Short Communication

Screening and identification of xylanase-producing bacteria from Thai soils

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Xylan, the most abundant of the hemicelluloses containing heteropolysaccharides, consists of a backbone of β -1,4-linked xylopyranose residues with substitutions of *O*-acetyl, arabinosyl and methylglucuronosyl (Chang et al., 2004; Rawashdeh et al., 2005). The complete hydrolysis of xylan requires the combined action of various enzymes such as endoxylanase and several accessory enzymes to hydrolyze substituted xylan. The endoxylanase attacks internal xylosidic linkages on the backbone and the β -xylosidase releases xylosyl residues by endwise attack of xylooligosaccharide (Wong et al., 1988). Many microorganisms including bacteria, yeasts and fungi are known to produce different type of xylanases and the nature of the enzymes varies among different organisms. Among xylan degrading bacteria, the strains of *Aeromonas*, *Bacillus*, *Bacteroides*, *Cellulomonas*, *Microbacterium*, *Paenibacillus*, *Ruminococcus* and *Streptomyces* have been reported (Rapp and Wagner, 1986). This work deals with the screening and identification of xylanase-producing bacteria isolated from Thai soils based on their phenotypic and chemotaxonomic characteristics, including DNA-DNA similarity and 16S rRNA gene se-

quence similarity.

A total of 28 soil samples were collected from Viengsa and Muang districts, Nan Province, Thailand and were isolated for xylanase producing bacteria by an enrichment culture method. The soil sample (0.1 g) was put into 10 ml of XC broth in a 25×250 mm test tube and incubated on a rotary shaker at 200 rpm at 40°C for 2 days. The XC medium contained 10 g oat spelt xylan, 5 g peptone, 1 g yeast extract, 4 g K₂HPO₄, 1 g MgSO₄·7H₂O, 0.2 g KCl, 0.02 g FeSO₄·7H₂O and 1,000 ml distilled water and was adjusted pH to 7.0. One milliliter of the culture was transferred to fresh XC broth and incubated as mentioned above. The cultures were repeated to cultivate in fresh XC broth one more time. The diluted enriched cultures (0.1 ml) were dropped and spreaded on the XC agar plate and incubated at 40°C for 2 days. Their colonies grown on XC agar plate were flooded with 0.1% (w/v) Congo red solution and kept for 1 min, then were washed by 0.1 M NaCl (Teather and Wood, 1982). The colonies surrounded by a clearance zone were selected as xylanase-producing isolates and they were purified on a C agar plate (in the XC medium of which oat spelt xylan was omitted).

Xylanase-producing isolates were inoculated into 10 ml of XC broth in a 25×250 mm test tube and incubated on a rotary shaker at 200 rpm at 40°C for 2 days. Three milliliters of the cultures were transferred into 30 ml of XC broth in a 250 ml Erlenmeyer flask

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and incubated at 40°C for 2 days. Supernatant obtained after centrifugation of the culture broth at 4°C, 10,000 rpm for 15 min was used as crude enzyme. The xylanase activity was assayed as described by Nakajima et al. (1984). A reaction mixture composed of 0.1 ml of 10 mg/ml oat spelt xylan in 100 mM sodium phosphate buffer pH 7.0, 0.8 ml of 100 mM sodium phosphate buffer pH 7.0 and 0.1 ml of crude enzyme were incubated at 40°C for 10 min. The reaction was stopped in boiling water for 10 min and put on ice immediately. The amount of reducing sugar released was quantified by the Somogyi and Nelson method (Somogyi, 1952) using xylose as authentic sugar. After the addition of the enzyme solution, the reactions were stopped immediately and used as a reaction blank. Protein concentration was analyzed by the Lowry method (Lowry et al., 1951) using bovine serum albumin as the standard protein. One unit of xylanase was defined as the amount of enzyme yielding 1 μ mol of xylose within 1 min under the assay conditions.

