J. Gen. Appl. Microbiol., **49**, 257–262 (2003)

Short Communication

Characterization and identification of *Acinetobacter* strains from clinical specimens in Thailand

Pintip Pongpech,* Chutima Sereekul, Somboon Tanasupawat, and Chertsak Dhiraputra[1]

Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[1] Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

(Received March 3, 2003; Accepted June 28, 2003)

Key Words—*Acinetobacter*; *A. baumannii*; *A. calcoaceticus*; *A. junii*; *A. lwoffii*; clinical specimens

The genus *Acinetobacter* has recently been shown to be comprised of many species and genomic species including *A. baumannii*, *A. calcoaceticus*, *Acinetobacter* genospecies 3, *A. haemolyticus*, *A. junii*, *Acinetobacter* genospecies 6, *A. johnsonii*, *A. lwoffii*, *Acinetobacter* genospecies 9, *Acinetobacter* genospecies 10, and 11, *A. radioresistens*, *Acinetobacter* genospecies 13TU, 14TU, 14BJ, 15BJ, 15TU, 16, and 17, *A. ursingii*, and *A. schindleri* as defined by DNA-DNA similarities and 16S rRNA gene sequence analysis (Berlau et al., 1999; Nemec et al., 2001). Many strains described so far based on phenotypic characteristics and the Biolog system have remained unclassified (Bernards et al., 1995; Bouvet and Grimont, 1986; Bouvet and Jeanjean, 1989; Gerner-Smidt et al., 1991; Juni, 1984; Tjernberg and Ursing, 1989). The phylogenetic analysis using protein-encoding genes in *Acinetobacter* species was also reported (Yamamoto et al., 1999; Yamamoto and Harayama, 1996). However, there is still a limitation to the identification of *Acinetobacter* strains from clinical specimens in our country. In this study, photobiotin labeling DNA-DNA hybridization including phenotypic characteristics and ubiquinone analysis were used in order to identify *Acinetobacter* isolates.

Of 125 isolates, 46 isolates were kindly provided from the Department of Microbiology, Faculty of Medicine, King Chulalongkorn Memorial Hospital and 79 isolates were obtained from the Division of Bacteriology, Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University. They were obtained during a 3-month period between July to September 1999 from clinical specimens, which were urine, sputum, blood pus, body fluid and cerebrospinal fluid as listed in Table 1. Furthermore, the type strains of *A. baumannii* JCM 6841^T, *A. lwoffii* JCM 6840^T (Japan Collection of Microorganisms, Saitama, Japan), *A. calcoaceticus* DMST 2270^T, *Acinetobacter* genospecies 3 DMST 2272^T, and *A. junii* DMST 2274^T (Department of Medical Sciences, National Institute of Health, Nontaburi, Thailand) were used. All the isolates were cultivated on trypticase soy agar (Difco, Laboratories, Detroit, MI, USA) supplemented with 5% sheep blood at 37°C for 24 h.

The isolates on blood agar or MacConkey agar plate were examined for cell form, cell arrangement, colonial appearance and Gram stain. Catalase, oxidase, triple sugar iron agar reactions, motility, indole production, nitrate reduction, utilization of citrate, and oxidative-fermentative in glucose were tested as reported (Baron et al., 1994). The ability to grow at 37°C, 41°C, and 44°C, and at pH 3.5, 4.0, 4.5 and 5.0 in Tryptic Soy Broth (TSB) were determined as described by Bouvet and Grimont (1986). A utilization test of the different carbon sources of each kind at the final concentration of 0.2% (w/v) was performed as described by Barrow and Feltham (1993) and Juni (1984). Acid production from

* Address reprint requests to: Dr. Pintip Pongpech, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail: pintip.p@chula.ac.th

Table 1. Isolate number, sources, DNA groups and identification.

