

Diversity of Xylanolytic Bacteria Isolated from Thai Sources

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Abstract

Twenty-three xylanolytic bacteria were isolated from soils, sediments and buffalo faeces collected in Thailand. They were divided into 10 groups based on the phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence analyses. Eleven isolates were Gram-positive, facultatively anaerobic, spore-forming, rod-shaped bacteria. They contained *meso*-diaminopimelic in cell wall peptidoglycan. Two isolates (Group IA) were identified as *Bacillus subtilis*, 4 isolates (Group IB) were *B. licheniformis*, 2 isolates (Group IC) were *B. niabensis*, one (Group ID) was *B. nealsonii*, and 2 isolates (Group IE) were *B. cereus*. Seven isolates were Gram-positive, non-spore-forming, rod-shaped bacteria and were identified as *Isoptericola variabilis* (2 isolates in Group II), as *Jonesia denitrificans* (2 isolates in Group III), as *Microbacterium natoriense* (2 isolates in Group IV), and one isolate as *Nocardioides simplex* (Group V). Five isolates were Gram-negative; facultatively anaerobic, non-spore-forming, rod-shaped bacteria and each of them were identified respectively as *Acinetobacter junii* (Group VI), *Aeromonas enteropelogenes* (Group VII), *Pseudomonas stutzeri* (Group VIII), *Stenotrophomonas maltophilia* (Group IX) and *Zobellella denitrificans* (Group X). The isolates produced xylanase activity ranged from 1.03 to 17.65±0.25 unit/ml.

Keywords: *Acinetobacter*, *Aeromonas*, *Bacillus*, *Isoptericola*, *Jonesia*, *Microbacterium*, *Nocardioides*, *Pseudomonas*, *Stenotrophomonas*, *Zobellella*, xylanolytic bacteria

1. Introduction

Xylan, a major component 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; Collins et al., 2005; Rawashdeh et al., 2005). The complete hydrolysis of xylan requires the combined action of various enzymes such as endoxylanase and several accessory enzymes to hydrolyse 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 bacterial strains of *Acidobacterium*, *Aeromonas*, *Bacillus*, *Bacteroides*, *Cellulomonas*, *Microbacterium*, *Paenibacillus*, *Ruminococcus* and *Streptomyces*, *Thermoanaerobacterium* and *Thermotoga*; the yeast strains of *Aureobasidium*, *Cryptococcus* and *Trichosporon*; and the fungal strains of *Acrophialophora*, *Aspergillus*, *Cephalosporium*, *Fusarium*, *Geotrichum*, *Paecilomyces*, *Penicillium*, *Thermomyces* and *Trichoderma* are known to produce different type of xylanases and the nature of the enzymes varies between these different organisms (Rapp & Wagner, 1986; Beg et al., 2001; Abdelwahed et al., 2011).

In recent years, xylanases have received attractable research interest due to their potential for industrial applications, e.g. pretreatment of pulp to boost the bleaching process (Viikari et al., 1994), pretreatment of forage crops and other lignocellulosic biomasses to improve nutrient utilization, flour improvement for bakery products (Butt et al., 2008), saccharification of hemicellulosic wastes (Gilbert & Hazlewood, 1993), pulp and fiber processing (Yang et al., 1995), clarification of juices and wines, extraction of plant oils and coffee (Kulkarni et al., 1999; Maheswari & Chandra, 2000). This work deals with the screening and identification of the

xylanase-producing bacteria isolated from soils and related materials in Thailand based on their phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence similarity.

2. Materials and Methods

2.1 Isolation and Screening of Xylanase Activity

Twenty-three xylanolytic bacteria were isolated from soils, muddy shore sediments, hot spring sediments and buffalo faeces samples collected in Thailand (Table 1), by the spread plate method on XC agar medium as previous report (Kinengam et al., 2007). In this screening step for the thermotolerant strains, the agar plates were incubated at 40°C for 2 days. Xylanase-producing capacity of the cultures was detected by using a Congo red overlay method, as reported previously (Teather & Wood, 1982; Ruijsenaars & Hartsmans, 2000). Isolates showing xylanase-producing capacity were transferred to C agar medium. This medium had the same composition of XC medium apart from the omission of the oat spelt xylan. They were assayed for xylanase activity by using dinitrosalicylic acid (DNS) method and using 1% oat spelt xylan as substrate (Miller, 1959).

2.2 Identification Methods

Cells grown on C agar medium were examined for their morphological and cultural characteristics, including cell shape, colony appearance, endospore formation and pigmentation, after incubation at 37°C for 2 days. Physiological and biochemical characterization was performed using the API 20NE and API 50CH (combined with API 50CHB/E medium) strips (bioMérieux), in accordance with the manufacturer's directions. Catalase and oxidase; hydrolysis of casein, DNA, starch, Tween 80, L-tyrosine and urea; the methyl red/Voges-Proskauer (VP) reactions, indole production, citrate utilization and hydrogen sulfide (H₂S) production were determined as described by Barrow and Feltham (1993). Growth at different pH (5, 6, 7, 8 and 9), in 3 and 5 % (w/v) NaCl and at different temperatures (10, 15, 20, 25, 30, 40, 45, 50, 55 and 60°C) was tested by using C agar medium. All tests were carried out by incubating the cultures at 37°C, except for investigations into the effect of temperature on growth. Diaminopimelic acid in the cell wall and quinone system were determined as described by Komagata and Suzuki (1987). DNA was prepared by the method of Saito and Miura (1963). DNA base composition was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). The 16S rRNA genes of the strains were amplified by PCR with primers, 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACCTTGTTACGACTT-3') and PCR products were purified and sequenced as described previously (Tanasupawat et al., 2004). The sequences of strains were aligned with selected sequences obtained from GenBank by using CLUSTAL_X version 1.83 (Thompson et al., 1997). The alignment was edited manually to remove gaps and ambiguous nucleotides prior to the construction of phylogenetic trees. The phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) method in MEGA4 software (Tamura et al., 2007). The confidence values of branches of the phylogenetic trees were determined using bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets.

