	+	+	F
P-25	M	+	+	F
P-26	P	+	+	P
P-27	P	+	+	P
P-28	R	+	+	P
P-29	R	+	+	P
P-30	S	-	-	P
P-31	R	+	+	P
P-32	S	+	+	P
P-33	P	+	+	P
P-34	P	+	+	F

Nutrient broth: 25°C, 7 days stationary culture.

Surface: S, sediment; P, pellicle; M, membranous; R, ring.

Turbidity: #, strong; +, moderate; (+), slight.

Nutrient agar stab: 25°C, 5 days.

Form: F, Filiform; P, papillate; V, villous.

Table 4. Morphological characteristics

Strain	Form	Size(nm)	Motiity	Flagella	Gram stain	Spore
P-1	R	0.8×3-8	+	P	—	
P-2	R	0.8×2-2.5	+	L	—	
P-3	R	1 ×2-2.	+	L	—	
P-4	R	0.8×6-9	(+)	L	+	—
P-5	R	0.8×3-3.5	(+)	—	+	—
P-6	DC	1	—	—	—	
P-7	CR	1 ×1.2	+	L	—	
P-8	R	1 ×2-3.5	(+)	L	+	—
P-9	SC	1	+	S	+	+
P-10	R	1 ×6-8	—	—	+	+
P-11	CR	1 ×1.2-1.5	+	L	—	
P-12	CR	1 ×1.2-1.5	+	L	—	
P-13	CR	0.8×1	+	L	—	
P-14	R	1 ×2-2.5	+	L	—	
P-15	OC	1	+	S	+	+
P-16	DC	1	+	S	+	+
P-17	R	0.4×1-3	+	P	—	
P-18	SR	1 ×1.5-2	+	L	—	
P-19	CR	1 ×1.2-1.5	+	L	—	
P-20	R	0.5×3-3.5	(+)	—	+	—
P-21	R	1 ×3-3.5	+	M	—	
P-22	R	1 ×3-3.5	+	M	—	
P-23	R	1 ×1.5-2	+	L	—	
P-24	R	1 ×2.5-3.5	+	L	—	
P-25	R	0.8×2-3	+	L	—	
P-26	R	1.2×1.5-3	+	L	—	
P-27	R	1 ×2.5-3	+	L	—	
P-28	R	1 ×1.2-2	+	M	—	
P-29	DC	1	—	—	—	
P-30	DC	1	+	S	+	+
P-31	DC	1	—	—	—	
P-32	R	1 ×2-6	+	L	—	
P-33	R	1 ×1.5-3	+	L	—	
P-34	R	0.8×1.5-2				

Nutrient agar slant: 25°C, 20 hrs.

Form: R, rod; CR, coccoid-rod; DC, diplococcus; OC, octococcus; SC, staphylococcus.

Flagella: P, peritrichous; L, lophotrichous; M, monotrichous; S, single.

Discussions

The characteristics of strains included in each Group are summarized as follows.

In the case of P-4, P-5, P-8, P-10 and P-20 in

Group I were all Gram positive, (long) rod type, a little or no motile, with lophotrichous flagella or no flagella, positive in catalase activity, fermentation from glucose, no action type of O-F test, the optimum growth temperature of 28-32°C, good

Table 5. Optimum temperature and Growth at 2-3°C, 40°C and 45°C

Strain	Opt. temp (°C)	Growth at :		
		2-3°C	40°C	45°C
P-1	26	—	(+)	—
P-2	29	—	—	—
P-3	28	—	—	—
P-4	28	+	(+)	—
P-5	28	+	(+)	—
P-6	27	+	(+)	(+)
P-7	30	—	—	—
P-8	32	+	—	—
P-9	28	—	(+)	(+)
P-10	28	+	—	—
P-11	29	(+)	—	—
P-12	29	(+)	—	—
P-13	30	—	#	(+)
P-14	33.5	—	—	—
P-15	35	(+)	—	—
P-16	30	(+)	—	—
P-17	31	—	#	(+)
P-18	28	#	—	—
P-19	30	(+)	#	—
P-20	29	—	#	#
P-21	31	#	—	—
P-22	28	#	(+)	—
P-23	29	#	—	—
P-24	29	#	—	—
P-25	32	+	(+)	—
P-26	23	#	(+)	—
P-27	28	#	—	—
P-28	25	#	—	—
P-29	27	#	—	—
P-30	32	+	—	—
P-31	24	#	—	—
P-32	29	+	(+)	—
P-33	26	+	(+)	—
P-34	30	#	+	—

