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BIODIVERSITY OF THERMOTOLERANT XYLANASE-PRODUCING BACTERIA FROM SOIL IN NAN PROVINCE

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การคัดแยกและคัดกรองแบคทีเรียทนร้อนที่สามารถผลิตเอนไซม์ไซลาเนสจากดินจังหวัดน่าน พบว่าสามารถแยกแบคทีเรียได้จำนวน 60 ไอโซเลต จากผลการศึกษาลักษณะทางฟีโนไทป์ อนุกรมวิธานเคมีและความคล้ายคลึงของดีเอ็นเอ รวมทั้งการวิเคราะห์ลำดับเบสในช่วง 16S rDNA สามารถพิสูจน์เอกลักษณ์ของแบคทีเรียทนร้อนเหล่านี้ได้เป็น *Microbacterium* 25 ไอโซเลต, *Paenibacillus* 20 ไอโซเลต, *Bacillus* 8 ไอโซเลต, *Rhodococcus* 2 ไอโซเลต, *Cohnella* 3 ไอโซเลต, *Pseudoxanthomonas* 1 ไอโซเลต และ *Cupriavidus* 1 ไอโซเลต ผลการศึกษาอนุกรมวิธานเคมี พบว่ามีกรด *meso*-diaminopimelic เป็นองค์ประกอบในผนังเซลล์ของแบคทีเรียสกุล *Paenibacillus, Bacillus*, *Rhodococcus* และ *Cohnella* แบคทีเรียไอโซเลตที่ทดสอบของ *Microbacterium, Pseudoxanthomonas* และ *Cupriavidus* มีปริมาณ G+C ของ DNA อยู่ในช่วง 70.9- 71.4 โมล% *Paenibacillus, Rhodococcus*, และ *Cohnella* มีปริมาณ G+C ของ DNA อยู่ในช่วง 41.7-61.6 mol% และ *Bacillus* มีปริมาณ G+C ของ DNA อยู่ในช่วง 36-43.9 mol% ตามลำดับ แบคทีเรียในสกุล *Microbacterium* มี menaquinones เป็น MK-11 และ MK-12 *Paenibacillus, Bacillus,* และ *Cohnella* มี MK-7 และ *Rhodococcus* มี MK-8 (H_2) ส่วนแบคทีเรียในสกุล *Pseudoxanthomonas* และ *Cupriavidus* มี ubiquinone-8 (Q-8) ในการศึกษาครั้งนี้พบแบคทีเรียที่รู้สปีชีส์แล้วเป็น *Microbactetrium barkeri* (22 ไอโซเลต), *Paenibacillus favisporus* (1 ไอโซเลต), *P. naphthalenovorans* (1 ไอโซเลต), *Bacillus funiculus* (6 ไอโซเลต), *B. niabensis* (1 ไอโซเลต), *B. megarterium* (1 ไอโซเลต), *Rhodococcus rhodochrous* (2 ไอโซเลต), *Pseudoxanthomonas suwonensis* (1 ไอโซเลต) และ *Cupriavidus gilardii* (1 ไอโซเลต) โดยมีความคล้ายคลึงของลำดับเบสในช่วง 16S rDNA 99.0-99.8% และพบแบคทีเรียทนร้อนสปีชีส์ใหม่ในสกุล *Paenibacillus* (10 species) และ *Cohnella* (3 species) ซึ่งมีความแตกต่างจาก type strains ของแต่ละสกุลทั้งทางด้านลักษณะทางฟีโนไทป์และความคล้ายคลึงของลำดับเบสในช่วง 16S rDNA (93.9-99.2%)

จากแบคทีเรียทนร้อน 60 ไอโซเลตที่แยกได้ พบว่าไอโซเลตที่สร้างไซลาเนสเพื่อย่อยไซแลน (Oat spelt xylan)ได้สูงที่สุดให้วงใสขนาดเส้นผ่านศูนย์กลาง 3.5 ซม. ได้เลือกไอโซเลต S3-4A ที่พิสูจน์เอกลักษณ์ว่าเป็นสปีชีส์ใหม่ในสกุล *Paenibacillus* มาหาภาวะที่เหมาะสมที่สุดของการสร้างไซลาเนส พบว่าไอโซเลต S3-4A สร้างไซลาเนสสูงที่สุด 0.43 หน่วยเอนไซม์/มล.ในน้ำเลี้ยงเชื้อเมื่อเจริญในอาหาร XC ที่ประกอบด้วย (w/v) 1.0% oat spelt xylan, 0.5% peptone, 0.1 % yeast extract, 0.4% K_2HPO, และ 0.05% $MgSO_4.7H_2O$ บ่มที่ 55°C, pH 7.5 เป็นเวลา 5 วัน และภาวะที่เหมาะสมที่สุดต่อกิจกรรมของเอนไซม์ คือ 50 °C, pH 7 โดยมีความเสถียรที่อุณหภูมิช่วง 35- 50°C

ภาควิชา	จุลชีววิทยา	ลายมือชื่อนิสิต……..……..
สาขาวิชา	จุลชีววิทยาอุตสาหกรรม	ลายมือชื่ออาจารย์ที่ปรึกษา……
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SAOWAPAR KINEGAM: BIODIVERSITY OF THERMOTOLERANT XYLANASE-PRODUCING BACTERIA FROM SOIL IN NAN PROVINCE. THESIS ADVISOR: ASSOC. PROF. ANCHARIDA ACHARACHARANYA, PH. D., THESIS CO-ADVISOR: ASSOC. PROF. SOMBOON TANASUPAWAT, PH. D. AND MR. TEERAPATR SRINORAKUTARA, PH. D. 143 pp. ISBN: 974-14-1969-4

Isolation and screening of thermotolerant xylanase producing bacteria, sixty isolates were isolated from soil samples collected in NAN province. On the basis of their phenotypic and chemotaxonomic characteristics, DNA-DNA similarity and the phylogenetic analysis using 16S rDNA sequences, 25 isolates were identified as *Microbacterium*, 20 as *Paenibacillus,* 8 as *Bacillus*, 2 as *Rhodococcus*, 3 as *Cohnella*, 1 as *Pseudoxanthomona*s and 1 as *Cupriavidus*. The tested isolates of *Paenibacillus, Bacillus, Rhodococcus, Cohnella* contained *meso*-diaminopimelic in cell wall-peptidoglycan. The DNA G+C contents of *Microbacterium, Pseudoxanthomonas* and *Cupriavidus* isolates ranged from 70.9 to 71.4 mol%. The DNA G+C contents of *Paenibacillus, Rhodococcus,* and *Cohnella* isolates ranged from 41.7 to 61.6 mol%. *Bacillus* isolates contained 36-43.9 mol% of the DNA G+C content. Predominant menaquinones (MK) of the tested isolates in *Microbacterium* was MK11 and MK-12; *Paenibacillus, Bacillus,* and *Cohnella* were MK-7, and *Rhodococcus* was MK-8 (H_2). The tested isolates of *Pseudoxanthomonas* and *Cupriavidus* contained ubiquinone with eight isoprenoid units (Q-8). This study, the known species were identified as *Microbacterium barkeri* (22 isolates), *Paenibacillus favisporus* (1 isolate), *P. naphthalenovorans* (1 isolate); *Bacillus funiculus* (6 isolates), and *B. niabensis* (1 isolate); *Rhodococcus rhodochrous* (2 isolates), *Pseudoxanthomonas suwonensis* (1 isolate), *Cupriavidus gilardii* (1 isolate) based on 99.0 to 99.8% similarity of 16S rDNA sequences. The novel species were found in *Paenibacillus* (10 new species) and *Cohnella* (3 new species) that they were differentiated from the type strains by several phenotypic characteristics and 16S rDNA sequence similarity (93.9 to 99.2 %).

Among 60 isolates screened, a maximum xylanolytic activity exhibited 3.5 cm in diameter of clear zone. The isolate S3-4A identified as a new species of *Paenibacillus* was selected for xylanase optimization. This isolate produced 0.43 units of xylanase/ ml of culture broth when grown in XC medium containing (w/v) 1.0% oat spelt xylan, 0.5% peptone, 0.1 % yeast extract, 0.4% K_2HPO, and 0.05% $MgSO_4.7H_2O$ at 55 °C, pH 7.5 for 5 days. Optimum activity of this enzyme was at 50 °C and pH 7, and was highly stable at 35-50 °C .