3. Results and Discussion

3.1 Isolation and Screening of Xylanase Activity

Twenty-three isolates showed xylanase clear zone with 1.0-12.0 mm in diameter, surrounded their colonies. The xylanase producing bacteria of Group I isolates showed clear zone with 1.7-7.5 mm in diameter and produced xylanase activity ranged from 1.03 to 3.89±0.31 unit/ml while Group II to Group X isolates showed clear zone with 1.0-12.0 mm in diameter and produced xylanase activity ranged from 1.16 to 17.65±0.25 unit/ml. It was found that the isolate CR2-1 in Group II was produced biggest clear zone with 12.0 mm in diameter and had highest xylanase activity (17.65±0.25 unit/ml) as shown in Table 1. In this study, the isolates showed a wide ranges of xylanolytic activity and were better than as reported in the non spore-forming, Gram-positive irregular rods (0-0.13 units/ml) and the isolates of Gram-positive spore-forming rods; the isolates of Gram-negative rods; and isolate of Gram-positive rods/cocci (0-0.17 units/ml) by Kinengam et al. (2007).

3.2 Identification and Characterization of Isolates

Twenty-three isolates were divided into ten groups and were identified based on their phenotypic characteristics and the 16S rRNA gene sequence analyses.

Group I contained 11 isolates, TH2-2, P2-2, SK1-3, PJ1-2, SRC2-3, K3-6, PHC3-3, FCN3-4, NS1-1, K1-6A and K1-6B (Table 1). They were Gram-positive, motile rod-shaped (approximate 0.5-2.0 x 1.8-6.0 µm). Central or subterminal ellipsoidal endospores were observed in swollen sporangia. All isolates showed positive for catalase, growth at pH 7-9, at 25-45°C, hydrolysis of aesculin and DNA but showed negative for Voges-Proskauer (VP), H₂S production, indole production and hydrolysis of Tween 80 and acid production from sorbose. They contained *meso*-diaminopimelic in cell wall peptidoglycan and menaquinones with seven isoprene units (MK-7). They were divided into Group IA to IE based on their phenotypic characteristics (Table 2) and were clustered within a clade of the genus *Bacillus* (Figure 1) based on 16S rRNA gene sequence and phylogenetic analyses.

Group IA contained 2 isolates, K3-6 and SRC2-3. Colonies were 3-6 mm in diameter, round, smooth, raise,

opaque and creamy or white after 2 days of incubation at 37°C on C medium. They grew in 3-5% NaCl, at pH 5-9 and at 25-50°C but no growth at 10 and 60°C. Their differential and variable characteristics were shown in Table 2. On the basis of 16S rRNA gene sequence, isolates K3-6 (926 nt) and SRC2-3 (963 nt) were closely related to *B. subtilis* subsp. *subtilis* KCTC 3135^T (Figure 1) with 100% sequence similarity. Therefore, they were identified as *B. subtilis* subsp. *subtilis* (Nakamura et al., 1999).

Group IB contained 4 isolates, PJ1-2, SK1-3, P2-2 and TH2-2. Colonies were 3-12 mm in diameter, irregular or round, lobate or entire, wrinkled, raise, opaque and creamy or yellow after 2 days of incubation at 37°C on C medium. All isolates grew in 3-5% NaCl, at pH 5-9 and at 25-45°C but no growth at 10 and 60°C. Their differential and variable characteristics were shown in Table 2. On the basis of 16S rRNA gene sequence, the isolates TH2-2 (1,488 nt), P2-2 (971 nt), SK1-3 (927 nt) and PJ1-2 (947 nt) were closely related to *B. licheniformis* KCTC 1918^T (Figure 1) with 99.7, 99.2, 99.1 and 99.6% sequence similarity, respectively. Therefore, they were identified as *B. licheniformis* (Daffonchio et al., 1998; Palmisano et al., 2001).

Group IC contained 2 isolates, K1-6B and K1-6A. Colonies were 3-5 mm in diameter, irregular, curled, concentric, flat, opaque and creamy or white after 2 days of incubation at 37°C on C medium. They grew in 3-5% NaCl, at pH 7-9 and at 25-50°C, but did not grow at pH 5-6, at 10-15, 55 and 60°C. Their differential and variable characteristics were shown in Table 2. On the basis of 16S rRNA gene sequence, the isolates K1-6A (981 nt) and K1-6B (944 nt) were closely related to *B. niabensis* 4T19^T (Figure 1) with 99.9 and 100% sequence similarity, respectively. Therefore, they were identified as *B. niabensis* (Kwon et al., 2007).

Table 1. Location, sample, isolate number, group, xylanase activity and identification of the isolates