Isolate number	Source	Hospital	DNA group	Identification
SU1-1, SU7-1, SU7-2, SU9-3, SU11-12, SU1-2, SU1-4, SU13-9, SU2-1, SU11-16, SU11-10, SU6-22, SU11-6, SU13-2, SU11-25, SU11-27	Urine	Siriraj	I	*A. baumannii*
SU11-33			II	*A. calcoaceticus*
CU3-22, CU3-21, CU3-3		King Chulalongkorn Memorial	I	*A. baumannii*
CU3-11			IV	*A. junii*
SS5-3, SS6-5, SS6-6, SS6-7, SS4-2, SS4-7, SS4-9, SS6-3, SS4-3, SS11-20, SS1-18, SS11-19, SS1-3, SS4-8, SS5-8, SS6-8	Sputum	Siriraj	I	*A. baumannii*
SS6-4			IV	*A. junii*
SB7-1, SB4-1, SB6-15, SB1-3, SB4-5, SB7-2, SB6-13, SB6-14, SB7-3, SB1-4	Blood	Siriraj	I	*A. baumannii*
SB5-4			II	*A. calcoaceticus*
SP5-6, SP8-2, SP8-5, SP9-9, SP1-1, SP1-2, SP13-12, SP11-5, SP3-5, SP3-6	Pus	Siriraj	I	*A. baumannii*
SP9-8A, SP9-8B	Pus	Siriraj	IV	*A. junii*
CP3-4, CP5-1, CP4-9, CP5-2, CP5-3, CP5-5, CP3-5, CP5-10, CP5-16, CP5-7, CP5-9, CP4-3, CP5-13, CP4-1, CP4-2, CP5-17, CP2-4, CP3-8, CP4-4, CP4-15, CP1-5, CP4-27, CP3-25, CP3-1, CP3-24, CP4-21, CP3-13, CP2-10, CP1-15, CP4-10, CP5-4	Pus	King Chulalongkorn Memorial	I	*A. baumannii*
CP5-20, CP4-8, CP4-24	Pus	Siriraj	II	*A. calcoaceticus*
CP4-12, CP4-13, CP3-12	Pus	Siriraj	IV	*A. junii*
CP3-2, CP3-20	Pus	Siriraj	III	*A. lwoffii*
Un3-4, Un3-1, Un11-6, Un11-20, Un11-34, Un11-36, Un12-1, Un12-4, Un12-5, Un10-5, Un10-31, Un10-35, Un10-3, Un10-19, Un10-25, Un10-30, Un10-39A	Pus	Siriraj	I	*A. baumannii*
Un10-21, Un10-22, Un11-27	Pus	Siriraj	IV	*A. junii*
Un10-39B, Un3-10	Pus	Siriraj	V	*Acinetobacter* genospecies 3
CBF4-26, CBF4-25	Body fluid	King Chulalongkorn Memorial	I	*A. baumannii*
CCSF3-23	Cerebro-spinal fluid	King Chulalongkorn Memorial	V	*Acinetobacter* genospecies 3

carbohydrates was also determined (Baron et al., 1994).

DNAs were isolated from cells grown in 50 ml TSB shaken at 200 rpm for 24 h and were purified as described by Tamaoka and Komagata (1984). Photobiotin labeling DNA-DNA hybridization was carried out in 2× SSC (saline-trisodium citrate) and 50% formamide solution and incubated overnight (15 h) at 43°C (Ezaki et al., 1989). DNA-DNA similarity was determined by the colorimetric method (Tanasupawat et al., 2000). Quinones were purified and examined by HPLC (Shimadzu model LC-3A, Shimadzu, Kyoto, Japan) (Collins et al., 1977; Tamaoka et al., 1983). The abbreviation (e.g., Q-9, Q-8, etc.) used for ubiquinone indicated the number of isoprene units in the side chain.

All isolates were Gram-negative rods or coccobacilli, 0.5–0.8 μm in diameter and 1.0–1.9 μm in length. They occurred singly, in pairs or in short chains. They were nonmotile and nonsporing. Colonies on blood agar were gray to white, convex and entire, 2–3 mm in diameter, and on MacConkey agar were slightly bluish tint colonies. Among the 125 isolates, 111 isolates

Table 2. Characteristics of *Acinetobacter* isolates.