Department	Microbiology	Student' s signature……
Field of study	Industrial Microbiology	Advisor' s signature……
Academic year	2005	Co-Advisor' s signature…….
		Co-Advisor' s signature…….

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LIST OF ABBREVIATIONS AND SYMBOLS

α	=	Alpha
ATCC	=	American Type Culture Collection, Manassas
°C	=	Degree celsius
$CaCl_2$	=	Cacium chloride
CIP	=	Pasteur Institute Collection, Biological resource Center of Pasteur Institute (CRBIP)
cm	=	Centimeter
DAP	=	Diaminopimelic acid
DDBJ	=	DNA Data Bank of Japan
DNase	=	Deoxyribonuclease
DSMZ	=	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EDTA	=	Disodiumethylenediaminetetraacetate
EMBL	=	European Molecular Biology Laboratory
g	=	Gram
Gal	=	Galactose
Glu	=	Glucose
Gly	=	Glycine
GenBank	=	National Institute of Health genetic sequence database
h	=	Hour
HCl	=	Hydrochloric acid
HPLC	=	High performance liquid chromatograhphy
H_2O	=	Water
H_2O_2	=	Hydrogen peroxide
HSCC	=	Research Laboratory, Higeta Shoyu Co., Ltd
H_2SO_4	=	Sulfuric acid

IAM	=	IAM Culture Collection, Center for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, University of Tokyo
IFM	=	Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University
IFO	=	Institute for Fermentation
JCM	=	Japan Collection of Microorganisms
KH_2PO_4	=	Potassium hydrogenphosphate
KNO_3	=	Potassium nitrate
KOH	=	Potassium hydroxide
L	=	Liter
LMG	=	Laboratorium voor Microbiologie, Univversiteit Gent
MEGA	=	Molecular Evolutionary Genetics Analysis
MeOH	=	Methanol
meso-DAP	=	*meso*-Diaminopimelic acid
Min	=	Minute
µg	=	Microgram
mg	=	Milligram
$MgCl_2$	=	Magnesium chloride
µL	=	Microliter
mL	=	Milliliter
µm	=	Micrometer
mm	=	Millimeter
NaCl	=	Sodium chloride
$NaHCO_3$	=	Sodium hydrogencarbonate
NaOH	=	Sodium hydroxide
Na^+	=	Sodium ion
NAG	=	N-acetly glucose amine

NAM	=	N-acetyl muramic acid
NCIMB	=	National Collection of Industrial, Food and Marine Bacteria, NCIMB Ltd., Aberdeen
nm	=	Nanometer
O_2	=	Oxygen
%	=	Percent
PBS	=	Phosphate buffer saline
rDNA	=	Ribosomal deoxynucleic acid
rRNA	=	Ribosomal ribonucleic acid
rpm	=	Round per minute
sec	=	Second
SEM	=	Scanning electron microscope
SDS	=	Sodium dodecylsulfate
sp.	=	Species
SSC	=	Standard sodium citrate
TCA	=	Trichloroacetic acid
TLC	=	Thin layer chromatography
TCA	=	Trichloroacetic acid
UV	=	Ultraviolet
v/v	=	volume / volume
v/w	=	volume / weight

CHAPTER I

INTRODUCTION

Lignocellulose comprises of average 40% cellulose, 33% hemicellulose and 23% lignin by dry weight (Sa-Pereira *et al*., 2002) . Xylan is the most abundant of the hemicelluloses which are heteropolysaccharides having a linear backbone of β-1, 4-linked xylopyranose residues that often have side chains of O-acetyl, arabinosyl and methylglucuronosyl substituents (Rawashdeh *et al*., 2005). The complete hydrolysis of xylan requires the combined action of various enzymes such as endoxylanase (endo-1, 4-β-xylanase, 1, 4-β-D- xylan xylanohydrolase, E.C. 3.2.1.8), β-xylosidase (1,4-β-D-xylan xylohydrolase, E.C. 3.2.1.37), 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).

(Endo)xylanases occur widely in bacteria, yeasts and fungi. Many microorganisms are known to produce different type of xylanases, the nature of the enzymes varies between 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). In addition, xylanase-producing bacteria showing optimal activity at different values of pH and temperature. Several extracellular xylanases from bacteria have been studied and characterized *e.g*., *Bacillus firmus* is capable of growth at pH 10-12 and at above 55°C (Tseng *et al*., 2002), *B. thermantarcticus* , a thermophillic bacterium growth at 80°C (Lama *et al*., 2004), including *B. coagulans* (Womg *et al*., 1988), *B. circulans* (Kyu *et al*., 1994), *B. pumilus* (Duarte *et al*., 2000), *B. subtilis* (Yuan *et al*., 2005), and *B. polymyxa* (Sandhu and Kennedy, 1943). Recently the novel species of *Paenibacillus, P. xylanilyticus* (Rivas *et al*., 2005) and *P. favisporus* (Valazquez *et al*., 2004), *Microbacterium, M. xylanilyticum* (Kim *et al*., 2005) and *M.*

ulmi (Rivas *et al*., 2004); *Cellulomonas xylanticus* (Rivas *et al*., 2004), 1984) and *Ruminococus flavefaciens* (Cotta and Zeltwanger, 1995) were proposed as xylanase producer.

In recent years, xylanases have received attractable research interest due to their potential 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, saccharification of hemicellulosic wastes (Gilbert and Hazlewood, 1993), pulp and fibre processing (Yang *et al*, 1995), clarification of juices and wines, extraction of plant oils and coffee (Kulkarni and Shendye, 1999; Uma Maheswari and Chandra, 2000). However, such applications require xylanase (s) with particular properties, *e.g.* active under high temperature and/or alkaline condition. Bacterial xylanases are generally higher thermostable than fungal xylanases. Most xylanases from fungi have pH optima between 4.5 and 5.5, while bacterial xylanases active at alkaline pH have been reported from *Bacillus* and *Streptomyces* strains (Blanco and Zueco, 1999). Most of industrial processes are carried out at high temperature, so that thermostable enzymes would give an advantage. Nan is a province located in northern part of Thailand. Seventy-five percent of the area is covered by enriched forests where several important rivers of the country are originated. Therefore, soil samples in Nan is interesting and challenging resources for a discovery of novel xylanase-producing bacteria.

The main objectives of this present study are as follows:

1. To isolate and screen thermotolerant xylanase-producing bacteria from soil in Nan province, Thailand.

2. To identify and characterize the xylanase-producing thermotolerant bacteria from soil based on the phenotypic and chemotaxonomic characteristics including DNA-DNA similarity and 16S rDNA sequencing.

3. To optimize the xylanase production of the selected isolate based on the media compositions and cultivation conditions.

CHAPTER II

LITERATURE REVIEW

Xylanase

Lignocellulose, the most abundant renewable resources in nature, is composed of three major groups of polymers, cellulose, hemicellulose and lignin. Cellulose is a linear polymer of β-1, 4-linked D-glucose residues. Hemicellulose is a heteropolymer of pentoses (xylose, aribinose), hexoses (mannose, glucose, galactose), and sugar acids. Lignin is a complex polyphenolic polymer. Hemicellulose represents about 20-35% of lignocellulosic biomass. Xylan, the most abundant hemicellulose, is a heteropolysaccharide with homopolymeric backbone chain of 1, 4-linked β-D-xylopyranose units. About 80% of the xylan backbone is highly substituted with monomeric side-chains of arabinose or glucuronic acid linked to 0-2 and/or 0-3 of xylose residues, and also with oligomeric side chains containing arabinose, xylose, and sometimes galactose residues (Fig.1). The frequency and composition of branches are dependent on the source of xylan. However, unsubstituted linear xylan has been isolated from guar seed husk, esparto grass and tobacco stalk (Saha, 2003).