Location (Province)	Sample	Isolate no.	Group	Xylanase (unit/ml)*	Identification
Kanchanaburi	Soil	K3-6	IA	2.22±0.35	<i>B. subtilis</i>
Suratthani	Soil	SRC2-3	IA	6.07±0.55	<i>B. subtilis</i>
Prachuapkhirikhan	Muddy shore sediment	PJ1-2	IB	1.21±0.16	<i>B. licheniformis</i>
Samutsongkhram	Soil	SK1-3	IB	1.60±0.18	<i>B. licheniformis</i>
Phetchaburi	Soil	P2-2	IB	2.89±0.02	<i>B. licheniformis</i>
Trat	Soil	TH2-2	IB	3.89±0.31	<i>B. licheniformis</i>
Kanchanaburi	Soil	K1-6B	IC	1.22±0.24	<i>B. niabensis</i>
Kanchanaburi	Soil	K1-6A	IC	1.49±0.17	<i>B. niabensis</i>
Nakhonsithammarat	Soil	NS1-1	ID	1.07±0.03	<i>B. nealsonii</i>
Phetchaburi	Muddy shore sediment	PHC3-3	IE	1.34±0.02	<i>B. cereus</i>
Nakhonnayok	Buffalo faeces	FCN3-4	IE	1.03±0.03	<i>B. cereus</i>
Chiangrai	Hot spring sediment	CR1-2	II	17.65±0.25	<i>I. variabilis</i>
Chiangrai	Hot spring sediment	CR5-1	II	8.10±0.12	<i>I. variabilis</i>
Phetchaburi	Muddy shore sediment	PHX2-5	III	1.21±0.16	<i>J. denitrificans</i>
Nakhonnayok	Buffalo faeces	FXN1-1B	III	1.16±0.13	<i>J. denitrificans</i>
Suratthani	Soil	SRC1-1	IV	1.53±0.03	<i>M. natoriense</i>
Suratthani	Soil	SRC3-3	IV	1.18±0.05	<i>M. natoriense</i>
Suratthani	Soil	SRX2-3	V	1.52±0.02	<i>N. simplex</i>
Suratthani	Soil	SRX2-1	VI	1.30±0.03	<i>Ac. junii</i>
Suratthani	Soil	SRX2-2	VII	1.21±0.10	<i>A. enteropelogenes</i>
Phetchaburi	Muddy shore sediment	PHX3-1	VIII	1.28±0.07	<i>P. stutzeri</i>
	Muddy shore sediment	PHX2-7	X	9.27±0.19	<i>Z. denitrificans</i>
Nakhonnayok	Buffalo faeces	FXN3-1	IX	1.28±0.07	<i>St. matophila</i>

*One unit of xylanase activity was defined as 1 µmol of xylose released per min under the condition assayed.

Group ID contained NS1-1. Colonies were 2-3 mm in diameter, round, concentric, flat, opaque and white after 2 days of incubation at 37°C on C medium. The isolate grew in 3- 5% NaCl, at pH 5-7 and at 15-55°C but did not grow at pH 8-9 and at 10, 60°C. The differential and variable characteristics were shown in Table 2. On the basis of 16S rRNA gene sequence, isolate NS1-1 (1,053 nt) was closely related to *B. nealsonii* FO-092^T (Figure 1) with 99.7% sequence similarity. Therefore, the isolate NS1-1 was identified as *B. nealsonii* (Venkateswaran et al., 2003).

Group IE contained 2 isolates, PHC3-3 and FCN3-4. Colonies were 2.5-6 mm in diameter, round, smooth or

curled, flat, opaque and yellow or white after 2 days of incubation at 37°C on C medium. They grew in 3-5% NaCl, at pH 5-9 and at 10-45°C, but did not grow at 55-60°C. Their differential and variable characteristics were shown in Table 2. On the basis of 16S rRNA gene sequence, isolates FCN3-4 (854 nt) and PHC3-3 (895 nt) were closely related to *B. cereus* IAM 12605^T (Figure 1) with 100 and 99.8% sequence similarity, respectively. Therefore, they were identified as *B. cereus* (Daffonchio et al., 1998).

Table 2. Differential characteristics of *Bacillus* isolates in Group I (A to E)

Characteristics	IA	IB	IC	ID	IE
Growth at pH 5 & 6	+	+	-	w	+
Growth at 10°C	-	-	-	-	+
Growth at 15°C	+(-1)	-	-	+	+
Growth at 50°C	+	+	+	+	-(1w)
Growth at 55°C	+	+	-	w	-
Oxidase	+(-1)	+(-1)	+(-1)	-	-
Citrate utilization	+	+(-2)	-	-	-
Methyl red	-	-	-	+	-
Nitrate reduction	-	-(+1)	-	-	+
L-Arginine hydrolysis	+	+	-	w	+
Casein hydrolysis	+	+	+(-1)	+	+
Gelatin hydrolysis	+	+	+(-1)	w	+(-1)
Starch hydrolysis	+	+(-1)	-	-	-(+1)
L-Tyrosine hydrolysis	-	-	-	-	-(+1)
Urea hydrolysis	+	+	+(-1)	+	+
Acid from					
D-Amygdalin	+(-1)	+	+(-1)		-
L-Arabinose	-	+	+	+	-
D-Cellobiose	+	+(-1)	+(-1)	+	-
D-Fructose	+	+(-1)	-	+	-
D-Galactose	-	-	-	+	-
D-Glucose	+	-(+1)	-	+	+
Gluconate	-	-	-	+	-(+1)
Glycerol	+	-(+1)	-(+1)	+	+
Inositol	-	-	-	+	-
Inulin	+(-1)	-	-	-	-
Lactose	-	-	-	+	-
D-Maltose	+	+	-	+	+
D-Mannitol	+	+	-	+	-
D-Mannose	+	-(+1)	-	+	+(-1)
D-Melibiose	-	-	-	+	-
D-Melezitose	-	-	-	+	-
α -Glucopyranoside	-	+(-1)	-	+	-
Raffinose	-	-	-	+	-
L-Rhamnose	-	-	-	+	-
D-Ribose	-	-(+1)	-	+	+
Salicin	+(-1)	+(-1)	-	+	+
Sorbitol	-	-	+(-1)	+	-
Sucrose	+(-1)	+(-1)	-	+	-(+1)
D-Trehalose	+(-1)	+(-2)	-	+	+
D-Xylose	-	-(w1)	+(-1)	+	-

IA, K3-6 & SRC2-3; IB, PJ1-2, SK1-3, P2-2 & TH2-2; IC, K1-6A & K1-6B; ID, NS1-1; IE, FCN3-4 & PHC3-3. +, positive; -, negative; w, weak positive. Number in parentheses indicates the number of isolate shows positive or negative reaction.