Among 31 isolates screened, a maximum xylanolytic activity with 2.8 cm in diameter of clear zone was ex-

hibited. Twenty isolates of non spore-forming, Gram-positive irregular rods produced 0–0.13 units/ml while 8 isolates of Gram-positive spore-forming rods; 2 isolates of Gram-negative rods; and 1 isolate of Gram-positive rods/cocci produced 0–0.17 units/ml (Fig. 1, A and B).

All the selected isolates were examined for their cell shape and colonial appearance, spore formation, motility, and pigmentation after incubating at 37°C for 1 day. Catalase, oxidase, urease, hydrolysis of L-arginine, aesculin, casein, gelatin, starch, tyrosine, MR-VP, deoxyribonuclease (DNase), indole test, nitrate reduction, Simmon citrate test, Triple Sugar Iron agar (TSI), dihydroxyacetone from glycerol, and acid from carbohydrates were determined as described by Barrow and Feltham (1993). Growth at different pHs (5, 6, 8 and 9), in 3 and 5% NaCl and at different temperatures (10°C, 15°C, 20°C, 37°C, 45°C, 50°C, 55°C and 60°C) were performed. All tests were carried out by using C medium as a basal component and incubating the cultures at 37°C, except for the investigation of the effect of temperatures.

Fig. 1. Xylanase production capability of the isolates in Group 1 (A) and in Groups 2 to 7 (B).

Diaminopimelic acid in the cell wall, and menaquinone and ubiquinone were determined as described by Komagata and Suzuki (1987). DNAs were isolated from cells grown on a C agar plate for 18 to 48 h, and purified by the method of Saito and Miura (1963). DNA base composition was determined by the method of Tamaoka and Komagata (1984). Photobiotin labelling DNA-DNA hybridization was determined in $2\times$ SSC (saline trisodium citrate) and 50% formamide solution at 39 or 52°C for 15 h (Ezaki et al., 1989). The 16S rRNA gene of the isolate was amplified, purified, and sequenced as described previously (Tanasupawat et al., 2004). The sequences determined were aligned with the selected sequences obtained from the GenBank/EMBL/DDBJ database employing CLUSTAL_X version 1.81 (Thompson et al., 1997). The alignment was manually edited to remove gaps and ambiguous nucleotides prior to construction of phylogenetic trees. The phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987) in the MEGA program version 2.1 (Kumar et al., 2001). The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1,000 resamplings. The DDBJ accession number for the 16S rRNA gene sequences of strains MXC4-2-1, MXC4-6-2, MX1-2, MX2-1, MX3-2, MX12-2, MXC1-3, MXC3-4-2, S2-1, MX1-1, MX8-2, MXC3-9 and SF are AB271156, AB271131, AB271132, AB271133, AB271134, AB271135, AB271136, AB271137, AB271138, AB271139, AB271140, AB271141, and AB271142, respectively.

On the basis of their phenotypic and chemotaxo-

nomic characteristics, DNA-DNA similarity and the phylogenetic analysis using 16S rRNA gene sequences, thirty-one isolates were divided into 7 different groups (Tables 1, 2, 3 and 4, Figs. 2 and 3). Twenty isolates of non spore-forming, Gram-positive irregular rods in Group 1 were identified as *Microbacterium* (Lee et al., 2006; Takeuchi and Hatano, 1998a). Eight isolates of Gram-positive spore-forming rods in Groups 2, 3 and 4 were identified as *Bacillus* (Heyrman et al., 2004; Venkateswaran et al., 2003). The Gram-negative rods of Group 5 was identified as *Pseudoxanthomonas* (Weon et al., 2006) and one in Group 6 was *Cupriavidus* (Vandamme and Coenye, 2004). One Gram-positive rod/coccal isolate in Group 7 was identified as *Rhodococcus* (Yoon et al., 2000).