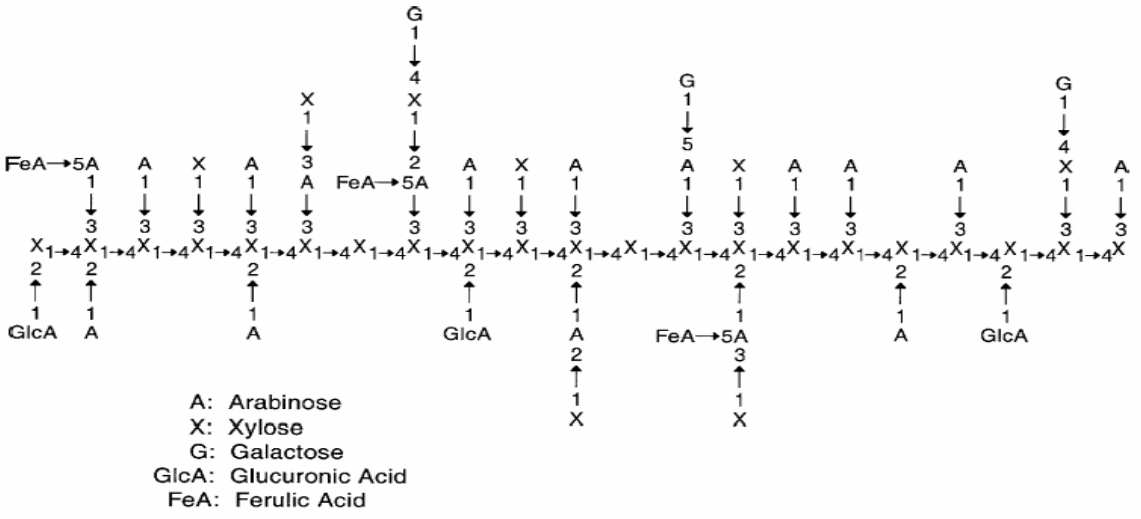

Fig. 1 Schematic structure of corn fiber xylan (Saha, 2003)

1. Classification of xylanase

Due to the heterogeneity and complex chemical nature of xylan, its complete breakdown requires the action of a complex of several hydrolytic enzymes with diverse specificity and mode of action. The xylanolytic enzyme system carrying out the xylan hydrolysis is usually composed of hydrolytic enzymes : β-1,4-endoxylanase (1, 4-β-D-xylan xylohydrolase, E.C. 3.2.1.8), β-xylosidase (1, 4-β-D-xylan xylohydrolase, E.C. 3.2.1.37), α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, and phenolic acid (ferulic and *p*-coumaric acid) esterase (Fig.2). All these enzymes act cooperatively to convert xylan into its constituent sugars.

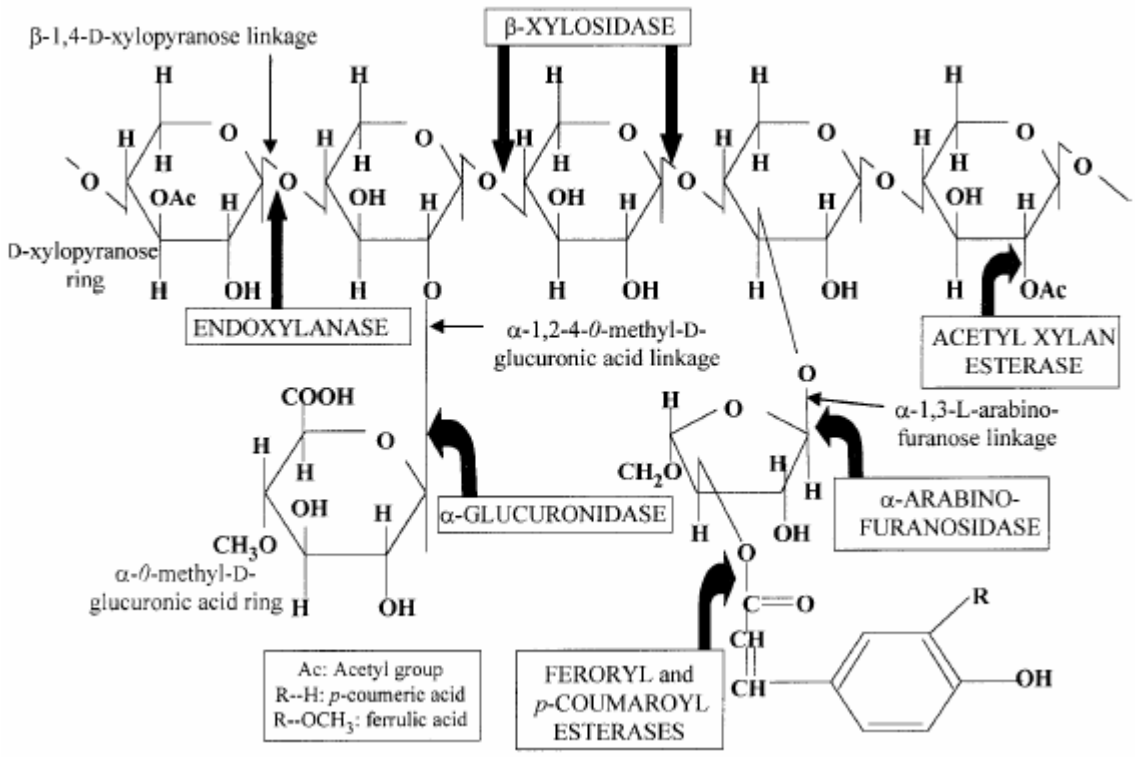

Fig 2. A hypothetical plant xylan structure showing different substituent groups with sites of attack by microbial xylanases (Beg *et al*., 2001)

2. Sources of xylanases from microorganisms

A wide variety of bacteria, fungi, yeasts, and actinomycetes are known to produce xylan-degrading enzymes (Table 1) (Beg *et al*., 2001)

Table 1 Characteristics of xylanases from different microorganisms (*kDa* kilodaltons)

Microorganism	Molecular weight (kDa)	Optimum		Stability		pI	K_m (mg/ml)	V_{max} (μM/mine per mg)	References
		pH	Temperature (°C)	pH	Temperature (°C)				
Bacteria									
Acidobacterium capsulatum	41	5	65	3–8	20–50	7.3	3.5	403	Inagaki et al. 1998
Bacillus sp. W–1	21.5	6	65	4–10	40	8.5	4.5	–	Okazaki et al. 1985
Bacillus circulans WL–12	15	5.5–7	–	–	–	9.1	4	–	Esteban et al. 1982
Bacillus stearothermophilus T–6	43	6.5	55	6.5–10	70	7, 9	1.63	288	Khasin et al. 1993
Bacillus sp. strain BP-23	32	5.5	50	9.5–11	55	9.3	–	–	Blanco et al. 1995
Bacillus sp. strain BP-7	22–120	6	55	8–9	65	7–9	–	–	Lopez et al. 1998
Bacillus polymyxa CECT 153	61	6.5	50	–	–	4.7	17.1	112	Morales et al. 1995
Bacillus sp. strain K-1	23	5.5	60	5–12	50–60	–	–	–	Ratannaka-nokchai et al. 1999
Bacillus sp. NG-27	–	7, 8.4	70	6–11	40–90	–	–	–	Gupta et al. 1992
Bacillus sp. SPS-0	–	6	75	6–9	85	–	–	–	Bataillon et al. 1998
Bacillus sp. strain AR-009	23, 48	9–10	60–75	8–9	60–65	–	–	–	Gessesse 1998
Bacillus sp. NCIM 59	15.8, 35	6	50–60	7	50	4, 8	1.58, 3.50	0.017, 0.742	Dey et al. 1992
Cellulomonas fimi	14–150	5–6.5	40–45	–	–	4.5–8.5	1.25–1.72	–	Khanna and Gauri 1993
Cellulomonas sp. N.C.I.M. 2353	22, 33, 53	6.5	55	–	–	8	1.7, 1.5	380, 690	Chaudhary and Deobagkar 1997
Micrococcus sp. AR-135	56	7.5–9	55	6.5–10	40	–	–	–	Gessesse and Mamo 1998
Staphylococcus sp. SG-13	60	7.5, 9.2	50	7.5–9.5	50	–	4	90	Gupta et al. 2000
Thermoanaerobacterium sp. JW/SL–YS 485	24–180	6.2	80	–	–	4.37	3	–	Shao et al. 1995
Thermotoga maritima MSB8	40, 120	5.4, 6.2	92–105	–	–	5.6	1.1, 0.29	374, 4760	Winterhalter and Liebel 1995
Fungi									
Acrophialophora nainiana	17	6	50	5	50	–	0.731, 0.343	–	Ximenes et al. 1999
Aspergillus niger	13.5–14.0	5.5	45	5–6	60	9	–	–	Frederick et al. 1985
Aspergillus kawachii IFO 4308	26–35	2–5.5	50–60	1–10	30–60	3.5–6.7	–	–	Ito et al. 1992
Aspergillus nidulans	22–34	5.4	55	5.4	24–40	–	–	–	Fernandez-Espinar et al. 1992
Aspergillus fischeri Fxn1	31	6	60	5–9.5	55	–	4.88	5.88	Raj and Chandra 1996
Aspergillus sojae	32.7, 35.5	5, 5.5	60, 50	5–8, 5–9	50, 35	3.5, 3.75	–	–	Kimura et al. 1995