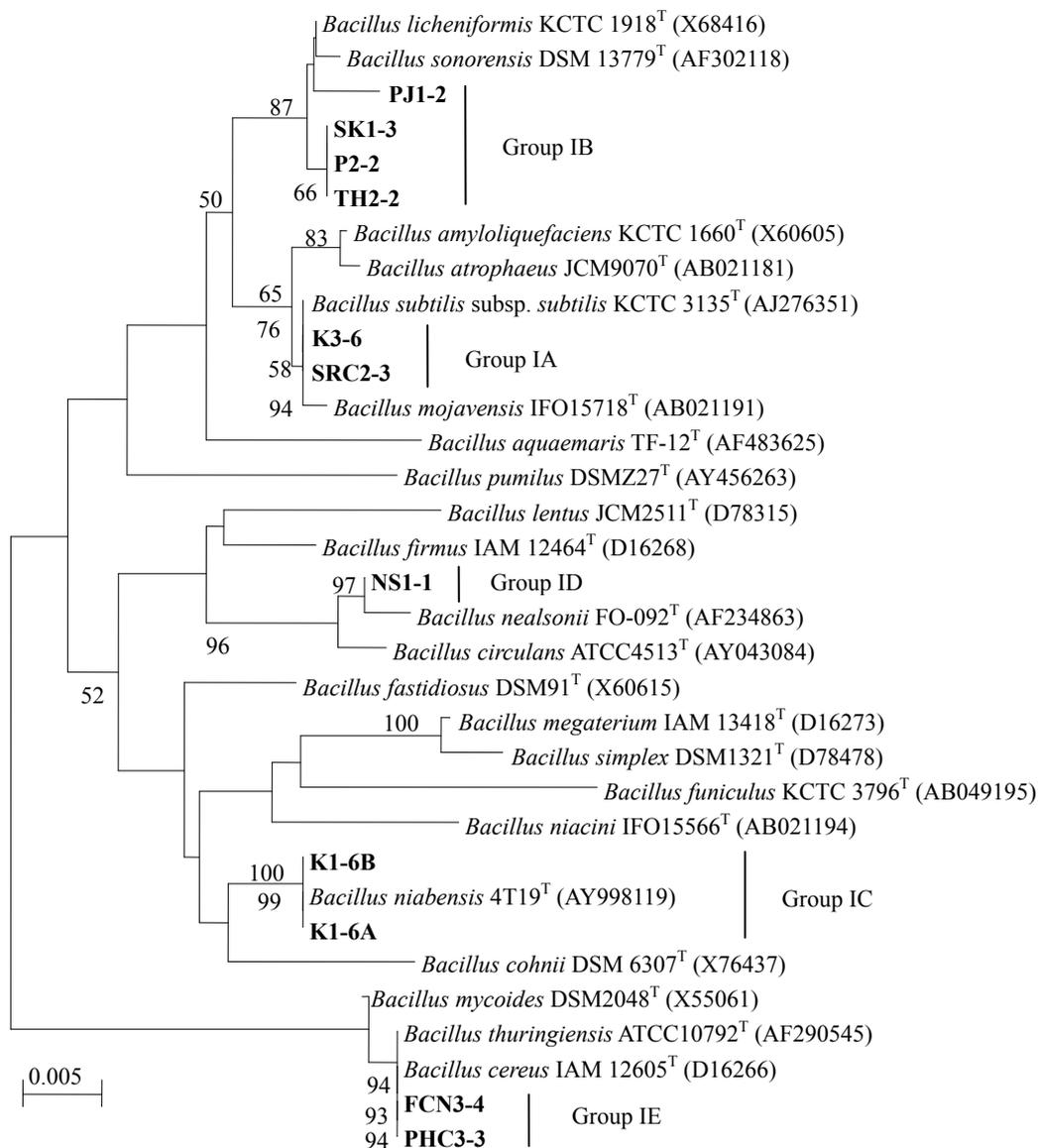


Figure 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between *Bacillus* isolates and *Bacillus* species. Based on 1000 resamplings, bootstrap percentages above 50% are shown. Bar, 0.005 substitutions per nucleotide position

On the basis of 16S rRNA gene-based phylogenetic tree, as shown in Figures 2 and 3, seven isolates were Gram-positive, non-spore-forming, rod-shaped bacteria and were belonged to *Isoperitcola* (2 isolates in Group II), *Jonesia* (2 isolates in Group III), *Microbacterium* (2 isolates in Group IV), and one isolate in *Nocardioidea* (Group V). Five isolates were Gram-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacteria and each of them were belonged respectively to *Acinetobacter* (Group VI), *Aeromonas* (Group VII), *Pseudomonas* (Group VIII), *Stenotrophomonas* (Group IX) and *Zobellella*. All 12 isolates showed catalase positive except Group X isolate. All showed negative for VP, H₂S production, and acid production from D-amylgdalin, gluconate, inositol, inulin, L-rhamnose, sorbitol and sorbose. Their differential characteristics are listed below and in Table 3.