Group 1 contained 20 isolates. They were Gram-positive, non spore-forming, motile rods measuring from 0.5 to 1.0 by 2.0–4.0 μm . Colonies were white to yellowish, low convex, opaque, and circular with entire margins. Catalase, VP, DNase, urease, hydrolysis of aesculin, L-arginine and starch were positive but negative for oxidase, MR, indole production, TSI, nitrate reduction, hydrolysis of gelatin and Tween 80. All grew in 3 and 5% NaCl, at pH 6–9 and at 15–45°C, and in anaerobic conditions but not at pH 5 or at 60°C (Tables 1 and 2). The variable characteristics and acid production from carbohydrates are shown in Tables 2 and 3. The tested strains had no *meso*-diaminopimelic acid in the cell wall. MXC3-1 contained MK-11 (65.6%) and MK-12 (34.4%). MXC3-1 had 71.5 mol% of DNA G+C content. MXC 4-2-1 (1,478 bp) and MXC4-6-2 (1,443 bp) showed 99.3 and 99.4% of 16S rRNA se-

Table 1. Source location, Isolate no., Group, and identification.

District in Nan Province	Isolate no.	Group	Identification
Viengsa Muang	X9-2	1	<i>M. barkeri</i>
	MX16-2, MX17-2, MX18, MXC3-1, MXC3-2, MXC3-4-1, MXC3-5-1, MXC3-5-2, MXC3-7-1, MXC3-7-2, MXC3-10-2, MXC4-1-1, MXC4-1-2, MXC4-2-1, MXC4-5-1, MXC4-5-2, MXC4-6-2, MXC4-9-3, ST1	1	<i>M. barkeri</i>
	MX1-2, MX2-1, MX3-2, MX12-2, MXC1-3, MXC3-4-2	2	<i>B. funiculus</i>
	S2-1	3	<i>B. niabensis</i>
	MX1-1	4	<i>B. megaterium</i>
	MX8-2	5	<i>Px. suwonensis</i>
	MXC3-9	6	<i>C. gilardii</i>
	SF	7	<i>R. rhodochrous</i>

Table 2. Characteristics of xylanase-producing isolates.

Characteristics	Group 1 (20) ^a	Group 2 (6)	Group 3 (1)	Group 4 (1)	Group 5 (1)	Group 6 (1)	Group 7 (1)
Catalase	+	—	—	+	—	+	+
Oxidase	—	+	—	+	+	+	—
Anaerobic growth	+	+	+	+	+	+	+
Growth at 10°C	— (w4)	—	—	—	—	—	w
15°C	+	— (+3)	+	+	+	+	+
45°C	+	+	+	+	+	+	+
50°C	+	—	+	+	+	+	—
55°C	— (+4)	—	+	+	—	—	—
Growth at pH 5.0	—	—	—	—	—	+	—
pH 6.0	+	—	—	—	+	+	+
Growth in 3% NaCl	+	—	+	+	+	+	+
5% NaCl	+	—	+	+	+	—	+
Methyl red	—	+	—	—	—	—	—
Voges-Proskauer	+	+	+	w	+	+	w
DNase	+	+	+	—	—	+	+
Urease	+	—	+	+	+	+	+
Citrate	+	—	—	—	—	+	—
Nitrate reduction	—	— (+3)	+	+	—	—	+
Aesculin	+	— (+3)	+	+	+	w	w
L-Arginine	+	— (w2)	—	—	+	+	+
Dihydroxyacetone	+	—	—	—	—	—	—
Hydrolysis of:							
Casein	— (+3)	+	—	+	—	—	—
Starch	+	+	+	+	—	—	—
L-Tyrosine	— (+3)	—	—	—	—	+	+
Tween80	—	— (+3)	+	—	—	+	+
DNA G+C content (mol%)	71.5–72.8	36.6–37.2	39.3	37.3	65.4	71.4	61.4

+, positive; w, weak positive; —, negative reaction.

^a Number of strains.

Numbers in parentheses indicate the number of strains showing the reaction. All grew in anaerobic conditions, at 20°C, pH 8.0 and 9.0, but did not grow at 60°C. None formed indole and H₂S, or hydrolyzed gelatin.

quence similarity to *Microbacterium barkeri* DSM 20145^T (Fig. 2). MXC3-1 showed a high degree of DNA–DNA similarity with *M. barkeri* JCM 1343^T, MXC 4-2-1, MXC4-6-2, and related strains over 72.4% (Table 4). They were included in the same species and identified as *M. barkeri* (Lee et al., 2006; Takeuchi and Hatano, 1998b; Wayne et al., 1987).