Characteristics	*A. baumannii* JCM 6841^T	Group I (105)[a]	*A. calcoaceticus* DMST 2270^T	Group II (5)	*A. lwoffii* JCM 6840^T	Group III (2)	*A. junii* DMST 2274^T	Group IV (10)	*Acinetobacter* genospecies 3 DMST 2272^T	Group V (3)
Cell form	Coccobacilli									
Cell arrangement	in singly or in pairs									
Oxidative	+	+(−4)	+	+	−	−	−	−(+3)	+	+(−1)
Growth at 41°C	+	+(−1)	−	−	−	−	+	+(−4)	+	+(−1)
44°C	+	+(−12)	+	−	+	−	−	−	−	−(+1)
pH 5	+	+	−	−	−	−	−	−	+	+
Utilization of										
Acetate	+	+	+	+(−1)	+	+	−	+(−1)	+	+
Aspartate	−	−	−	−(+1)	−	+	−	−	−	−
Benzoate	+	−(+24)	−	−	−	−	−	−(+2)	−	−
Citrate	+	+(−4)	+	+	−	−	−	+(−1)	−	+
Glutarate	+	+(−9)	+	+(−2)	−	+(−1)	−	+(−2)	+	+(−1)
Hippurate	+	+(−1)	−	−	−	−	−	−	+	+(−1)
DL-Lactate	+	+(−2)	+	+	+	+	+	+	+	+
D-Malate	+	+(−5)	+	+(−1)	+	+	+	+(−2)	+	+(−1)
Malonate	+	+(−24)	−	+(−2)	−	−	−	−(+2)	+	−(+1)
Phenylacetate	+	+(−44)	−	−	−	−	−	−	−	−
Propionate	+	+(−25)	−	−	−	−	−	−	+	+
Succinate	+	+(−8)	−	+	−	+	−	+(−1)	+	+
L-Tartrate	−	−(+10)	−	+(−1)	−	−	+	−	+	−(+1)
β-Alanine	+	+(−14)	+	+	−	+	−	+(−5)	+	+
L-Arginine	+	+(−6)	+	−(+2)	−	−	+	+(−1)	+	+
L-Histidine	+	+(−8)	+	+	−	+(−1)	+	+	+	+
L-Leucine	+	−(+1)	−	−	−	−	−	−	+	−
L-Ornithine	+	−(+2)	−	−	−	−	−	−	−	−
L-Phenylalanine	+	+(−52)	+	+(−2)	−	−	−	−(+2)	+	−(+1)
Threonine	+	−(+32)	−	−	−	−	−	−	−	−
Acid from										
D-Glucose	+	+	+	+(−1)	−	−	−	−	+	+(−1)
Maltose	−	−(+12)	−	−	−	−	−	−	−	−(+1)
D-Mannitol	−	−(+11)	−	−	−	−	−	−	−	−(+1)
Lactose	−	−(+19)	−	−	−	−	−	−	−	−(+1)
Sucroce	−	−(+13)	−	−	−	−	−	−	−	−(+1)
Ribose	+	+	+	+	−	−	+	+	+	+
L-Arabinose	+	+(−2)	+	+	−	−	−	−	+	+
D-Xylose	+	+(−2)	+	+	−	−	+	+(−3)	+	+

[a] Number of tested strains. Numbers in parentheses indicate the number of strains showing positive or negative reaction.

Table 3. DNA-DNA similarity of *Acinetobacter* isolates.

DNA group	Number of strains	JCM 6841^T	DMST 2270^T	JCM 6840^T	DMST 2274^T	DMST 2272^T
I	105	**70.1–99.2**				
II	5		**71.2–99.4**			
III	2			**70.3–70.5**	22.6–30.5	
IV	10				**72.1–98.7**	
V	3	20.4–57.5	20.2–31.7			**81.2–84.9**
A. baumannii JCM 6841^T		**100**				
A. calcoaceticus DMST 2270^T			**100**			
A. lwoffii JCM 6840^T				**100**		
A. junii DMST 2274^T					**100**	
Acinetobacter genospecies 3 DMST 2272^T						**100**

(groups I, II, V and IV) were glucose oxidizers and 14 isolates (groups I, III and V) were nonoxidizers. All showed negative reaction to oxidase, nitrate reduction, utilization of L-valine and acid production from fructose, but showed a positive reaction to catalase and utilization of pyruvate. They could not grow at pH 3.5, 4.0 or 4.5. All grew at 37°C and most of the isolates of group I grew at 41°C and 44°C. A utilization test of carbon sources was variable and few isolates produced acid from carbon sources as shown in Table 2.