Group II contained 2 isolates, CR1-2 and CR5-1. Cells were Gram-positive, rod or coccoid shaped (approximate 0.8-1.0 x 0.8-4.5 µm), non-spore forming, facultative anaerobic and non-motile. Colonies were 0.5-2.0 mm in diameter, circular, convex, smooth, opaque and yellow or white after 2 days of incubation at 37°C on C medium. They grew in 3-5% NaCl, at pH 7-9 (optimally at 7) and 25-45°C (optimally at 37°C). They did not grow at pH 5 and at 10-15 and 50-60°C. Variable characteristics were shown in Table 3. Their phenotypic characteristics are almost the same as *I. variabilis* MX5^T (data not shown). The isolates CR1-2 (1,006 nt) and CR5-1 (930 nt) were

closely related to each other with 100% 16S rRNA gene sequence similarity and to *Is. variabilis* MX5^T with 99.6% sequence similarity (Figure 2). The isolate CR1-2 contained MK-9(H₄) of menaquinone and 70.0 mol% of DNA G+C content. Based on the phenotypic properties, chemotaxonomic characteristics and 16S rRNA gene sequence, therefore, they were identified as *Isoptericola variabilis* (Stackebrandt et al., 2004).

Group III contained 2 isolates, PHX2-5 and FXN1-1B. Cells were Gram positive, rod shaped (approximate 0.5-1.0 x 1.5-2.0 µm), facultative anaerobic and non-motile. Colonies were 0.5-1.0 mm in diameter, circular, convex, smooth, translucent and yellow after 2 days of incubation at 37°C on C medium. They grew in 3-5% NaCl, at pH 7-9 (optimally at 7) and 15-37°C (optimally at 30°C). They did not grow at pH 5 and at 50-60°C.

Their phenotypic characteristics are almost the same as *J. denitrificans* ATCC 14870^T (data not shown). The isolates FXN1-1B (922 nt) and PHX2-5 (983 nt) were closely related to each other with 99.8% 16S rRNA gene sequence similarity and to *J. denitrificans* ATCC 14870^T with 99.2 and 99.1% sequence similarity, respectively (Figure 2). The isolate PHX2-5 contained MK-9 of menaquinone and 58.4 mol% of DNA G+C content. Variable characteristics were shown in Table 3. Based on the phenotypic properties, chemotaxonomic characteristics and 16S rRNA gene sequence, isolates FXN1-1B and PHX2-5 were identified as *J. denitrificans* (Rocourt et al., 1987).

Group IV contained 2 isolates, SRC1-1 and SRC3-3. Cells were Gram positive, rod shaped (approximate 0.5-1.0 x 0.6-1.5 µm), non-spore-forming and non-motile. Colonies were 0.5-2.5 mm in diameter, circular, convex, smooth, translucent and white after 2 days of incubation at 37°C on C medium. They grew in 3-5% NaCl, at pH 5-9 and 30-45°C, but no growth at 10-25 and 50-60°C. Major menaquinones were MK-11 and MK12. Variable characteristics were shown in Table 3. Their phenotypic and chemotaxonomic characteristics are almost the same as *Microbacterium natoriense* TNJL143-2^T (data not shown). The isolates SRC1-1 (1,401 nt) and SRC3-3 (1,397 nt) were closely related to each other with 100% 16S rRNA gene sequence similarity and to *M. natoriense* TNJL143-2^T with 99.0% sequence similarity (Figure 2). Therefore, based on the results mentioned above and phenotypic properties indicated that SRC1-1 and SRC3-3 were identified as *M. natoriense* (Liu et al., 2005).

Group V contained SRX2-3. Cells were Gram positive, rod or coccoid shaped (approximate 1.0-1.2 x 1.5-6.0 µm), motile, non-spore-forming and strictly aerobic. Colonies were 0.7-1.0 mm in diameter, irregular, flat, smooth, glistening, yellowish-white and opaque after 2 days of incubation at 37°C on C medium. Grew at pH 6-9 (optimally at 7) and 25-37°C (optimally at 30°C), but not growth in 3-5% NaCl, at pH 5 and at 10-15, 45-60°C. Isolate SRX2-3 contained meso-diaminopimelic in cell wall peptidoglycan. MK-8(H₄) was the predominant menaquinone. DNA G+C content was 72.0 mol %. Their phenotypic and chemotaxonomic characteristics are almost the same as *Nocardioides simplex* DSM 20130^T (data not shown). The isolate SRX2-3 (900 nt) was closely related to *N. simplex* DSM 20130^T with 99.3% sequence similarity (Figure 2). Based on the results mentioned above and phenotypic properties indicated in Table 3, the isolate SRX2-3 was identified as *N. simplex* (Yoon et al., 1997).

Group VI contained SRX2-1. Cells were Gram negative, coccobacilli shaped (approximate 0.4-0.7 x 0.5-0.9 µm), facultative anaerobic, non-motile. Colonies were 0.5-1.5 mm in diameter, circular, flat, smooth, yellow and opaque after 2 days of incubation at 37°C on C medium. Grew in 3% NaCl, at pH 5-9 (optimally at 7) and 10-50°C (optimally at 30°C), but not growth in 5% NaCl and at 55-60°C. Isolate SRX2-1 was different in hydrolysis of gelatin, acid production from L-arabinose, glucose, and D-mannose to *Acinetobacter junii* LMG 998^T (data not shown). Predominant ubiquinone of SRX2-1 was Q-9. DNA G+C content was 42.1 mol%. The isolate SRX2-1 (973 nt) was closely related to *A. junii* LMG 998^T with 99.8% sequence similarity (Figure 3). Based on the results mentioned above and phenotypic properties indicated in Table 3, the isolate SRX2-1 was identified as *A. junii* (Bouvet & Grimont, 1986).

Group VII contained SRX2-2. Cells were Gram negative, rod (approximate 0.4-1.0 x 1.0-4.0 µm), facultative anaerobic, motile. Colonies were 1-1.6 mm in diameter, irregular, lobate, flat, yellow and opaque after 2 days of incubation at 37°C on C medium. The isolate grew in 3% NaCl, at pH 5-9 (optimally at 7) and 30-45°C (optimally at 30°C), but did not grow in 5% NaCl, at 10-25 and 50-60°C. Predominant ubiquinone of SRX2-2 was Q-8. DNA G+C content was 57.7 mol%. The isolate SRX2-2 (1,053 nt) was closely related to *A. enteropelogenes* DSM 6394^T with 99.4% sequence similarity (Figure 3). Based on the results mentioned above and phenotypic properties indicated in Table 3, the isolate SRX2-2 was identified as *A. enteropelogenes* (Collins et al., 1993).