Opt. temp: Nutrient broth in temperatural gradient incubator for 12-18 hrs.

Tra smitance (at 660 nm): #, 0-4%; +, 40-70%; (+), 70-90%; —, 90-100%.

Table 6. Physiological Characteristics

Strain	Oxidase	Catalase	Acid formation from glucose	O-F test
P-1	—	+	—	—
P-2	—	+	—	—
P-3	—	+	—	—
P-4	—	#	—	—
P-5	—	#	—	—
P-6	+	#	—	—
P-7	—	+	—	—
P-8	(+)	(+)	—	—
P-9	—	#	—	—
P-10	—	#	—	—
P-11	—	#	—	—
P-12	—	#	—	—
P-13	+	+	—	—
P-14	+	+	—	—
P-15	—	+	—	—
P-16	—	#	—	—
P-17	+	+	—	—
P-18	+	+	+	O
P-19	+	+	—	—
P-20	—	#	—	—
P-21	+	#	+	O
P-22	+	#	+	O
P-23	+	+	+	O
P-24	+	#	+	O
P-25	+	#	—	—
P-26	+	#	+	O
P-27	+	#	+	O
P-28	+	#	+	O
P-29	+	#	(+)	—
P-30	—	#	—	—
P-31	+	#	+	O
P-32	—	#	+	F
P-33	+	#	+	O
P-34	+	#	+	O

Symbols: #, strong; —, good; (+), weak; —, none.

O-F test: O, oxidation; F, fermentation; —, no action

Table 7. Grouping o experimented strains (1)

	Group I					Group II				Group III*	
	P-4	P-5	P-8	P-10	P-20	P-9	P-15	P-16	P-30	P-17	P-25
Gram reaction	+	+	+	+	+	+	+	+	+	—	—
Morphology	R	R	R	R	R	SC	OC	DC	DC	R	R
Motility	(+)	(+)	(+)	—	(+)	+	+	+	+	#	#
Flagella	L	—	L	—	—	S	S	S	S	P	L
Spore	—	—	—	—	—	+	+	+	+		
Oxidase	+	+	+	+	+	—	—	—	—	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
Glucose, acid from	—	—	—	—	—	—	—	—	—	—	—
O-F test	—	—	—	—	—	—	—	—	—	—	—
Colony color	W	W	PB	W	W	O	O	O	O	PY	PY
Opt. temp. (°C)	28	28	32	28	29	28	35	30	32	31	32
Growth at:											
2-3°C	+	+	+	+	—	—	(+)	(+)	+	—	+
40°C	(+)	(+)	—	—	#	—	—	—	—	#	(+)
45°C	—	—	—	—	#	—	—	—	—	(+)	—
Sources	wet soil	wet soil	fresh fish	mud (small river)	mud (+1000 m)	sea water	algae (salt field)	algae (salt field)	salt (salt field)	salted fish	Pindang fish

Morphology: R, rod; CR, coccoid rod; DC, diplococcus; OC, octococcus; SC, staphylococcus.

Flagella: P, peritrichous; L, lophotrichous; M, monotrichous; S, single.

Colony color: PB, pale brown; W, white; PW, pale white; MW, milky white; O, orange; PP, pale pink; PY, pale yellow.

* Rough and rhizoid colony.

growth at 2-3°C excepting P-20, a little or no growth at 40°C and 45°C excepting P-20, and all strains were from wet soil or fresh water fish.