Groups 2, 3 and 4 contained 8 isolates. They were Gram-positive, spore-forming, motile rod measuring from 0.8 to 2.0 by 2.0–11.0 µm. Colonies were cream or light brown, irregular, and opaque. VP was positive but negative for indole production, citrate utilization, TSI, dihydroxyacetone formation, hydrolysis of gelatin and L-tyrosine. No acid production from gluconate or L-

sorbose. These strains grew at pH 7–9, and at 20–40°C, and in anaerobic conditions but not at pH 5–6, 10 or 60°C (Tables 2 and 3). The tested strains had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as the major menaquinone. The DNA G+C content was 36.6 and 37.2 mol% for MX1-2 and MX12-2 in Group 2 and 39.3 mol% for S2-1 in Group 3 and 37.3 mol% for MX1-1 in Group 4. MX1-2 (1,483 bp), MX2-1 (1,476 bp), MX3-2 (1,491 bp), MX12-2 (1,475 bp), MXC1-3 (1,463 bp) and MXC3-4-2 (1,474 bp) showed 99.6, 99.3, 99.5, 99.5, 99.8 and 99.8% of 16S rRNA sequence similarity to *Bacillus funiculus* NAF001^T (Fig. 3). MX12-2 showed a high degree of DNA–DNA similarity with *B. funiculus* JCM 11201^T and

Table 3. Acid production from carbohydrates of the isolates.

Acid from carbohydrate	Group 1 (20) ^a	Group 2 (6)	Group 3 (1)	Group 4 (1)	Group 5 (1)	Group 6 (1)	Group 7 (1)
D-Amygdalin	—	—	+	+	—	—	—
L-Arabinose	— (1w)	— (+2)	+	—	—	—	—
D-Cellobiose	— (+5)	—	+	+	—	—	—
D-Fructose	—	— (+3)	+	—	+	—	+
D-Galactose	—	+	+	—	—	—	—
D-Glucose	— (+2)	+	+	—	+	—	+
Glycerol	— (+5)	—	+	+	—	—	+
Inositol	— (+8)	—	+	—	—	—	—
Inulin	— (+4)	—	—	+	—	—	—
Lactose	—	—	+	+	—	—	—
D-Maltose	+	—	+	+	+	—	+
D-Mannitol	— (+1)	—	+	+	—	—	+
D-Mannose	—	—	+	—	—	—	—
D-Melibiose	— (+2)	— (+1)	+	+	—	—	—
D-Melezitose	— (+1)	—	—	+	—	—	—
α-Methyl-D-glucoside	—	—	+	+	—	—	—
Raffinose	+	—	+	+	—	—	—
L-Rhamnose	—	—	+	—	—	—	—
D-Ribose	—	— (+1)	+	—	—	—	—
Salicin	+	—	+	+	+	—	+
D-Sorbitol	—	—	+	—	—	—	—
Sucrose	+	—	—	+	+	—	+
D-Trehalose	—	—	+	+	+	—	+
D-Xylose	—	— (+1)	+	—	—	—	—

+, positive; w, weak positive; —, negative reaction.

^aNumber of strains.

Numbers in parentheses indicate the number of strains showing the reaction. None produced acids from gluconate or L-sorbose.

5 related strains over 70.4% (Table 4). Therefore, the 6 isolates in Group 2 were identified as *B. funiculus* (Ajithkumar et al., 2002; Wayne et al., 1987). The strain S2-1 in Group 3 showed 99.4% of 16S rRNA sequence similarity to *Bacillus niabensis* 4T19^T as shown in Fig. 3; therefore it was identified as *Bacillus niabensis* 4T19^T. The strain MX1-1 in Group 4 showed 99.3% of 16S rRNA sequence similarity to *Bacillus megaterium* IAM13418^T as shown in Fig. 3; therefore, it was identified as *Bacillus megaterium* IAM13418^T (Suzuki and Yamasato, 1994).