Group VIII contained PHX3-1. Cells were Gram negative, straight rod shaped (approximate 0.3-0.5 x 1.5-2.5 µm), facultative anaerobic, motile. Colonies were 0.5-1.0 mm in diameter, circular, raise, smooth, yellowish brown and opaque after 2 days of incubation at 37 °C on C medium. The isolate grew in 3-5% NaCl, at pH 6-9

(optimally at 7) and 10-37°C (optimally at 30°C), but did not grow at pH 5 and at 45-60°C. Predominant ubiquinone of PHX3-1 was Q-9. DNA G+C content was 60.6 mol%. The isolate PHX3-1 (962 nt) was closely related to *Ps. stutzeri* ATCC 17588^T with 99.8% sequence similarity (Figure 3). Based on the results mentioned above and phenotypic properties indicated in Table 3, the PHX3-1 was identified as *P. stutzeri* (Döhler et al., 1987).

Group IX contained FXN3-1. Cells were Gram negative, straight rod shaped (approximate 0.4-0.5 x 1.5-2.0 µm), facultative anaerobic, motile. Colonies were 0.5-1.0 mm in diameter, circular, flat, smooth, yellowish brown and opaque after 2 days of incubation at 37°C on C medium. The isolate grew in 3-5% NaCl, at pH 5-9 (optimally at 7) and 10-37°C (optimally at 30°C), but did not grow at 45-60°C. Isolate FXN3-1 contained Q-8 as predominant ubiquinone and 65.4 mol% of DNA G+C content. The isolate FXN3-1 (923 nt) was closely related to *St. maltophilia* IAM 12423^T with 99.4% sequence similarity (Figure 3). Based on the results mentioned above and phenotypic properties as shown in Table 3, the isolate FXN3-1 was identified as *St. maltophilia* (Palleroni & Bradbury, 1993).

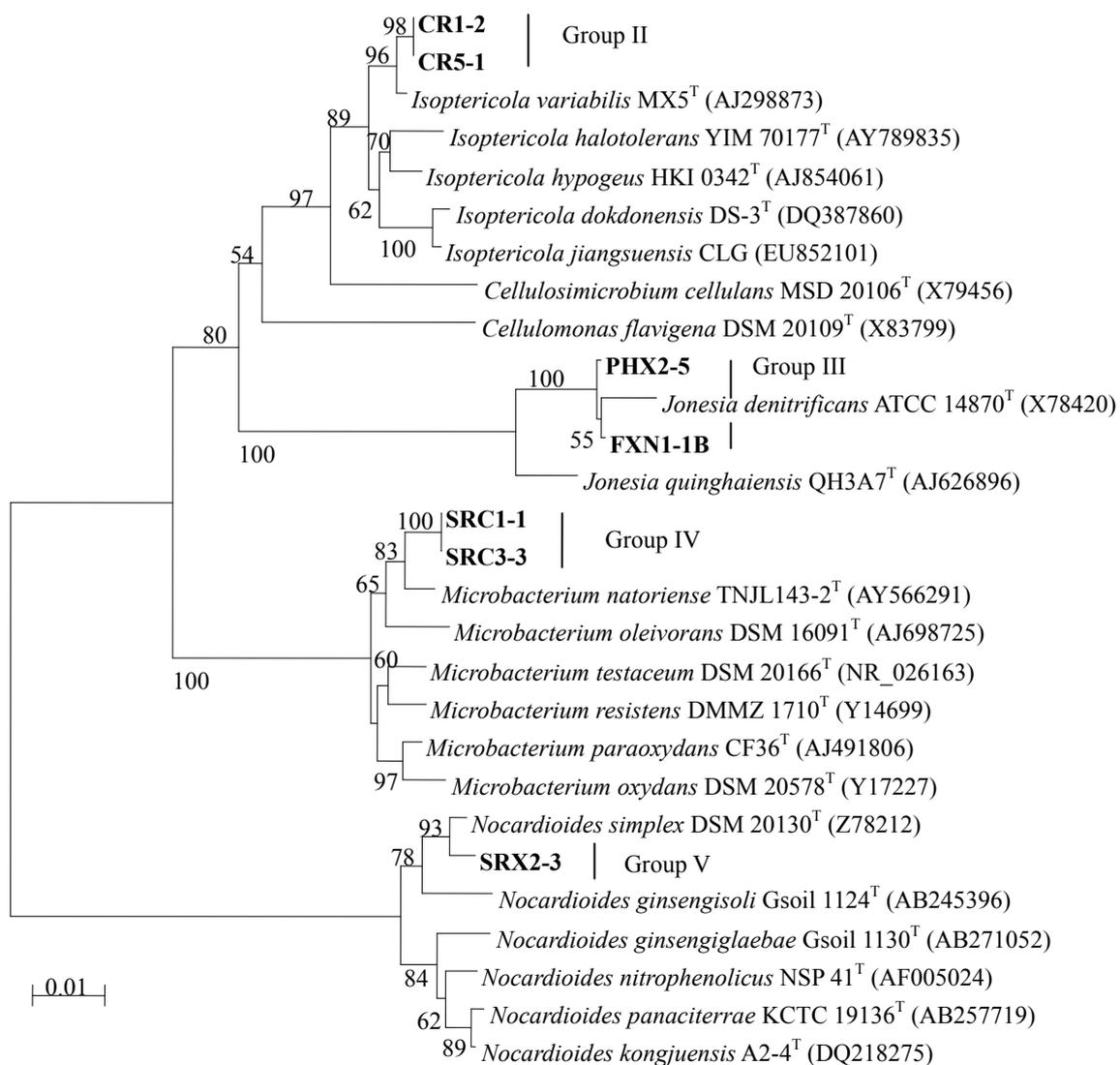


Figure 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between isolates in Group II to V. Based on 1000 resamplings, bootstrap percentages above 54% are shown. Bar, 0.01 substitutions per nucleotide position