Table 1 (continued)

Microorganism	Molecular weight (kDa)	Optimum		Stability		pI	K_m (mg/ml)	V_{max} (μM/mine per mg)	References
		pH	Temperature (°C)	pH	Temperature (°C)				
Aspergillus sydowii MG 49	30	5.5	60	–	–	–	–	–	Ghosh and Nanda 1994
Cephalosporium sp.	30, 70	8	40	8–10	–	–	0.15	–	Bansod et al. 1993
Fusarium oxysporum	20.8, 23.5	6	60, 55	7–10	30	–	9.5; 8.45, 8.7	0.41, 0.37	Christakopolous et al. 1996
Geotrichum candidum	60–67	4	50	3–4.5	45	3.4	–	–	Radionova et al. 2000
Paecilomyces varioti	20	4	50	–	–	5.2	49.5	–	Kelly et al. 1989
Penicillium purpurogenum	33, 23	7, 3.5	60, 50	6–7.5, 4.5–7.5	40	8.6, 5.9	–	–	Belancic et al. 1995
Thermomyces lanuginosus DSM 5826	25.5	7	60–70	5–9	60	4.1	7.3	–	Cesar and Mrsa 1996
Thermomyces lanuginosus–SSBP	23.6	6.5	70–75	5–12	60	3.8	3.26	6300	Lin et al. 1999
Trichoderma harzianum	20	5	50	–	40	–	0.58	0.106	Tan et al. 1985
Trichoderma reesei	20, 19	5–5.5, 4–4.5	45, 40	3–8.5, 2.5–8.5	–	9, 5.5	3–6.8, 14.8–22.3	–	Tenkanen et al. 1992
Yeast									
Aureobasidium pullulans Y-2311–1	25	4.4	54	4.5	55	9.4	7.6	2650	Li et al. 1993
Cryptococcus albidus	48	5	25	–	–	–	5.7, 5.3	–	Morosoli et al. 1986
Trichosporon cutaneum SL409	–	6.5	50	4.5–8.5	50	–	–	–	Liu et al. 1998
Actinomycete									
Streptomyces sp. EC 10	32	7–8	60	–	–	6.8	3	–	Lumba and Pennickx 1992
Streptomyces sp. B–12–2	23.8–40.5	6–7	55–60	–	–	4.8–8.3	0.8–5.8	162–470	Elegir et al. 1994
Streptomyces T7	20	4.5–5.5	60	5	37–50	7.8	10	7610	Kesker 1992
Streptomyces thermoviolaceus OPC–520	33, 54	7	60–70	–	–	4.2, 8	–	–	Tsujibo et al. 1992
Streptomyces chattanoogensis CECT 3336	48	6	50	5–8	40–60	9	4, 0.3	78.2, 19.1	Lopez-Fernandez et al. 1998
Streptomyces viridisporus T7A	59	7–8	65–70	5–9	70	10.2–10.5	–	–	Magnuson and Crawford 1997
Streptomyces sp. QG-11-3	–	8.6	60	5.4–9.2	50–75	–	1.2	158.85	Beg et al. 2000a
Thermomonospora curvata	15–36	6.8–7.8	75	–	–	4.2–8.4	1.4–2.5	–	Stutzenberger and Bodine 1992

3. Industrial applications

Xylanolytic enzymes from microorganisms have attracted a great deal of attentions, because of their biotechnological potential in various industrial processes such as the following (Beg *et al*., 2001) :

- Biobleaching of cellulosic pulp.
- Cellulose recovery from dissolving pulp in textile industry.
- Pretreatment of forage crops to improve the digestibility of ruminant feeds.
- Saccharification of agricultural, industrial and municipal wastes.
- Ethanol and xylitol production from lignocellulosic biomasses.
- Clarification of must and juices, and liquefying fruits and vegetables.
- Flour improvement in bread and bakery industry.
- Production of alkyl glycosides, a new surfactant, by direct transglycosylation of xylan.

The use of xylanases in biotechnological applications has stimulated the search for enzymes with high temperature optima and/or alkaline pH optima. Fungal xylanases are generally less thermostable than bacterial xylanases. Most xylanases from fungi have pH optima between 4.5 and 5.5. Xylanases from actinobacteria are active at pH 6.0-7.0. However, xylanases with alkaline pH optima have been described from *Bacillus* sp. and *Streptomyces viridosporus.* (Perez *et al*., 2002). The recent works on isolation of xylanase-producing bacteria and actinobacteria, their xylanase-properties, and optimal conditions for the xylanase-production are listed below.

Costa-Ferreira *et al*. (2002) isolated xylanase producing *Bacillus subtilis* from hot-spring. Oat spelt xylan was used as xylanase inducer in culture medium. Optimal xylanase production of about 12 units/ml was achieved at pH 6.0, 50^{o}C within 18 h fermentation. Xylanase production decreased as function of time when xylan was used as substrate. But with trehalose as carbon source, xylanase production maintained constant for at least 80 hrs. Optimal xylanolytic activity was reached at 60^{o}C in

phosphate buffer pH 6.0. The xylanase was completely stable at 60°C for 3 hrs. Under optimized fermentation conditions, no cellulolytic activity was detected. Protein disulfide reducing agents, *e. g.* DTT, enhanced xylanolytic activity about 2.5 fold.

Sasaki *et al*. (2003) isolated cellulase-free xylanase producing actinobacteria, *Streptomyces* sp., from agricultural wastes. Their xylanase produced from cane bagasse was active and stable at temperature of 50-80°C, active at alkaline pH (pH 7-9), and half-life at 70°C, pH 9.0 was 5 hrs.

Uddin and Roy (2004) isolated xylanase producing bacteria, *Paenibacillus* sp., from soil in Bangladesh. The molecular weight of the purified xylanase was 48 kDa. The optimum temperature and pH of the purified enzyme were 55°C and pH 7.0, respectively.

Roy (2004) isolated xylanase-producing *Bacillus* sp. from soil in Bangladesh. The *Bacillus* sp., grown in xylan medium at pH 7.0, produced xylanase at 55 units/ml. Maximal enzyme activity was obtained by cultivation in oat spelt xylan, but high enzyme production was also obtained on wheat bran. The pH optimum and temperature optimum of the xylanase were between pH 6 and 7, and at 50°C (pH 7.0), respectively. The enzyme could not hydrolyse cellulose, carboxymethyl-cellulose and starch.

Mahasneh *et al*. (2005) isolated xylanase producing actinobacteria, *Streptomyces* sp., from soil in Jordan, and studied the effect of some cultural conditions on the xylanase production. Maximal xylanase production was obtained when oat spelt xylan was used as a carbon source. When tomato pomace was used as carbon and nitrogen source, the maximal xylanase production was 1,447 units/ml. The crude enzyme was maximally active at pH 6.5 and 60°C.

Naik *et al*. (2005) isolated thermostable alkaline cellulose-free xylanase producing bacteria, *Bacillus* sp., from sugarcane molass. Xylanase production on various agricultural wastes (wheat bran, rice bran, sugarcane bagasse, ragi hask, gram bran, corncob) in solid-state fermentation by the *Bacillus* sp. was studied. Maximal

xylanase production was observed in rice bran moistened with mineral salt solution at a substrate-to-moisturizing agent ratio of 1:2 (w/v) at 50^{o}C for 72 h. Yeast extract, beef extract and xylan enhanced enzyme production, while glucose, lactose and fructose strongly repressed the production process.

Xylanase-producing of bacteria

Generally, growth of a given species of bacterium occurred most rapidly at a particular temperature : the optimum growth temperature. The rate of growth tails off at temperature above and below the optimum, and for each species there were maximum and minimum temperatures beyond which growth did not occur. Bacteria whose optimum growth temperature was higher than 45^{o}Cwere called thermophiles (Note that some bacteria can tolerate high temperature even though their optimum growth temperature was below 45^{o}C). Bacteria which grew optimally in the temperature range 20-45^{o}C were called mesophiles; they occur in a wide rang of habitats while bacteria which could grow at very low temperature, *e.g*. 0^{o}C and below, were called psychrophile (Brock *et al*, 1984). Although, a wide variety of bacteria were known to produce xylanases, that were involved in the hydrolysis of xylan (Cordeiro *et al*., 2002).