Group 5 contained 1 isolate, MX8-2. This isolate was Gram-negative, non spore-forming, motile rods measuring from 0.5 to 0.8 by 0.9–1.4 µm. Colonies were yellow orange, convex, and circular with entire margins. Oxidase, VP, urease, hydrolysis of aesculin and L-arginine was positive but negative for catalase, MR, DNase, indole production, citrate, TSI, nitrate re-

duction, dihydroxyacetone, hydrolysis of casein, gelatin, starch, L-tyrosine and Tween 80. This isolate grew in 3 and 5% NaCl, at pH 6–9, 15–50°C, and in anaerobic conditions but not at pH 5, 10 or at 55–60°C (Table 2). No acid production from D-amygda- lin, L-arabinose, D-cellobiose, D-galactose, gluconate, glycerol, inositol, inulin, lactose, D-mannitol, D-mannose, D-meli- biose, D-melezitose, α-methyl-D-glucoside, raffinose, L- rhamnose, D-ribose, D-sorbitol, L-sorbose or D-xylose (Table 3). It had no meso-diaminopimelic acid in the cell wall. Ubiquinone Q-8 was a major component. DNA G+C contents of MX8-2 were 65.4 mol%. The isolate MX8-2 showed 99.6% of 16S rRNA sequence similarity to *Pseudoxanthomonas suwonensis* 4M1^T. Therefore, it was identified as *Pseudoxanthomonas suwonensis* (Weon et al., 2006).

Group 6 contained 1 isolate, MXC3-9. This isolate was Gram-negative, non spore-forming, motile rods

Table 4. DNA G + C contents and DNA-DNA similarity of Group 1 and Group 2 isolates.

Isolate no.	DNA G + C (mol%)	%DNA similarity with labeled strains			
		MXC3-1	MXC4-2-1	MXC4-6-2	JCM 1343 ^T
Group 1					
MXC3-1	71.5	100.0	89.7	91.1	99.6
MXC3-10-2		98.3	103.2	80.1	ND
MXC3-7-1		99.5	ND	ND	98.8
MXC3-5-1		95.0	ND	ND	115.1
MXC4-5-1		94.3	ND	ND	88.8
MXC4-9-3		92.0	ND	88.9	90.1
MX17-2		91.3	ND	ND	111.3
MX18		89.8	ND	88.4	93.9
X9-2		89.2	ND	ND	90.9
MXC3-7-2		87.3	ND	ND	72.0
ST1		88.0	ND	81.1	104.6
MXC3-2		83.0	104.0	ND	117.0
MXC3-5-2		81.8	ND	ND	92.8
MXC4-5-2		81.4	ND	ND	80.5
MX16-2		80.7	ND	ND	86.6
MXC3-4-1		80.0	ND	ND	92.9
MXC4-1-1		75.5	81.0	ND	95.5
MXC4-6-2	72.8	73.9	87.3	100.0	114.0
MXC4-1-2		ND	72.4	ND	75.0
MXC4-2-1	72.4	94.4	100.0	83.4	116.0
<i>M. barkeri</i> JCM1343 ^T	68.7 ^a	83.0	97.0	78.1	100.0
<i>M. aborescens</i> JCM 5884 ^T	71.0 ^a	17.0	4.0	5.6	23.4
Group 2					
MX 12-2	37.2			100.0	
MXC1-3				100.0	
MX1-2	36.6			80.1	
MX2-1				78.0	
MX3-2				70.4	
MXC3-4-2				70.4	
<i>B. funiculus</i> JCM 11201 ^T	37.2 ^a			77.6	

ND, not determined.