In the case of P-9, P-15, P-16 and P-30 in Group II, all were Gram positive, diplo- or octococcus, good motile, single polar flagella, spherical spore, negative oxidase activity, positive catalase activity, no acid formation from glucose, no action type of O-F test, orange color colony, the optimum growth temperature of 28-35°C (a fairly wide range), no growth over 40°C. The strains in this group were supposed very near to the genus *Sporosarcina*. The sources of the group were sea water or algae in salt field, which shows that salt seems to be one requirement for growth in this group.

In the case of P-17 and P-25 in Group III, both strains were Gram negative, rod type, good motile, with peritrichous or lophotrichous flagella, posi-

tive in both oxidase and catalase activities, no acid formation from glucose, no action in O-F test, faint yellow, rhizoid and rough colony formation. The optimum growth temperature was 31-32°C, and the growth at 40°C was a little or weak. From characteristics above mentioned, these strains were concluded to be in the genus *Alcaligenes* or *Flavobacterium*. The main source of these strains was salted fish.

P-34, P-23, P-18, P-28, P-13, P-19, P-15, P-22, P-24 and P-14 were assembled into Group IV. These strains were rod or coccoid rod, Gram negative and good motile with lophotrichous or monotrichous flagella, and were positive in both oxidase and catalase activities. 8 strains among them were oxidative and remaining 2 strains were fermentative in O-F test. The acid formation from glucose was possible in 7 strains except for P-14,

Table 8. Grouping of experimented strains (2)

	Group IV									
	P-34	P-18	P-23	P-28	P-13	P-19	P-15	P-22	P-24	P-14
Gram reaction	—	—	—	—	—	—	—	—	—	—
Morphology	R	R	R	R	CR	CR	R	R	R	R
Motility	+	+	+	+	+	+	+	+	+	+
Flagella	M	L	L	M	L	L	M	M	L	L
Oxidase	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Glucose, acid from	+	+	+	+	—	—	+	+	+	—
O-F test	O	O	O	O	—	—	O	O	O	—
Colony color	PB	PB	PP	PB	MW	PW	PP	PB	PB	PP
Opt. temp.(°C)	30	28	29	25	30	30	31	28	29	33.5
Growth at :										
2-3°C	+	+	+	+	—	(+)	+	+	+	—
40°C	+	—	—	—	+	+	—	(+)	—	—
45°C	—	—	—	—	(+)	—	—	—	—	—
Sources	rice field	shell fish	shell fish	surface mud (salt field)	water	rice field mud (+700 m)	fresh water fish	fresh fish	shell fish	algae (salt field)

Morphology : R, rod ; CR, coccoid rod ; DC, diplococcus ; OC, octococcus ; SC, staphylococcus.

Flagella : P, peritrichous ; L, lophotrichous ; M, monotrichous ; S, single.

Colony color : PB, pale brown ; W, white ; PW, pale white ; MW, milky white ; O, orange ; PP, pale pink ; PY, pale yellow.

Table 9. Grouping of experimented strains (3)

	Group V							Group VI*			Group VII		
	P-7	P-11	P-12	P-1	P-2	P-32	P-3	P-6	P-31	P-29	P-26	P-27	P-33
Gram reaction	—	—	—	—	—	—	—	—	—	—	—	—	—
Morphology	CR	CR	CR	R	R	R	R	DC	DC	DC	R	R	R
Motility	+	+	+	+	+	+	+	—	—	—	+	+	+
Flagella	L	L	L	P	L	L	L	—	—	—	L	L	L
Oxidase	—	—	—	—	—	—	—	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose, acid from	—	—	—	—	—	+	—	—	+	(+)	+	+	+
O-F test	—	—	—	—	—	F	—	—	O	—	O	O	O
Colony color	PW	PB	MW	PB	W	MW	PW	MW	MW	MW	PB	PB	PB
Opt. temp. (°C)	30	29	29	26	29	29	28	27	24	27	23	28	26
Growth at :													
2-3°C	—	(+)	(+)	—	—	+	—	+	+	+	+	—	+
40°C	—	—	—	(+)	—	(+)	—	(+)	—	—	(+)	—	(+)
45°C	—	—	—	—	—	—	—	—	—	—	—	—	—
Sources	fresh fish	mud (river)	mud (river)	sand	rice field water	salted fish (Sisik)	rice field water	fresh fish	fresh fish (Emas fish)	salt (salt field)	algae (coastal area)	running water (salt field)	algae (rice field)