Bacillus

Bacillus species including *B. firmus* (Tseng *et al*., 2002), *B. pumilus* (Duarte *et al*., 2000), *B. subtilis* (Yuan *et al*., 2005), *B. polymyxa* (Sandhu and Kennedy, 1984), *B. coagulans* (Womg *et al*., 1988), *B. thermantarcticus* (Lama *et al*., 2004), *B. circulans* (Kyu *et al*., 1994), *B. licheniformis* (Archana and Satyanarayana, 1997) and *B. stearothermophilus* (Khasin *et al*., 1993) were reported to produce xylanase. They were rod-shaped and straight, 0.5-2.5 × 1.2-10 μm, and arranged in pairs or chains, with rounded or squared ends. Cells were Gram-positive and were motile by peritrichous flagella. Endospores were oval or sometimes round or cylindrical and were

very resistant to many adverse conditions. There was not more than one spore per cell, and sporulation was not repressed by exposure to air. They were aerobic or facultatively anaerobic, with wide diversity of physiological abilities with respect to heat, pH, and salinity. There was chemoorganotrophs, with a fermentative or respiratory metabolisms. Usually, catalase was positive. Found in a wide range of habitats; a few species were pathogenic to vertebrates or invertebrates (Holt *et al*., 1994). The organisms of these genera were characterized by the presence of DAP in the cell wall, by having major menaquinone(MK-7), and by G+C contents of 37-47 mol% (Takeuchi and Hatano, 1998)

Table 2 Characteristics of *Bacillus* species (Venkateswaran *et al*., 2003)

Strain: 1, *B. licheniformis* ATCC 14580T; 2, *B. subtilis* IAM 1026T; 3, *B. pumilus* ATCC 7061T; 4, *B. mycoides* ATCC 6462T; 5, *B. circulans* ATCC 4513T; 6, *B. firmus* ATCC 14575T; 7, *B. nealsonii* FO-92T.

Test	1	2	3	4	5	6	7
Enzyme production:							
β-Galactosidase	+	−	−	+	+	+	+
Arginine dihydrolase	+	−	−	−	−	−	−
Cytochrome oxidase	+	+	+	−	−	−	−
Acetoin production	−	−	+	−	−	−	−
Gelatin liquefication	−	+	−	−	−	−	−
Utilization of:							
Mannitol	−	−	−	−	+	+	−
Amygdalin	−	−	+	−	+	−	−
Fermentation of:							
Glycerol	+	+	+	−	+	+	+
Ribose	+	+	+	−	−	−	−
D-Xylose	−	−	+	+	+	−	+
Adonitol	−	+	−	−	−	−	−
Galactose	−	−	+	−	+	−	+
Rhamnose	−	−	−	−	−	−	+
Inositol	+	+	+	−	+	−	+
Sorbitol	+	+	−	−	+	−	+
N-Acetylglucosamine	+	−	+	+	+	+	+
Lactose	−	−	+	−	−	−	+
Melibiose	−	+	+	−	+	−	+
Melezitose	−	−	−	−	+	−	+
Raffinose	−	+	−	−	−	−	+
Starch	−	+	−	−	+	−	+
Glycogen	−	+	−	−	+	−	−
Gentiobiose	−	+	+	−	+	−	+
D-Turanose	−	+	+	−	+	+	+
D-Lyxose	−	−	−	−	+	−	+
D-Tagatose	+	−	+	+	−	−	+
D-Arabitol	−	−	−	−	−	−	+
Gluconate	+	−	−	−	+	−	+
2-Ketogluconate	−	−	−	−	−	−	+

Bacteriodes

Bacteroides xylanolyticus produced xylanase (Scholten-Koerselman *et al*., 1988). *Bacteroides* strains were rod-shaped organisms of variable size with pleomorphic and showed terminal or central swellings, vacuoles, or filaments, nonmotile, anaerobic, chemoorganotrophic, metabolizing carbohydrates, peptone, or metabolic intermediates. Especially with strongly saccharolytic species, fermentation products include acetate, succinate, lactate, formate, or propionate. Butyrate was not usually a major product, but when it was formed it was accompanied by isobutyrate and isovalerate. Many species contain high level of branched chain fatty acids, generally anteiso-C_{15} acids, and also sphingolipids. Hemin and Vitamin K were highly stimulatory for the growth of many species and were generally added to media for growth of habitats: gingival crevice, intestinal tract (cecum and rumen), sewage sludge, and infective and purulent conditions in human and animals (Holt *et al*., 1994). The organisms of this genus were characterized by having major menaquinone (MK-10) and (MK-11), and by G+C contents of 39-42 mol% (Miyamoto and Itoh, 2000).

Cellulomonas

The strain of *Cellulomonas xylanticus* (Rivas *et al*., 2004), *C. persica* (Elberson *et al*., 2000), *C. terrae* (An *et al*., 2005) and *C. uda* were reported to produce xylanase (Rapp and Wagner, 1986). They were slender, irregular rods, 0.5-0.6 × 2.0-5.0 μm, straight or slightly curved in young cultures; some rods were in pairs at an angle to each other giving V formation; rod occasionally showed branching, but no mycelium was formed. In old cultures, the rods were usually short, and a few cocci occurred. Stain were Gram positive but were easily decolorized, often motile by one or a few flagella, nonsporing, non-acid-fast, facultative anaerobes, but some grew very poorly anaerobically. Growth on peptone-yeast extract agar gives usually convex, yellow colonies. Chemoorganotrophic, the metabolisms are respiratory and also fermentative, giving acid from glucose and various other carbohydrates, both

aerobically and anaerobically. Catalase and cellulolytic were positive. Nitrate was reduced to nitrite. The optimum temperature was 30° C. Widely distributed in soils and decaying vegetable matters (Holt *et al*., 1994). The organisms of these genera were characterized by the presence of L- Orn-D-Glu in the cell wall, by having major menaquinone (MK-8(H_4) and (MK-9(H_4), and by G+C contents of 72-76 mol% (Rivas *et al*., 2004).

Table 3 Characteristics of *Cellulomonas* (Rivas *et al*., 2004)

Species: 1, *C. xylanilytica*; 2, *C. humilata*; 3, *C. biazotea*; 4, *C. cellasea*; 5, *C. fimi; 6*, *C. hominis.*

Characteristic	1	2	3	4	5	6
Shape	Curved rods or coccoid	Diphtheroid or coccoid	Straight or curved rods	Straight or curved rods	Straight or curved rods	Regular short rods
Mycelium	−	+	−	−	−	−
Motility	−	−	+	−	+	+
Catalase	+	−	+	+	+	+
Growth in:						
Acetate	−	−	+	+	−	ND
Gluconate	−	+	−	−	−	+
Lactose	+	+	+	−	+	+
Mannitol	−	+	−	+	−	−
Rhamnose	+	+	+	−	+	+
Hydrolysis of gelatin	W	W	+	−	+	+
Peptidoglycan type*	L-Orn–D-Glu	L-Orn–D-Glu†	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn
Cell-wall sugars‡	Rha, Man, Fuc	Rha, Glc, Fuc	Rha, Gal, Man, 6-deoxy-Tal	Rha, Man, 6-deoxy-Tal	Rha, Fuc, Glc	ND
Principal fatty acids	ai-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$	ND	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{16:0}$	ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$

Clostridium

Clostridium algidixylanolyticum (Broda *et al*., 2000), *C. xylanovorans* (Mechichi *et al*., 1999), *C. xylanolyticum*(Chamkha *et al*., 2001), *C. acetobutylicum* and *C. stercorarium* strains (Wong *et al*., 1988) were reported to produce xylanase. They were rod-shaped, 0.3-2.0 × 1.5-20.0 μm, and were often arranged in pairs or short chains, with rounded or sometimes pointed end, commonly pleomorphic. They were

Gram positive in young cultures, usually motile by peritrichous flagella, form oval or spherical endospores usually distend the cell. Most species were chemoorganotrophic; some were chemoautotrophic or chemolithotrophic as well. May be saccharolytic, proteolytic, neither, or both. Usually they produced mixtures of organic acids and alcohols from carbohydrates or peptones. Did not carry out a dissimilatory sulfate reduction. Usually, catalase was negative and obligately anaerobic; if growth occurred in air, it was scanty and sporulation was inhibited. Metabolically they were very diverse, with optimum temperatures of 10-65°C. Many species produce potent exotoxins, and some were pathogenic for animals because of either wound infections or the absorption of toxins (Holt *et al*., 1994). The organisms of these genera were characterized by the presence of the *meso*-diaminopimelic acid in the cell wall, and by G+C contents of 39-43 mol% (Fardeau *et al*., 2001).