^aData from Ajithkumar et al., 2002 and Takeuchi and Hatano, 1998a.

measuring from 0.5 to 1.0 by 1.0–2.0 µm. Colonies were white, convex, and circular with entire margins. Catalase, oxidase, VP, DNase, urease, citrate, aesculin and L-arginine hydrolysis were positive but negative for MR, indole production, TSI, nitrate reduction, dihydroxyacetone, hydrolysis of casein, gelatin and starch. This isolate grew in 3% NaCl, at pH 5–9 and 15–50°C, and in anaerobic conditions but not at 10 or 55–60°C (Table 2). No acid production from D-amygda-
lin, L-arabinose, D-cellobiose, D-fructose, D-galac-
tose, D-glucose, gluconate, glycerol, inositol, inulin,

lactose, D-maltose, D-mannitol, D-mannose, D-meli-
biose, D-melezitose, α-methyl-D-glucoside, raffinose, L-
rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, su-
crose, D-trehalose or D-xylose (Table 3). It had no
meso-diaminopimelic acid in the cell wall. Ubiquinone
Q-8 was a major component. DNA G+C contents of
MXC3-9 were 71.4 mol%. The isolate MXC3-9 in
Group 6 showed 99.0% of 16S rRNA sequence simi-
larity to *Cupriavidus gilardii* LMG5886^T; therefore, it
was identified as *C. gilardii* (Coenye et al., 1999).

Group 7 contained 1 isolate, SF. This isolate was

Gram-positive, non-motile, non-spore-forming rods/cocci measuring from 0.2 to 0.5 by 1.5–3.0 μm . Colonies were light orange, raised, circular, undulate and opaque. Catalase, DNase, urease, nitrate reduction, hydrolysis of L-arginine, L-tyrosine and Tween 80 were positive but negative for oxidase, MR, indole production, citrate, TSI, dihydroxyacetone, hydrolysis of casein, gelatin and starch. This isolate grew in 3 and 5% NaCl, pH 6–9, and at 15–45°C and in anaerobic conditions but not at pH 5, or at 50–60°C (Table 2). No

acid production from D-amygdalin, L-arabinose, D-cellobiose, D-galactose, gluconate, inositol, inulin, lactose, D-mannose, D-melibiose, D-melezitose, α -methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, or D-xylose (Table 3). The tested isolate had *meso*-diaminopimelic acid in the cell wall and contained MK-8(H₂) as the major menaquinone. The DNA G+C content of SF was 61.4 mol%. SF (1,490 bp) showed 99.6% of 16S rRNA sequence similarity to *Rhodococcus rhodochrous* DSM43241^T. Therefore, the isolate SF was identified as *Rhodococcus rhodochrous* (Yoon et al., 2000).

Recently, the xylanase from the strains of *Cellulomonas uda*, *Microbacterium ulmi*, *M. xylanilyticum*, *Bacillus amyloliquefaciens*, *B. firmus*, *B. thermoleovorans*, *B. flavothermus*, *B. pumilus*, *Bacillus* sp., and *Streptomyces* sp. has been reported (Archana and Satyanarayana, 2003; Beg et al., 2001; Breccia et al., 1999; Chang et al., 2004; Duarte et al., 2000; Kim et al., 2005; Rapp and Wagner, 1986; Rawashdeh et al., 2005; Rivas et al., 2004; Sunna et al., 1997). In this study, a lot of *Microbacterium barkeri*, *Bacillus niabensis*, *B. funiculus*, *B. megaterium*, *Pseudoxanthomonas suwonensis*, *Cupriavidus gilardii*, and *Rhodococcus rhodochrous* strains could produce xylanase as mentioned above. However, our isolated xylanase-producing bacteria were found to be diverse species in soil samples collected in Nan Province, Thailand. *M. barkeri* strain was found in soils collected in Viengsa dis-

Fig. 2. Phylogenetic relationships between MXC4-2-1, MXC4-6-2 and related taxa, based on 16S rRNA gene sequences.

The branching pattern was generated by the neighbor-joining method. Bootstrap percentages above 61%, based on 1,000 replications, are shown at the nodes. Bar, 1 substitution per 100 nucleotide positions.

Fig. 3. Phylogenetic relationships between the tested strains and related *Bacillus* species based on 16S rRNA gene sequences.

The branching pattern was generated by the neighbor-joining method. Bootstrap percentages above 59%, based on 1,000 replications, are shown at the nodes. Bar, 1 substitution per 100 nucleotide positions.

strict while *M. barkeri*, *B. niabensis*, *B. funiculus*, *B. megaterium*, *Px. suwonensis*, *C. gilardii*, and *R. rhodochrous* strains were distributed in soils samples collected in Muang district.