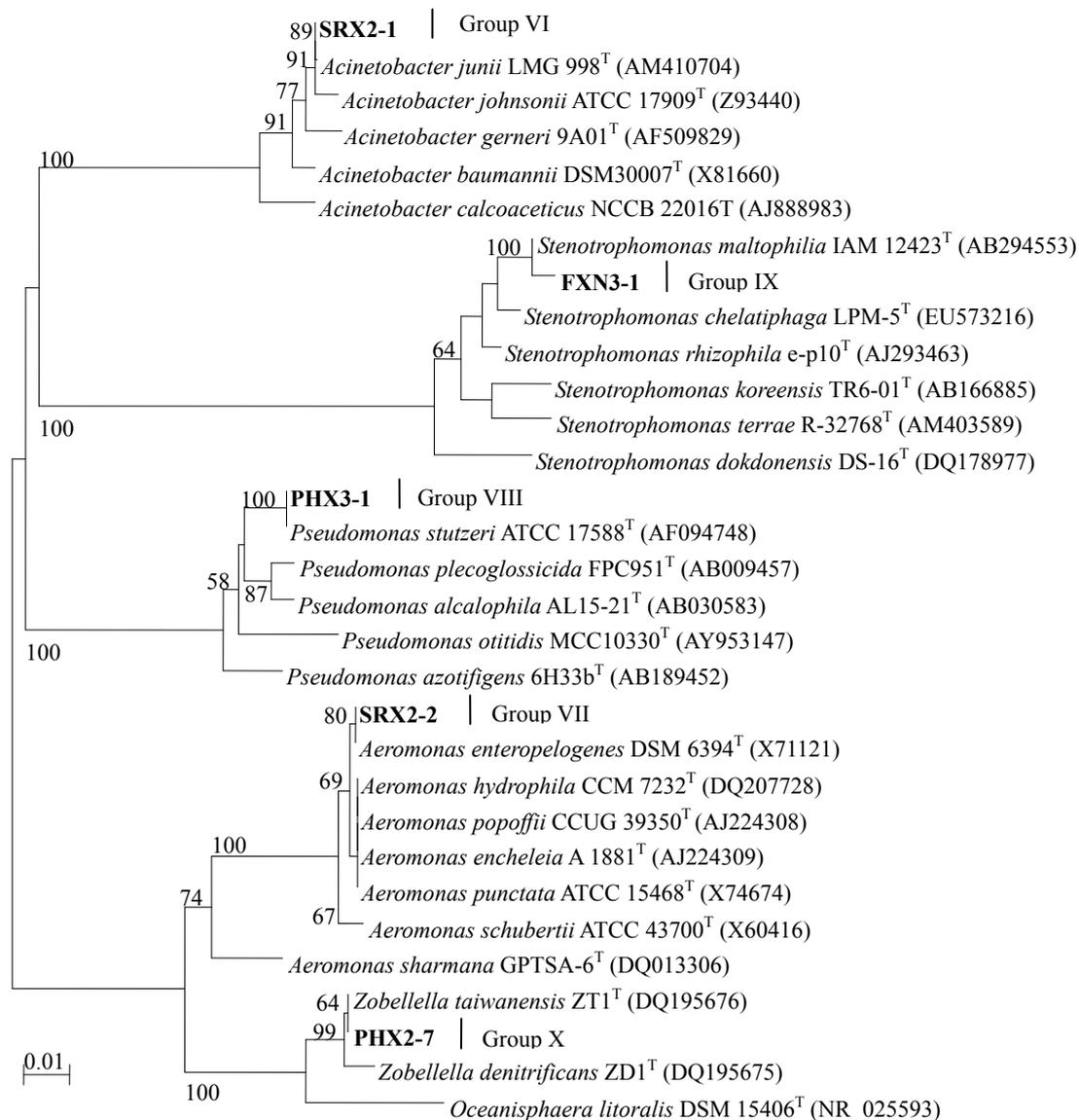


Figure 3. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between the isolates in Group VI to X. Based on 1000 resamplings, bootstrap percentages above 58% are shown. Bar, 0.01 substitutions per nucleotide position

Group X contained PHX2-7. Cells were Gram negative, rod shaped (approximate 0.6-0.7 x 1.5-2.5 μm), facultative anaerobic, motile. Colonies were 1.0-4.0 mm in diameter, circular, raise, smooth, yellow and opaque after 2 days of incubation at 37°C on C medium. The isolate grew in 3-5% NaCl, at pH 5-9 (optimally at 7) and 25-45°C (optimally at 30°C), but did not grow at 10-15 and 50-60°C. This isolate showed negative for catalase. It contained Q-8 of ubiquinone and 61.9 mol% of DNA G+C content. The isolate PHX2-7 (911 nt) was closely related to *Z. denitrificans* ZD1^T and *Z. taiwanensis* ZT1^T with 99.2% and 98.6% sequence similarity, respectively (Figure 3). Based on the results mentioned above and phenotypic properties indicated in Table 3, the isolate PHX2-7 was identified as *Z. denitrificans* (Lin & Shieh, 2006).