Morphology : R, rod ; CR, coccoid rod ; DC, diplococcus ; OC, octococcus ; SC, staphylococcus.

Flagella : P, peritrichous ; L, lophotrichous ; M, monotrichous ; S, single.

Colony color : PB, pale brown ; W, white ; PW, pale white ; MW, milky white ; O, orange ; PP, pale pink ; PY, pale yellow.

* Diffusible brown pigments production

Table 10. Physiological characteristics of Group VI and Group VII

Strain no.	P-6	P-31	P-29	P-26	P-27	P-33
Pigment production	—	—	—	+	+	—
Growth in air	+	+	+	+	+	+
Growth under anaerobic	±	±	—	—	—	—
Denitrification	—	(+)	—	(+)	(+)	(+)
Nitrate reduced	—	—	—	—	—	—
Nitrite reduced	—	—	—	—	—	—
O-F test (/O/—)	—	O	—	O	O	O
Acid from :						
Glucose	—	+	+	+	+	+
Lactose	—	—	—	—	—	—
Maltose	—	—	—	—	—	—
Sucrose	—	—	—	—	—	—
Fructose	—	—	—	—	—	—
Starch	—	—	—	—	—	—
Xylose	(+)	+	+	+	+	+
Salicine	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—
Trehalose	—	—	—	—	—	—
PHB accumulation	—	+	+	+	+	+
Growth factor requirement	—	—	—	—	—	—
Citrate as C source	—	(+)	(+)	+	+	+
Haemolysis	+	+	+	—	—	—
H ₂ S	—	—	—	(+)	—	(+)
Indole	—	—	—	—	—	—
Penicillin sensitivity	+	+	+	—	—	—
Growth :						
2-3°C	+	+	+	+	+	+
40°C	(+)	—	—	(+)	—	(+)
45°C	—	—	—	—	—	—
S-S agar	—	—	—	+	+	+
MacConkey agar	—	+	+	+	+	+
5% NaCl	+	+	+	+	+	+
10% NaCl	+	+	+	—	—	—
15% NaCl	—	—	+	—	—	—
20% NaCl	—	—	—	—	—	—
Hydrolysis :						
Aesculin	—	—	—	—	—	—
Starch	—	—	—	—	—	—
Gelatin	—	—	—	—	—	—
Casien	+	+	+	—	—	—
Tween 80	—	(+)	(+)	—	—	—
Activity :						
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Urease	(+)	—	—	+	+	+
Arginine dihydrolase	—	—	—	+	+	+
Opt. temp. (°C)	27	24	27	23	28	26
Utilization as C source :						
Glucose	(+)	—	—	+	+	+
D-Xylose	+	—	—	(+)	(+)	(+)
L-Rhamnose	+	—	—	+	+	+
D-Ribose	±	—	—	+	+	+
Levulinic acid	—	—	—	—	—	—
Citraconic acid	—	—	—	—	—	—
Mesaconic acid	—	—	—	—	—	—
Meso-Tartaric acid	—	—	—	+	+	+
D-(—)-Tartaric acid	—	—	—	—	—	—
Erythritol	±	—	—	±	±	±
Adonitol	±	—	—	—	—	—
m-Hydroxy benzoic acid	—	—	—	—	—	—
2·3-Butylene glycol	±	—	—	+	+	+