Table 4 Characteristics of *Clostrdium*.

Species: 1, *C. acetobutylicum*; 2, *C. beijerinckii*; 3, *C. saccharoperbutylacetonicum*; 4, *C. saccharobutylicum*.

Characteristic	1	2	3	4
Number of strains	7	16	2	4
Susceptibility to rifampicin	s	(r)	r	s
Riboflavin (yellow pigment) produced in milk*	+	−	−	−
Gelatin liquefaction	+	(−)	+	+
Utilization of:				
Ribose	−	d	−	w
Glycerol	w	w	−	−
D-Arabitol	−	(+)	+	−/+
L-Arabitol	−	(+)	+	−/+
Dulcitol	−	(+)	d	−
Inositol	−	+	d	+
Mannitol	+	+	+	−/+
Sorbitol	d	+	d	−
Melezitose	(−)	+	+	−
Melibiose	(−)	(+)	+	+
Rhamnose	−	(w)	w	−
Trehalose	(−)	+	+	+
Turanose	(w)	+	+	+
Glycogen	+	(+)	+	+
Inulin	d	+	+	+/−
Pectin	+	+	+	−

Microbacterium

Microbacterium species that produced xylanase, such as the strains of *M. ulmi* sp. nov., *M. xylanilyticum* sp. nov. and *M. paludicola* sp. nov had reported (Rivas *et al*., 2004; Kim *et al*., 2005; Park *et al*., 2006). They were slender, irregular rods in young cultures, 0.4-0.8x1.0-4.0 μm, arranged singly or in pair, when some were arranged at an angle to give V formation. Primary branching was uncommon, and mycelia were not produced. In old cultures, rods were shorter and cocci, but there was no marked rod-coccus cycle, Gram-positive, non-acid-fast, nonsporing, nonmotile or motile by one to three flagella. Aerobic; weak anaerobic growth may occur. On yeast extract-peptone-glucose agar, colonies were opaque glistening, often with yellowish pigmentation. Chemoorganotrophic, metabolisms primarily respiratory but might be weakly fermentative. Acid was produced from glucose and some other carbohydrates. Nutritional requirements were complex. Catalase was positive. The optimum growth temperature was 30º C. Found in dairy products, sewage, and insects (Holt *et al*., 1994). The organisms of these genera were characterized by the presence of N-glycolyl residues in the cell wall, by having major isoprenoid quinones MK-11,MK-12 and MK-13 and/ or MK-14, and by G+C contents of 65-76 mol% (Takeuchi and Hatano, 1998).

Table 5 Differential characteristics of *Microbacterium* sp.(Takeuchi *et al*., 1998)

Species	Colour of colony	Motility	Growth		Hydrolysis of:		H_2S produ	VP test	ADH	Assimilation of:					Acid from Glc	Cell wall diamino	Major menaquinone acid
			37 °C	2% NaCl	GEL	STA				ARA	NAc-GlcN	MLT	CIT	PAC			
M. arabinogalactanolyticum	YW	–	–	+	+	+	+	–	+	+	+	+	–	+	–	Orn	MK-12,13
M. arborescens	O	+	–	ND	+	–	+	–	–	+	+	+	+w	–	+	Lys	MK-11,12
M. aurantiacum	O	–	+	+	–	+	+	–	–	d	–	d	–	–	d	Orn	MK-12
M. aurum	YW	–	+	ND	+	+	+	–	–	–	+w	–	–	–	+	Lys	MK-11, 12
M. barkeri	W	+	+	+	+	+	+	–	+	+	+	+	+	–	–	Orn	MK-11, 12
M. chocolatum	O	–	+	+w	–	+w	+	–	–	–	+w	–	–	–	–	Orn	MK-12
M. dextranolyticum	W	–	–	ND	–	–	+	+	–	+	–	+	–	–	+	Orn	MK-11, 12
M. esteraromaticum	YW	+	d	–	–	+	+	–	–	+	–	–	–	–	–	Orn	MK-12, 13
M. flavescens	Y	–	–	+	+	+	+	–	–	+	–	+	–	–	+	Orn	MK-13, 14
M. halophilum	Y	–	+	+	+w	+	–	–	–	–	–	–	–	–	+	Orn	MK-11, 12, 13
M. hominis	YW	–	+	+	–	–	+	+	–	+	+	+	+	–	+	Lys	MK-11, 12
M. imperiale	O	+	+	ND	–	+	+	–	–	+	+w	+	–	–	+	Lys	MK-11, 12
M. keratanolyticum	Y	+	–	+	+	–	+	–	+w	+w	+	–	–	–	–	Orn	MK-12, 13
M. ketosireducens	Y	–	–	+	+	+	+	–	–	+	–	–	d	–	+	Orn	MK-13
M. lacticum	Y	–	–	ND	–	+	–	–	–	+w	+	+	–	–	+	Lys	MK-11, 12
M. laevaniformans	Y/YW	–	+	ND	d	+	+	+	d	–	–	+	–	–	+	Lys	MK-11, 12
M. liquefaciens	Y	–	–	+	+	–	+	–	+	–	+	–	+	–	–	Orn	MK-11, 12
M. luteolum	YW	–	–	–	–	–	+	–	–	+	+	+	–	–	–	Orn	MK-12
M. maritypicum	Y	+	+	+	+	–	–	–	–	–	+	+	+	+	+	Orn	MK-12
M. saperdae	YW	+	–	–	–	+	+	–	+	+	+	+	–	–	+	Orn	MK-11, 12
M. schleiferi	YW	–	+w	+w	–	–	–	+	–	+w	+w	+	–	–	+	Orn	MK-11, 12
M. terrae	Y	–	–	+	+	+	+	–	–	+	–	–	–	–	+	Orn	MK-13, 14
M. terregens	Y	–	–	+w	–	–	–	–	–	–	–	+	–	–	+	Orn	MK-12, 13
M. testaceum	O	+	–	–	+	–	+	–	–	+	+	+	+	+w	–	Orn	MK-10, 11
M. thalassium	Y/LY	–	–	+	d	+	–	–	–	–	–	–	–	–	–	Orn	MK-11, 12
M. trichothecenolyticum	Y	–	–	+w	–	+	+	–	–	–	+	+	+	–	+	Orn	MK-12, 13

Abbreviations: +, all strains positive ; +w, weakly positive ; -, all strains negative; d, differs among strains ; nd, not determined;Y, yellow; YW, yellowish white; LY, light yellow; O, orange; GEL, gelatin ; STA, starch; VP test, Voges-Proskauer test ; ADH, arginine dihydrolase; ARA, arabinose; NAc-GlcN, *N*-acetylglucosamine; MLT, malate; CIT, citrate ; PAC, phenyl acetate; Glu, glucose.

Table 6 Differential characteristics of *M. ulmi* and *M. xylanilyticum*. (Kim *et al*., 2005 ; Rivas *et al*., 2004)

Characteristics	***M. ulmi***	***M. xylanilyticum***
Colony colour	White	Yellow
Motility	-	-
Catalase	-	+
Oxidase	-	+
Hydrogen sulfide	-	-
Chemotaxonomic characteristics:		
Cell wall sugars	Gal, Fuc, Xyl, Rha	Glc, Gal
Major fatty acids	ai-$C_{15:0}$, ai-$C_{17:0}$, ai-$C_{16:0}$	ai-$C_{14:0}$, ai-$C_{15:0}$, ai-$C_{17:0}$, ai-$C_{16:0}$
Major menaquinones	MK-12, MK-13, MK-11, M-14, MK-10	MK-12, MK-13, MK-11
% mol G+C	69	69.7

Paenibacillus

Paenibacillus species that produced xylanase, such as *Paenibacillus faviporus* (Velazquez, 2004), *Paenibacillus xylanilyticus* (Rivas *et al*., 2005) and *Paenibacillus barcinonensis* (Sanchez *et al*., 2005). The genus *Paenibacillus*, which was proposed belong to the family 'Paenibacillaceae' produced ellipsoidal spores in swollen sporangia, are facultatively anaerobic or strictly aerobic rod shaped, and have G+C contents ranging from 45 to 54 mol%. Some of these organisms excrete diverse assortments of polysaccharide-hydrolysing enzymes and produce antibacterial compounds such as polymyxin, octopytin baciphelacin and an antifungal compounds. Cells were Gram-variable, rod-shaped and motile with peritrichous flagella. They produced ellipsoidal spores in swollen sporangia. Strains formed circular, flat, convex, smooth colonies. The major isoprenoid quinone was menaquinone MK-7. The major cellular fatty acid was 12-methyltetradecanoic acid. Cell-wall peptidoglycan contained *meso*-diaminopimelic acid.(Berge *et al*., 2002 ; Lee *et al.,* 2002 ;Takeda *et al*., 2002).