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References

- Ajithkumar, V. P., Ajithkumar, B., Iriye, R., and Sakai, T. (2002) *Bacillus funiculus* sp. nov., novel filamentous isolates from activated sludge. *Int. J. Syst. Evol. Microbiol.*, **52**, 1141–1144.
- Archana, A. and Satyanarayana, T. (2003) Purification and characterization of cellulase-free xylanase of a moderate thermophile *Bacillus licheniformis* A99. *World J. Microbiol. Biotechnol.*, **19**, 53–57.
- 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.
- Beg, Q. K., Kapoor, M., Mahajan, L., and Hoondal, G. S. (2001) Microbial xylanase and their industrial applications: A review. *Appl. Microbiol. Biotechnol.*, **56**, 326–338.
- Breccia, J. D., Kaul, R. H., Castro, G. R., and Sineriz, F. (1999) Isolation of β -xylanase from whole broth of *Bacillus amyloliquefaciens* by adsorption on a matrix in fluidized bed with low degree of expansion. *Bioseparation*, **8**, 273–279.
- Chang, P., Tsai, W-S., Tsai, C-L., and Tseng, M-J. (2004) Cloning and characterization of two thermostable xylanases from an alkaliphilic *Bacillus firmus*. *Biochem. Biophys. Res. Commun.*, **319**, 1017–1025.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K., and Vandamme, P. (1999) Classification of some *Alcaligenes faecalis*-like isolates from the environment and human clinical sample as *Ralstonia gilardii* sp. nov. *Int. J. Syst. Bacteriol.*, **49**, 405–413.
- Duarte, M. C. T., Pellegrino, A. C. A., Ponezi, A. N., and Franco, T. T. (2000) Characterization of alkaline xylanases from *Bacillus pumilus*. *Braz. J. Microbiol.*, **31**, 90–94.
- 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.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.
- Heyrman, J., Vanparys, B., Logan, N. A., Balcaen, A., Rodrigue-Diaz, M., Felske, A., and De Vos, P. (2004) *Bacillus novalis* sp. nov., *Bacillus vireti* sp. nov., *Bacillus soli* sp. nov., *Bacillus bataviensis* sp. nov. and *Bacillus drentensis* sp. nov., from the Drentse A grasslands. *Int. J. Syst. Evol. Microbiol.*, **54**, 47–57.
- Kim, K. K., Park, H. Y., Park, W., Kim, I. S., and Lee, S-T. (2005) *Microbacterium xylanilyticum* sp. nov., a xylan-degrading bacterium isolated from a biofilm. *Int. J. Syst. Evol. Microbiol.*, **55**, 2075–2079.
- Komagata, K. and Suzuki, K. (1987) Lipid and cell wall analysis in bacteria systematics. In *Methods in Microbiology*, Vol. 19, ed. by Colwell, R. R. and Grigorava, R., Academic Press Limited, London, pp. 161–207.
- Kumar, S., Tamura, K., Jakobson, I. B., and Nei, M. (2001) MEGA 2: Molecular evolution analysis software. *Bioinformatics*, **17**, 1244–1245.
- Lee, J. S., Lee, K. C., and Park, Y. H. (2006) *Microbacterium koreense* sp. nov., from sea water in the South Sea of Korea. *Int. J. Syst. Evol. Microbiol.*, **56**, 423–427.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measured with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- Nakajima, T., Tsukamoto, K., Watanabe, T., Kainuma, K., and Matsuda, K. (1984) Purification and some properties of an endo-1,4- β -D-xylanase from *Streptomyces* sp. *J. Ferment. Technol.*, **62**, 269–276.
- Rapp, P. and Wagner, F. (1986) Production and properties of xylan-degrading enzymes from *Cellulomonas uda*. *Appl. Environ. Microbiol.*, **51**, 746–752.
- Rawashdeh, R., Saadoun, I., and Mahasneh, A. (2005) Effect of cultural conditions on xylanase production by *Streptomyces* sp. (strain Ib 24D) and its potential to utilize tomato pomace. *Afr. J. Biotechnol.*, **4**, 251–255.
- Rivas, R., Trujillo, M. E., Sánchez, M., Mateos, P. F., Martínez-Molina, E. M., and Velázquez, E. (2004) *Microbacterium ulmi* sp. nov., a xylanolytic, phosphate-solubilizing bacterium isolated from sawdust of *Ulmus nigra*. *Int. J. Syst. Evol. Microbiol.*, **54**, 513–517.
- Saito, H. and Miura, K. (1963) Preparation of transforming DNA by phenol treatment. *Biochim. Biophys. Acta*, **72**, 619–629.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.
- Somogyi, M. (1952) Notes on sugar determination. *J. Biol. Chem.*, **195**, 19–23.
- Sunna, A., Prowe, S. G., Stoffregen, T., and Antranikian, G. (1997) Characterization of the xylanase from the new isolated thermophilic xylan-degrading *Bacillus thermoleovorans* strain K-3D and *Bacillus flavothermus* strain LB3A. *FEMS Microbiol. Lett.*, **148**, 209–216.
- Suzuki, T. and Yamasato, K. (1994) Phylogeny of spore-forming lactic acid bacteria based on 16S rRNA gene sequences. *FEMS Microbiol. Lett.*, **115**, 13–17.
- Takeuchi, M. and Hatano, K. (1998a) Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins et al. in a redefined genus *Microbacterium*. *Int. J. Syst. Evol. Microbiol.*, **48**, 739–747.
- Takeuchi, M. and Hatano, K. (1998b) Proposal of six new