Table 11. Characters of Group VI & VII, and Gram-negative bacteria

Strain	Group VI			Moraxella*	Acinetobacter*	Group VII			Chromobacterium*	Pseudomonas*
	P-6	P-31	P-29			P-26	P-27	P-33		
Morphology	S/R	S/R	S/R	S/R	S/R	R	R	R	R	R
Motility	—	—	—	—	—	+	+	+	+	+
Growth in air	+	+	+	+	+	+	+	+	+	+
Growth anaerobically	±	±	—	—	—	—	—	—	—	+
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	—	+	+	+	+	+
Glucose (acid)	—	—	—	—	+	+	+	+	+	+
O-F test (O/F/—)	—	O	—	—	O	O	O	O	O	O

* Cowan & Steel (1974)

Table 12. Characters of Group VI, and Moraxella

	P-6	P-31	P-29	Moraxella* osloensis	Moraxella* bovis	Moraxella* urethralis
Catalase	+	+	+	+	d	+
Oxidase	+	+	+	+	+	+
O-F test (F/O/—)	— ⁽³⁾	O ⁽²⁾	—	—	—	—
PHB accumulation	—	+	+	+	—	+
Haemolysis	+	+	+	—	+	—
Growth at 42°C	—	—	—	—	—	—
Growth on nutrient broth/agar	+	+	+	+	+	+
Serum requirement	—	—	—	—	—	—
Growth on MacConkey	—	+	+	d ⁽¹⁾	—	•
Citrate as C source	—	w ⁽⁴⁾	w	w	—	d
Carbohydrates, and from:						
glucose	—	—	—	—	—	—
lactose	—	—	—	—	—	—
maltose	—	—	—	—	—	—
xylose	—	+	+	—	—	—
Nitrate reduced	—	—	—	d	d	—
Gelatin liquefaction	—	—	—	—	+	—
Urease	w	—	—	d	—	—

⁽¹⁾ d, Different reactions in different strains.⁽²⁾ o, Oxidation.⁽³⁾ —, No action.⁽⁴⁾ w, Weak reaction.

* Cowan & Steel (1974)

Table 13. Characters of Group VII, and genus *Pseudomonas*

Strain	P-26	P-27	P-33	<i>P. pseudomallei</i> *	<i>P. caryophylli</i> *	<i>P. cepacia</i> *
No. of flagella	>1	>1	>1	>1	>1	>1
Diffusible pigments	+	+	+	—	+	+
Arginine dihydrolase	+	+	+	+	+	—
Denitrification	(+) ⁽¹⁾	(+)	(+)	+	+	+
Hydrolysis of:						
Starch	—	—	—	+	—	—
Gelatin	—	—	—	+	—	d
Carbon source for growth:						
Glucose	+	+	+	+	+	+
D-Xylose	(+)	(+)	(+)	—	(+)	d
D-Ribose	+	+	+	+	+	+
L-Rhamnose	+	+	+	—	+	d
Levulinate	—	—	—	+	—	+
Citraconate	—	—	—	—	—	+
Mesaconate	—	—	—	—	—	—
D-(—)-Tartrate	—	—	—	—	—	—
Meso-Tartrate	+	+	+	—	+	+
Erythritol	+	+	+	+	—	—
Adonitol	—	—	—	d ⁽²⁾	—	+
2,3-Butylene glycol	+	+	+	—	+	+
meta-Hydroxybenzoate	—	—	—	—	—	+

* Buchanan & Gibbons, (1974)

⁽¹⁾ (+), Weak reaction.⁽²⁾ d, Different reactions in different strains.

P-13 and P-19. The optimum growth temperature were in the range of 25~33.5°C. The growth tendencies were good at 2~3°C except for P-13 and P-14. Over 40°C only P-34, P-13 and P-19 showed growth, but in the other strains a little or no growth was observed.

From the results above obtained, Group IV was considered in the genus *Alcaligenes* and the genus *Pseudomonas*. The sources were distributed widely, for example, soil, algae, crustacea and fresh water fish.