Table 7 Characteristics of *Paenibacillus*.

Species: 1, *Paenibacillus polymyxa* ATCC 842T; 2, *Paenibacillus azotofixans* ATCC 35681T; 3, *Paenibacillus peoriae* LMG 14832T; 4, *Paenibacillus macerans* ATCC 8244T; 5, *Paenibacillus lautus* NRRL NRS-666T; 6, *Paenibacillus amylolyticus* NRRL NRS-290T; 7, *Paenibacillus macquariensis* ATCC 23464T; 8, *Paenibacillus pabuli* NRRL NRS-924T; 9, *Paenibacillus campinasensis* KCTC 0364BPT; 10, *Paenibacillus dendritiformis* T168T; 11, *Paenibacillus illinoisensis* NRRL NRS-1356T; 12, *Paenibacillus chibensis* NRRL B-142T; 13, *Paenibacillus glucanolyticus* DSM5162T; 14, *P. azoreducens* DSM 13822T; 15, *Paenibacillus turicensis* MOL722T; 16, *Paenibacillus graminis* RSA19T; 17, *Paenibacillus odorifer* TOD45T; 18, *Paenibacillus borealis* KK19T;19, *Paenibacillus jam*ilae B.3T; 20, *Paenibacillus terrae* AM141T; 21, *Paenibacillus kribbensis* AM49T; 22, *Paenibacillus brasilensis* PB172T; 23, *Paenibacillus favisporus* GMP01T (Yoon *et al*., 2003)

Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15*	16†	17†	18‡	19§	20‖	21‖	22¶	23
Anaerobic growth	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Optimum growth temp.	30	30–37	30	30	28–30	28–30	20	28–30	40	37	37	37	NR	37	37–42	10–35	5–35	28	30	30	30–37	30–32	37
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	NR	+	−	+	+	+	+	+	+	+	+
Oxidase	−	−	−	NR	−	−	NR	−	−	+	−	−	+	−	−	−	−	−	−	−	−	NR	+
Nitrate reduction	+	−	+	+	+	+	−	−	NR	−	−	+	V	−	−	+	+	−	+	+	+	+	+
Production of:																							
Acetylmethylcarbinol	+	+	+	−	−	−	−	−	NR	−	−	−	NR	−	+	NR	NR	−	+	NR	NR	+	−
Indole	−	−	−	−	−	−	−	−	NR	+	NR	−	NR	−	−	NR	NR	−	NR	NR	NR	NR	−
Dihydroxyacetone	+	−	−	−	NR	NR	−	NR	NR	−	NR	−	NR	−	NR	NR	NR	−	NR	NR	NR	−	−
pH in V–P broth#	4·5–6·8	4·5–5·1	5·5–6·6	4·5–5·0	<5·5	<5·5	<6	<5·5	NR	<6	5·0–5·2	4·6–4·7	NR	4·6–4·7	NR	NR	NR	NR	NR	NR	NR	NR	5·0
Tyrosinase	−	−	−	−	−	−	−	−	NR	−	−	−	NR	−	NR	NR	NR	NR	NR	−	−	NR	−
Caseinase	+	−	+	−	−	−	−	V	+	+	+	−	V	−	NR	−	NR	+	+	+	+	+	−
Amylases	+	−	+	+	+	+	+	+	+	+	+	+	NR	+	NR	+	+	−	+	+	+	+	+
Citrate	−	−	+	V	−	−	−	−	NR	−	−	−	V	−	−	−	NR	−	−	W	W	+	−
Growth at/in:																							
pH 5·6	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	NR	NR	+	+	+	+	+	+
50 °C	−	−	−	+	−	−	−	−	−	−	+	+	NR	+	−	−	−	−	NR	−	−	−	−
5% NaCl	−	−	−	−	+	−	−	V	+	+	−	−	+	−	+	NR	NR	−	−	−	−	−	W
Utilization of:																							
L-Arabinose	+	−	+	+	+	+	−	+	+	−	+	+	V	−	+	+	+	+	+	+	−	−	−
Mannitol	+	+	+	+	+	+	+	+	−	−	+	+	NR	+	−	+	−	V	+	+	+	+	+
D-Xylose	+	−	+	+	+	+	+	+	+	−	+	+	NR	+	+	+	+	+	+	+	−	−	+

Thermotoga.

*Thermotoga maritime*strain was reported to produce xylanase (Beg *et al*., 2001). This bacterium was rod-shaped and had a characteristic outer sheath-like structure which could be observed under in situ conditions. Members of the recently described genera *Geotoga* and *Petrotoga* also possessed this morphological feature and, as determined by a 16S rRNA sequence analysis, were distantly related to members of the *Thermotogales*. Collectively, the five genera mentioned above represented one of the deepest phylogenetic branches in the domain *Bacteria*. These taxa could be differentiated on the basis of their optimum temperatures for growth; *Thermotoga* species were extreme thermophiles that have optimum temperatures for growth of around 80°C, *Thermosipho* and *Fervidobacterium* species had optimum temperatures for growth of 65 to 75°C and were regarded as thermophiles, and *Geotoga* and *Petrotoga* species were moderate thermophiles having optimum temperatures for growth of less than 60°C. Until recently, members of the three genera belonging to the order *Thermotogales* (*Thermotoga*, *Thermosipho*, and *Fervidobacterium*) had been isolated only from volcanic aquatic environments. Different species had different sodium chloride requirements and optimum temperatures for growth. These differences reflected the restricted ecological habitats (hydrothermal marine environments, hydrothermal terrestrial environments) from which the organisms were isolated. Round colonies (diameter, 1 mm) were present after 7 days of incubation at 60°C. Cells were rods (0.5 to 1 by 2 to 3 mm), and each cell had an outer sheath-like structure (toga). The cells occurred singly or in pairs and had peritrichous flagella. The cell wall was Gram negative, as determined by electron microscopy or Gram staining. Chemoorganotrophic and obligately anaerobic members of the domain *Bacteria*. The G+C contents of the DNA was 29-46 mol% (Ravot *et al*, 1995).

Table 8 Characteristics of *Thermotoga.* (Ravot *et al*, 1995).

Species	Type strain	Reference	Outer sheath	Source	Temp range (°C)	Optimum temp (°C)	pH range	Optimum pH	NaCl concn range (%)	Optimum NaCl concn (%)	Generation time (h)	G+C content (mol%)	Reduction of S^0	Flagella	Substrates	Metabolites from glucose fermentation
Thermotoga elfii	DSM 9442	This study	Toga	Oil well	50–72	66	5.5–8.7	7.5	0–2.4	1.2	2.8	39.6	–	Peritrichous	Arabinose, bio-Trypticase, fructose, glucose, lactose, maltose, ribose, sucrose, xylose	Acetate, CO_2, H_2
Thermotoga maritima	DSM 3109	17	Toga	Geothermal heated sea floor	55–90	80	5.5–9	6.5	0.25–3.75	2.7	1.25	46	+	One, subpolar	Galactose, glucose, glycogen, maltose, ribose, starch, sucrose, xylose, yeast extract	L-Lactate, acetate, CO_2, H_2[a]
Thermotoga neapolitana	DSM 4359	21	Toga	Submarine thermal vent	55–90	80	5.5–9	7	ND[b]	ND	0.75	41	+	–	Galactose, glucose, glycogen, lactose, maltose, ribose, starch, sucrose, xylose	ND
Thermotoga thermarum	DSM 5069	41	Toga	Solfataric spring	55–84	70	5.5–9	7	0.2–0.55	0.35	1.25	40	–	Lateral	Glucose, maltose, starch, yeast extract	ND
Petrotoga miotherma	ATCC 51224	9	Toga	Oil well	35–65	55	5.5–9	6.5	0.5–10	3	7	40	+	–	Galactose, glucose, lactose, maltodextrins, maltose, mannose, starch, sucrose, xylose	Acetate, ethanol, CO_2, H_2
Geotoga subterranea	ATCC 51225	9	Toga	Oil well	30–60	45	5.5–9	6.5	0.5–10	4	14	30	+	ND	Galactose, glucose, lactose, maltodextrins, maltose, mannose, starch, sucrose	Acetate, ethanol, CO_2, H_2
Fervidobacterium nodosum	ATCC 35602	35	Spheroid	New Zealand hot spring	41–79	70	6–8	7	Low	0.1	1.75	33.7	ND	ND	Arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, pectin, raffinose, sorbitol, sucrose	Lactate, acetate, ethanol, CO_2, H_2
Fervidobacterium islandicum	DSM 5733	19	Spheroid	Icelandic hot spring	50–80	65	6–8	7.2	Low	0.2	2.5	41	+	ND	Cellulose, glucose, maltose, pyruvate, raffinose, ribose, starch	L-Lactate, acetate, ethanol, CO_2, H_2
Thermosipho africanus	DSM 5309	18	Toga	Marine hydrothermal area	35–77	75	6–8	7.2	0.11–3.6	ND	0.5	29	+	ND	Peptone, yeast extract	ND