On the basis of DNA-DNA similarity, 125 isolates (Tables 1 and 3) were divided into five groups (groups I to V) when 70% or greater DNA similarity with the type strains of *Acinetobacter* species were shown (Wayne et al., 1987).

Group I (105 isolates) showed a high degree of DNA similarity (over 70.1%) with *A. baumannii* JCM 6841^T, and was identified as *A. baumannii*. Most isolates in this group oxidized glucose, and grew at 41°C and 44°C. However, a variable of growth at 44°C was found in 12 isolates (i.e., SS4-2, SP11-5, Un10-35, SU2-1, SU6-22, CP2-4, CP4-2, CP3-8, CP3-13, SS11-19, Un10-39A and CP4-10). All grew at pH 5 (Table 2). The utilization of citrate, glutarate, DL-lactate, malate, malonate, propionate, β-alanine, arginine and histidine, and acid produced from D-glucose were the same as previous reports (Bernards et al., 1995; Bouvet and Grimont, 1986; Gerner-Smidt et al., 1991). The growth at 41°C and 44°C were confirmed to be useful in the separation of *A. baumannii*, *A. calcoaceticus* and *Acinetobacter* genospecies 3 as reported by Kampfer et al. (1993) and Berlau et al. (1999). The tested strains in group I and *A. baumannii* JCM 6841^T had Q-9 as the major ubiquinone component (Table 4), which was different from *A. baumannii* ATCC 9955 (=ATCC 17961) (Yokota et al., 1992) that contained Q-8 as the major component. In this study, *A. baumannii* was isolated from many clinical specimens, particularly from pus. Of 105 isolates, 46 isolates were found in pus, 19 in urine, 16 in sputum, 10 in blood and 2 in body fluid (Table 1).

Group II consisted of 5 isolates (i.e., SU11-33, CP5-20, CP4-8, CP4-24 and SB5-4) (Table 1). They had a high degree of DNA similarity with *A. calcoaceticus* (over 71.2%) and were identified as *A. calcoaceticus*. They could not grow at 41°C or 44°C, which can be differentiated from group I isolates (Bouvet and Grimont, 1986; Gerner-Smidt et al., 1991). All isolates utilized citrate, DL-lactate, succinate, β-alanine and L-histidine, but did not utilize benzoate, hippurate, phenylacetate, propionate, L-leucine, L-ornithine or threonine. Variable reactions are shown in Table 2. The strains designated as CP5-20 and *A. calcoaceticus* DMST 2270^T had Q-9 as the major ubiquinone (Table 4). Three were isolated from pus, 1 from blood and 1 from urine (Table 1).

Group III isolates (CP3-20 and CP3-2) showed a high degree of DNA similarity with *A. lwoffii* JCM 6840^T (over 70.3%) and were identified as *A. lwoffii*. All isolates were nonoxidizers and could not grow at 41°C. They utilized acetate, aspartate, DL-lactate, D-malate, succinate and β-alanine, but did not utilize benzoate, citrate, hippurate, malonate, phenylacetate, propionate, L-tartrate, L-arginine, histidine, L-leucine, ornithine, L-phenylalanine or threonine (Table 2). In this study, some isolates could utilize glutarate, β-alanine and L-histidine, but the study by Bouvet and Grimont (1986) indicated that *A. lwoffii* could not utilize these

Table 4. Ubiquinone system of *Acinetobacter* isolates.

DNA group	Strain	Ubiquinone (%)			
		Q-7	Q-8	Q-9	Q-10
Group I	SU1-1	5.3	14.4	77.5	2.8
	SP9-9	2.6	10.4	82.5	4.3
	SS4-9	ND	19.1	79.2	1.7
	CP5-1	5.0	10.0	81.8	3.2
	CP1-5	3.8	10.3	81.6	4.1
	CBF4-25	1.0	11.2	85.4	2.4
	CP3-25	2.4	11.6	83.5	2.5
	SS1-3	6.3	6.2	83.0	4.5
	A. baumannii JCM 6841^T	3.2	16.3	77.6	2.9
Group II	CP5-20	6.1	11.0	79.8	3.0
	A. calcoaceticus DMST 2270^T	ND	10.4	86.4	3.2
Group III	CP3-20	15.7	5.6	78.6	ND
	A. lwoffii JCM 6840^T	8.2	7.5	82.8	1.3
Group IV	CP3-12	3.3	15.4	79.6	1.6
	A. junii DMST 2274^T	ND	39.5	60.5	ND
Group V	CCSF3-23	2.7	12.7	82.5	2.1
	Acinetobacter genospecies 3 DMST 2272^T	ND	10.1	82.5	7.4