In V Group including 9 strains (P-7, P-11, P-12, P-1, P-2, P-32 and P-3), nearly all strains were Gram negative, rod or coccoid, good motile, with lophotrichous or peritrichous flagella, negative in oxidase and positive in catalase activity, acid productive from glucose and no action type in O-F test except for fermentative P-32. The optimum

growth temperatures were generally low (20~30°C), and the growth at 2~3°C were very weak or no except for P-32. The growth at 40°C was also weak. The sources were widely distributed (fresh water fish, river mud, sand, paddy water, salted fish). In this stage, the determination of the genus of this group is difficult and more exact study is necessary.

P-6, P-31, and P-29 strains in Group VI were Gram negative, diplococcus rod, immotile and without flagella. Both oxidase and catalase activities were positive. P-31 was oxidative and P-29 was no action type in O-F test. The optimum growth temperatures were below 27°C, and good growth at 2~3°C and nearly no growth over 40°C were observed. The VI Group was concluded very near to the genus *Moraxella-Acinetobacter*.

Moraxella are compared in Table 12. In Table

Table 14-a. Comparisons of fatty acid compositions of Group VI and Group VII with known bacteria

bacteria	straight fatty acid* (% of total)																	
	10:0	11:0	12:0	13:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	19:0	20:0	21:0	22:0
P-6	1				2		3	10		11	5	58	T	1				
P-31	2		2		2		1	15		9		56						
P-29	2		1		2	T	T	12		11	61	T	T					
<i>M. bovis</i> (ATCC 10900-ref.)			T		T		4	26	T	7	T	52	9					
<i>M. lacunata</i> (ATCC 19975-ref.)			4		1		15	14	T	4	5	45	8					
M.-like-space (A. 4 bby Ito 1976)			T		T		2	6	T	14	1	61	3					
P-26			5		2	T	25	33				21				T		
P-27			4		2	T	26	27				20	T			T		
P-33			4		2	T	25	32				23				T	T	
<i>Ps. aeruginosa</i> (KS 0025-ref.)			3		1	1	29	8	T		T	27			2			
<i>Ps. aeruginosa</i> (IAM 1514 KS0025)			7		2	T	28	15			T	32			T			
<i>Ps. putida</i> (IAM 1506)			7		3	T	35	12				11			7			

* number before colon is carbon number, number after is unsaturation

T: ≤1%

13 the same comparison as Table 12 of Group VII and the genus *Pseudomonas* is shown. In Table 14 the fatty acid compositions of Group VI and VII are shown. In the former, they are compared with *M. bovis*, *M. lacunata* (ATCC 19975, ref) and *Moraxella*-like-space (A-4 by Ito 1976), and in the latter, with *P. aeruginosa* (KS0025-ref.), *P. aeruginosa* (IAM1514, KS0025) and *P. putida* (IAM1506). The results of fatty acid composition were analyzed by the calculation of similarity value. The high similarity values (1.00~0.99) were found in P-6, P-31 and P-29 (Group VI), and also the same conclusion was obtained in P-26, P-27 and P-33 (Group VII).

The formula of similarity value is as follows:

$$\cos\phi = \frac{\sum(x_i)(y_i)}{\sqrt{\sum(x_i)^2} \cdot \sqrt{\sum(y_i)^2}}$$

x_i : Peak area of i fatty acid component of X strain

y_i : peak area of i fatty acid component of Y strain

The characteristics of fatty acid compositions and component ratios in Group VI were high values of C18:1 (56-61%), C16:1 (10-15%) and C17:1 (9-11%), the total amount of which was 80-85%.