- species in the genus *Microbacterium* and transfer of *Flavobacterium marinotypicum* ZoBell and Upham to the genus *Microbacterium maritypicum* comb. nov. *Int. J. Syst. Bacteriol.*, **48**, 973–982.
- Tamaoka, J. and Komagata, K. (1984) Determination of DNA base comparison by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.*, **25**, 125–128.
- Tanasupawat, S., Thawai, C., Yukphan, P., Moonmangmee, D., Itoh, T., Adachi, O., and Yamada, Y. (2004) *Gluconobacter thailandicus* sp. nov., an acetic acid bacterium in the α -proteobacteria. *J. Gen. Appl. Microbiol.*, **50**, 159–167.
- Teather, R. M. and Wood, P. J. (1982) Use of Congo red polysaccharide interaction in enumeration of cellulolytic bacteria from bovine rumen. *Appl. Environ. Microbiol.*, **43**, 777–780.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **25**, 4876–4882.
- Vandamme, P. and Coenye, T. (2004) Taxonomy of the genus *Cupriavidus*: A tale of lost and found. *Int. J. Syst. Evol. Microbiol.*, **54**, 2285–2289.
- Venkateswaran, K., Kempf, M., Chen, F., Satomi, M., Nicholson, W., and Kern, R. (2003) *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are γ -radiation resistant. *Int. J. Syst. Evol. Microbiol.*, **53**, 165–172.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. J., Moore, L. H., Moore, W. E. C., Murry, 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. Evol. Microbiol.*, **37**, 463–464.
- Weon, H. Y., Kim, B. Y., Kim, J. S., Lee, S. Y., Cho, Y. H., Go, S. J., Hong, S. B., Im, W. T., and Kwon, S. W. (2006) *Pseudoxanthomonas suwonensis* sp. nov., isolated from cotton waste composts. *Int. J. Syst. Evol. Microbiol.*, **56**, 659–662.
- Wong, K. K. Y., Tan, L. U. L., and Saddler, J. N. (1988) Multiplicity of β -1,4-xylanase in microorganism: Functions and applications. *Microbiol. Rev.*, **52**, 305–317.
- Yoon, J. H., Kang, S. S., Cho, Y. G., Lee, S. T., Kho, Y. H., Kim, C. J., and Park, Y. H. (2000) *Rhodococcus pyridinivorans* sp. nov., a pyridine-degrading bacterium. *Int. J. Syst. Evol. Microbiol.*, **50**, 2173–2180.