Recently, Kinengam et al. (2007) reports that the isolates of *Microbacterium barkeri*, *Bacillus niabensis*, *B. funiculus*, *B. megaterium*, *Pseudoxanthomonas suwonensis*, *Cupriavidus gilardii*, and *Rhodococcus rhodochrous* strains isolated from soil samples collected in Nan Province, Thailand could produce xylanase. They are found to be diverse species. *M. barkeri* strain is found in soils collected in Viengsa district while *M. barkeri*, *B. niabensis*, *B. funiculus*, *B. megaterium*, *Px. suwonensis*, *C. gilardii*, and *R. rhodochrous* strains are distributed in soils samples collected in Muang district. However, in this study, the xylanolytic bacteria, *B. subtilis*, *B. niabensis* and

B. nealsonii isolates are found in soils and *B. licheniformis* isolates are distributed in soil and muddy shore sediment while *B. cereus* isolates are found in muddy shore sediment and buffalo faeces. In addition, the xylanolytic bacteria of *I. variabilis* from hot spring sediment, *J. denitrificans*, *M. natoriense*, *N. simplex*, *Ac. Junii*, *A. enteropelogenes*, *P. stutzeri*, *St. matophila* and *Z. denitrificans* from soils, muddy shore sediment and buffalo faeces are firstly isolated.

Table 3. Differential characteristics of the isolates in Group II to Group X

Characteristics	II	III	IV	V	VI	VII	VIII	IX	X
Growth in 3% NaCl	+	+	+	-	+	+	+	-	+
Growth in 5% NaCl	+	+	+	-	-	-	+	+	+
Growth at pH 5	-	-	+	-	+	+	-	+	w
Growth at pH 6	+(-1)	w (-1)	+	+	+	+	+	+	w
Growth at 10°C	-	+(-1)	-	-	+	-	+	+	-
Growth at 15°C	-	+(-1)	-	-	+	-	+	+	-
Growth at 25°C	+	+	-	+	+	-	+	+	+
Growth at 45°C	+	+	+	-	+	+	-	-	+
Growth at 50°C	-	-	-	-	+	-	-	-	-
Oxidase	w (-1)	w (-1)	-	-	-	+	+	-	+
Citrate utilization	-	-	w (-1)	-	+	-	+	+	+
Indole production	-	-	-	-	-	+	-	-	-
Methyl red	-	+	-	-	-	+	-	-	+
Nitrate reduction	+(-1)	+	-	-	-	-	-	-	-
L-Arginine hydrolysis	+	-	+	+	+	+	+	+	+
Casein hydrolysis	+	+(-1)	w (-1)	+	+	+	-	+	-
DNA hydrolysis	+	+(-1)	+	+	+	+	-	+	-
Gelatin hydrolysis	+	w (-1)	w	+	+	+	-	-	-
Starch hydrolysis	+	+	+	-	-	+	+	-	w
L-Tyrosine hydrolysis	+(-1)	-	+	-	+	+	+	+	+
Tween 80 hydrolysis	+(-1)	-	-	+	-	-	-	-	-
Urea hydrolysis	+	-	+	+	+	+	+	+	+
Acid from									
L-Arabinose	-	+(-1)	-	+	w	w	-	-	-
D-Cellobiose	w (-1)	+(-1)	+	-	-	+(-1)	-	-	-
D-Fructose	+(-1)	+(-1)	+	-	+	+	-	-	+
D-Galactose	-	+(-1)	+	-	+	+	-	-	+
D-Glucose	-	+(-1)	+(-1)	-	+	+	-	-	+
Glycerol	-	-	-	-	+	+	+	-	+
Lactose	-	+(-1)	-	-	-	+	-	-	-
D-Maltose	-	+(-1)	+(-1)	-	+	+	-	-	+
D-Mannitol	-	-	-	-	+	+	-	-	+
D-Mannose	-	+	+	-	+	+	-	-	-
D-Melibiose	-	+(-1)	+	-	-	-	-	-	+
D-Melezitose	-	-	w (-1)	-	-	-	-	-	+
α -Glucopyranoside	-	-	+(-1)	-	-	-	-	-	+
Raffinose	-	-	-	-	-	-	-	-	+
D-Ribose	-	+(-1)	-	-	-	-	-	-	-
Salicin	-	+	-	-	+	+	-	-	-
Sucrose	+(-1)	+(-1)	+	-	+	+	-	-	+
D-Trehalose	w (-1)	-	-	-	+	+	-	-	+
D-Xylose	-	+	w (-1)	-	-	-	-	-	-

II, CR1-2 & CR5-1; III, PHX2-5 & FXN1-1B; IV, SRC1-1 & SRC3-3; V, SRX2-3; VI, SRX2-1 ; VII, SRX2-2; VIII, PHX3-1; IX, FXN3-1; X, PHX2-7. +, positive; -, negative; w, weakly positive. Number in parentheses indicates the number of isolate shows positive or negative reaction.

4. Conclusion

The xylanolytic bacteria isolated from various samples collected in Thailand were identified based on the

analysis of 16S rRNA gene sequence. *B. subtilis*, *B. niabensis* and *B. nealsonii* isolates were found in soils. *B. licheniformis* isolates were distributed in soil and muddy shore sediment and *B. cereus* isolates were found in muddy shore sediment and buffalo faeces. This study, we reported the new finding of the xylanolytic bacteria of *I. variabilis* from hot spring sediment, *J. denitrificans*, *M. natoriense*, *N. simplex*, *Ac. junii*, *A. enteropelogenes*, *P. stutzeri*, *St. matophila* and *Z. denitrificans* from soils, muddy shore sediment and buffalo faeces. These isolates are the most likely source of enzymes and constitute a heterogeneous group of xylanase producing bacteria belonging to different genera. The isolated bacteria that be able to produce extracellular enzymes will provide the possibility to have optimal activities at different temperature and pH. Thus, the applications of the isolates are required for further study.

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