The fatty acid compositions of the genus *Moraxella* were obtained by (Nishimura *et al.*, 1979). Among these three reference species in the genus *Moraxella* in Table 14 very high similarity values were obtained. Moreover, the component ratios of fatty acid among Group VI and the genus *Moraxella* were very similar, which showed the high similarity among both groups. Group VII includes 3 strains (P-26, P-27, P-33). They are

Table 14-b. Comparisons of fatty acid compositions of Group VI and Group VII with known bacteria

bacteria	branched fatty acid						hydroxic fatty acid						cyclopropanoic fatty acid		unknown	number of peaks
	i-11	i-12	i-13	i-14	i-15	i-16	3.0 H 10:0	2.0 H 12:0	3.0 H 12:0	3.0 H 14:0	2.0 H 16:0	3.0 H 16:0	△17	△19		
P-6															11	(4)
P-31								3							9	(4)
P-29															9	(5)
<i>M. bovis</i> (ATCC 10900-ref.)															T	
<i>M. lacunata</i> (ATCC 19975-ref.)															4	
M.-like-space (A. 4 by Ito 1976)															12	
P-26								4	2				3		5	(4)
P-27								4	2				5		6	(6)
P-33								4	1				3		3	(2)
<i>Ps. aeruginosa</i> (KS 0025ref.)							3	8	2				4	7	4	(1)
<i>Ps. aeruginosa</i> (IAM 1514 KS0025)								5	2				1	2	4	(2)
<i>Ps. putida</i> (IAM 1506)								2	2				8		7	(3)

i : iso-brnched

T : ≤1%

Gram negative, rod, and motile with lophotrichous flagella, positive in both oxidase- and catalase-activities, acid productive from glucose, oxidative type in O-F test. As special characteristics, they formed brown diffusible pigments at 2~3°C. They had low optimum growth temperatures (23~38°C), and at 2~3°C they showed good growth. Above 40°C, their growth were weak. The sources were algae on seashore, water in paddy-field.

In the case of Group VI and Group VII, much more detail examinations were carried out, the physiological results of which are summarized in Table 10. For the more exact examination of growth characteristics of Group VI and VII, the growth curves at 25°C were obtained, which are shown in Fig. 2 and 3.

From the three growth curves of Group VI, it was apparent that, P-29 and P-31 showed neary equal

Fig. 2. Growth Curve of Group VI (P-6, P-29, P-31)

Fig. 3. Growth Curve of Group VII (P-26, P-27, P-33)

growth tendency, but P-6 was somewhat different. Fig. 3 showed that P-26, P-27 and P-33 had nearly equal curves.

In Fig. 4 the morphological change of the logarithmic and stationary growth phases of P-31 are shown, which show the change from short rod to coccoid. In Fig. 5 the cell shape of P-27 and flagella type of P-33 are shown, which are typical in the genus *Pseudomonas*. In Table 11 the characters of Group VI and Group VII are compared with typical gram-negative bacteria (*Moraxella* and *Acinetobacter*) and (*Chromobacterium* and *Pseudomonas*) respectively. In Table 12 the more detail characters of Group VI and three species of the genus are shown.

In the case of Group VII (P-26, P-27, P-33), the most typical characteristics were that these three strains had very high content of C16:1 (27–33%), C16:0 (25–26%) and, C-18:1 (20–23%), the total amount of which was 75–85%. Moreover, it must be pointed out that the chromatograms of these three strains showed the typical pattern of genus *Pseudomonas*. But, however, following the report of (Ikemoto *et al.*, 1978), the existence and

(a)

(b)

Fig. 4. Morphology of P-31 strain by negative stain (a): early logarithmic phase. (b): stationary phase

the ratio of typical minor fatty acid components (hydroximic acid, cyclopropanic acid, branched acid) are important factors for the classification and grouping of the genus *Pseudomonas*. In these points, our experimental results of fatty acid composition analysis were incomplete in comparison with (Ikemoto *et al.*, 1978), and the determination up to the species level was considered to be impossible in the present stage. But, it was concluded that Group VI is near to *M. bovis* and Group VII is near to *P. caryophylli*.

In conclusion, it was a very interesting result of

(a)

(b)

Fig. 5. (a): Morphology of P-27 strain by negative stain. (b): Flagella of strain by Nishizawa and Sugawara method

our study that there was no obligate psychrophile but facultative psychrophile are abundant in tropical districts. These results were a new information of psychrophilic bacteria in such a tropical region as Indonesia.

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