ND, Not detected.

three carbon sources. The tested strains, CP3-20 and *A. lwoffii* JCM 6840^T, contained Q-9 as a major ubiquinone component, which agreed with the results reported by Yokota et al. (1992). Both isolates were from pus.

Group IV consisted of 10 isolates (i.e., SP9-8A, CP3-12, SP9-8B, SS6-4, CU3-11, Un10-21, Un10-22, CP4-13, CP4-12 and Un11-27). They showed a high degree of DNA similarity (over 72.1%) with *A. junii* DMST 2274^T and were identified as *A. junii*. Most isolates did not produce acid from D-glucose. Six tested strains grew at 41°C. This group can be differentiated from group III by the utilization of L-arginine, citrate and growth at 41°C. In the study by Bouvet and Grimont (1986), *A. junii* was differentiated from the other glucose nonoxidizers by utilizing DL-lactate and L-histidine and the lack of utilizing glutarate. However, in the present study, the isolates failed to do so. The tested strains, CP3-12 and *A. junii* DMST 2274^T, had Q-9 as a major ubiquinone component (Table 4), which disagreed with the results reported by Yokota et al. (1992). Most of the isolates were also from pus (eight isolates), one from sputum, and one from urine.

Group V contained three isolates (i.e., Un3-10, Un10-39B and CCSF3-23) which were isolated from pus and cerebrospinal fluid (CSF). All isolates showed a high degree of DNA similarity (over 81.2%) with *Acinetobacter* genospecies 3 DMST 2272^T and were identified as *Acinetobacter* genospecies 3. In this study, their phenotypic characteristics could not be differentiated from the group II isolates except for the ability of growth at 41°C by two isolates. All three isolates utilized citrate, DL-lactate, propionate, β-alanine, L-arginine and histidine, which was similar to *Acinetobacter* genospecies 3 in the study by Bouvet and Grimont, 1986. The isolates CCSF3-23 and *Acinetobacter* genospecies 3 DMST 2272^T contained Q-9 as a major ubiquinone (Table 4).

In this study, the isolates belonging to the genus *Acinetobacter* could be divided into five groups. They were clearly identified as *A. baumannii* (105 isolates), *A. calcoaceticus*, *A. lwoffii*, *A. junii* and *Acinetobacter* genospecies 3 based on DNA-DNA similarity. This technique showed to be useful for differentiating the *Acinetobacter* strains as it has been recommended by other investigators including Bouvet and Grimont (1986), Tjernberg and Ursing (1989), and Fox et al. (1992). In addition, Stackebrandt and Goebel (1994) reported the resolution of 16S rRNA sequence analysis was insufficient to closely distinguish related ge-

nomic species and could not replace the DNA-DNA hybridization method. Therefore, we would like to recommend that DNA-DNA hybridization is the most appropriate method for differentiating *Acinetobacter* strains from clinical specimens in Thailand.

Acknowledgments

Part of this study was supported by a research budget from the Ministry of University Affairs. We thank Associate Professor Pongpun Nunthapisud, Department of Microbiology, Faculty of Medicine, King Chulalongkorn Memorial Hospital for kindly providing the cultures.

References

Baron, E. J., Peterson, L. R., and Finegold, S. M. (1994) Bailey and Scott's Diagnostic Microbiology, 9th ed., St. Louis, Baltimore, pp. 386–405.

Barrow, G. I. and Feltham, R. K. A. (1993) Cowan and Steel's Manual for the Identification of Medical Bacteria, 3rd ed., Cambridge University Press, Cambridge, 331 pp.

Berlau, J., Aueken, H. M., Malnick, H., and Pitt, T. L. (1999) The distribution of *Acinetobacter* spp. on skin of healthy humans. *Eur. J. Clin. Microbiol. Infect. Dis.*, **18**, 179–183.

Bernards, A. T., Dijkshoorn, L., van der Toorn, J., Bochner, B. R., and van Boven, C. P. A.(1995) Phenotypic characterization of *Acinetobacter* strains of 13 DNA-DNA hybridization groups by means of the Biolog system. *J. Med. Microbiol.*, **42**, 113–119.

Bouvet, P. J. M. and Grimont, P. A. D. (1986) Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov., and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int. J. Syst. Bacteriol.*, **36**, 228–240.

Bouvet, P. J. M. and Jeanjean, S. (1989) Delineation of new proteolytic genomic species in the genus *Acinetobacter*. *Res. Microbiol.*, **140**, 291–299.

Collins, M. D., Pirouz, T., and Goodfellow, M. (1977) Distribution of menaquinones in actinomycetes and corynebacteria. *J. Gen. Microbiol.*, **100**, 221–230.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.*, **39**, 224–229.

Fox, G. E., Wisotzkey, J. D., and Jurtshuk, P. J. (1992) How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int. J. Syst. Bacteriol.*, **42**, 166–170.

Gerner-Smidt, P., Tjernberg, I., and Ursing, J. (1991) Reliability of phenotypic test for identification of *Acinetobacter* species. *J. Clin. Microbiol.*, **29**, 277–282.

Juni, E. (1984) Genus II. *Acinetobacter* Brisou and Prevot. 1954. *In* Bergey's Manual of Systematic Bacteriology, Vol. 1, ed. by Krieg, N. R. and Holt, J. G., Williams & Wilkins, Baltimore, pp. 303–307.

Kampfer, P., Tjernberg, I., and Ursing, J. (1993) Numerical classification and identification of *Acinetobacter* genomic species. *J. Appl. Bacteriol.*, **75**, 259–268.

Nemec, A., De Baere, T., Tjernberg, I., Vaneechoutte, M., van der Reijden, T. J. K., and Dijkshoorn, L. (2001) *Acinetobacter ursingii* sp. nov and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int. Syst. Evol. Microbiol.*, **51**, 1891–1899.

Stackebrandt, E. and Goebel, B. M.. (1994) A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.*, **44**, 846–849.

Tamaoka, J., Katayama-Fujimura, Y., and Kuraishi, H. (1983) Analysis of bacterial menaquinone mixtures by high performances liquid chromotography. *J. Appl. Bacteriol.*, **54**, 31–36.

Tamaoka, J. and Komagata, K. (1984) Determination of DNA base composition by reverse phase high performances liquid chromotography. *FEMS Microbiol. Lett.*, **25**, 125–128.

Tanasupawat, S., Shida, O., Okada, S., and Komagata, K. (2000) *Lactobacillus acidipiscis* sp. nov. and *Weissella thailandensis* sp. nov., isolated from fermented fish in Thailand. *Int. J. Syst. Evol. Microbiol.*, **50**, 1479–1485.

Tjernberg, I. and Ursing, J. (1989) Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. *APMIS*, **97**, 595–605.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., Starr, M. P., and Trüper, H. G. (1987) Report of the *Ad Hoc* committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.*, **37**, 463–464.

Yamamoto, S., Bouvet, P. J. M., and Harayama, S. (1999) Phylogenetic structures of the genus *Acinetobacter* based on *gyrB* sequences: Comparison with the grouping by DNA-DNA hybridization. *Int. J. Syst. Bacteriol.*, **49**, 87–95.

Yamamoto, S. and Harayama, S. (1996) Phylogenetic analysis of *Acinetobacter* strain based on the nucleotide sequences of *gyrB* genes and on the amino acid sequences of their products. *Int. J. Syst. Bacteriol.*, **46**, 506–511.

Yokota, A., Akagawa-Matsushita, M., Hiraishi, A., Katayama, Y., Urakami, T., and Yamasato, K. (1992) Distribution of quinone systems in microorganisms: Gram-negative eubacteria. *Bull. JFCC*, **8**, 136–171.