Ruminococcus

Ruminococus albus and *Ruminococcus flavefaciens* were reported to produce xylanase (Cotta and Zeltwanger, 1995). They were spherical or slightly elongated, might have pointed ends 0.3-1.5x 0.7-1.8 μm, and arranged in pairs and chain. Might motile with 1-3 flagella per cell, non spore-forming, stain weakly, Gram-positive or Gram-negative, though cell wall structure is of the Gram-positive type. Strict anaerobes requiring special methods for study. Chemoorganotrophs with a fermentative metabolisms, utilizing carbohydrates with the production of mixed acids, ethanol, CO_2 , and H_2. Catalase negative; nitrate was not reduced, and ammonia was not produced from amino acids. Growth occurred at a temperature 20-45°C (optimum 40°C). In habit the rumen, large bowel, and cecum of mammals (Holt *et al*., 1994).

Thermobacillus

Thermobacillus xylanilyticus, a new aerobic thermophilic xylan-degrading bacterium isolated from farm soil situated underneath a manure heap in northern France was reported. This bacterium was aerobic, thermophilic, xylanolytic and spore-forming short rods which stained negative in the Gram test, occurred sometimes in chains. Its spores were ellipsoidal, central to subterminal and occurred in swollen sporangia. It grew at temperatures up to 63 °C and in the pH range 6.5–8.5. When grown on glucose in optimal conditions, its doubling time was found to be 33 min. CO_2 was observed to have a growth-stimulating effect at the start of the culture. In addition to glucose, the isolate utilized xylose, arabinose, mannose, cellubiose, galactose, maltose, sucrose, xylan and starch. Growth was inhibited by 5% NaCl. The G+C contents of strain was 57.5 mol% (Touzel *et al*., 2000)

In addition, Cotta and Zeltwanger (1995) reported the prodeominant species of xylanolytic ruminal bacteria included *Butyrivibrio fibrisolvens, Fibrobacter succinogenes, Eubacterium ruminantium* and *Prevotella ruminicola.* Prem *et al.* (2003) characterized the isolated strains biochemically and found to be *Proteus vulgaris, Proteus mirabilis*, *Citrobacter freundii, Serratia liquefaciens* and *Klebsiella oxytoca*. These bacteria did not digest cellulose and xylan in the diet of the bat. Beg *et al*. (2001) reported that *Acidobacterium capsulatum*, *Micrococcus* sp, AR-135, *Staphylococcus* SG-13 and *Thermoanaerobacterium* JW/SL-YS485 produced xylanases.

CHAPTER III

MATERIALS AND METHODS

Instruments, materials, chemical reagents, and media

Name list of all instruments, materials, chemical reagents were shown in Appendix A.

Methods

1. Screening of thermotolerant xylanase-producing bacteria

1.1 Screening of xylanase-producing bacteria on agar plate

A total of 86 soil samples were collected from Viengsa and Muang districts, Nan province, Thailand (Table 10). Xylanase producing bacteria were isolated from the soil samples not later than 24 hours after collection by an enrichment culture method. The soil sample (0.1 g) was put into a 10 ml of XC medium (Appendix B-2) in 25x250 mm test tube and incubated on a rotary shaker at 200 rpm at 40^{o}C for 2 days. One milliliter of the culture was transferred to fresh XC medium and incubated at the same above conditions for 2 more times. The enriched cultures or their dilutions (0.1 ml) were dropped and spreaded on the XC agar medium and incubated at 40^{o}C for 2 days. Xylanase producing capability of the cultures was screened qualitatively by method described by Teather and Wood (1982). Their colonies grown on XC agar medium at 40^{o}C for 2 days were flooded with 0.1% (w/v) Congo red solution for 1 minute and then washed by 0.1 M NaCl. Colonies surrounded by clearance zone were selected as xylanase producing isolates and then they were purified by streak plate method for further study.

1.2 Quantitative xylanase producing assay

Xylanase producing cultures were inoculated into 10 ml of XC medium in 25x250 cm test tube and incubated on a rotary shaker at 200 rpm at 40^{o}C for 2 days. Three milliliters of the cultures were transferred into 30 ml of XC medium in 250 ml Erlenmeyer flask and incubated at the same aboved conditions for 2 day. Supernatants obtained after centrifugation of the cultures at 4^{o}C, 10,000 rpm (13,300 g) for 15 min were used as crude enzyme for xylanase activity assay.

Xylanase activity assay was done by the method as described by Nakajima *et a.l* (1984). 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^{o}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 Somogyi and Nelson method (1952) using xylose as authentic sugar. After addition of enzyme solution, the reactions were stopped immediately and used as a reaction blank. Protein concentration was analysed by Lowry method (Lowry *et al*., 1951) using bovin serum albumin as standard protein. One unit of xylanase was defined as the amount of enzyme yielding 1 micromole of xylose within 1 min under the assay conditions. Details of the analytic method is described in AppendixC 1-4.

2. Identification methods

2.1 Cell morphology and cultural characteristics

The colonies grown on C agar medium (Appendix1) at 37^{o}C for 1 days were examined for their cell shape and colonial appearance, spore formation, motility, and pigmentation as described by Barrow and Feltham, 1993.

2.1.1 *Gram staining* Thin smear of bacterial colony on a clean slide was fixed by passing through flame and stained with Gram's crystal violet for 30 sec,

rinsed with water, followed by covering with Gram's iodine solution for 30 sec then rinsed with water, decolorized with 95% (v/v) ethanol and washed with water. Then, the smear was counter stained with safranin for 30 sec, blot dried and examined under microscope.

2.1.2 *Flagella staining* Standard microscopic slides, precleaned by the manufacturer, were used. The slide briefly flamed and drawn a thick line with a wax pencil across its width to confine a stain to be two-thirds of the slide surface. Three drops of sterile distilled water was added to this area and gently mixed with cells. There was no visible opalescence. The suspension was smeared over the staining area and then tapped off onto a disinfectant-soaked gauze sponge, and air dried on a level surface. Staining method by Forbes (1981), staining (Appendix C-28) was timed for 1 min with 1 ml of stain at ambient temperature. The slide was washed in tap water, counterstained with the Hucker modification of Gram crystal violet for 1 min, washed, blotted dry, and examined under oil immersion starting near the wax line.

2.2 Physiological and biochemical characteristics

2.2.1 *Oxidase test* A few drops of 1% tetramethyl-p-phenylenediamine were dropped on sterile filter paper disc. The culture tested was then smeared across the moist paper disc with sterile loop. The appearance of dark-purple colour on paper within 30 sec denoted a positive reaction.

2.2.2 *Catalase test* Cells were transferred onto slide, and immediately covered by 3% (v/v) hydrogen peroxide. The evolution of gas bubbles indicated a positive test.

2.2.3 *Growth at different temperature* Cells were inoculated on the C agar medium and incubated at 10^{o}C, 15^{o}C, 20^{o}C, 37^{o}C, 45^{o}C, 50^{o}C, 55^{o}C and 60^{o}C. Growth examination was performed after 5 days.

2.2.4 *Growth at different pH* Cells were inoculated into C broth which pH adjusted to 5, 6, 8, 9 and incubated at 37^{o}C for 5 days.

2.2.5 *Growth in different NaCl concentration* Cells were inoculated on the C agar medium containing 3 and 5% NaCl and incubated at 37°C for 5 days, then the growth was observed.

2.2.6 *L-Arginine hydrolysis* Cells were inoculated onto arginine agar slant (AppendixB-3) and incubated at 37°C for 5 days. A positive reaction is shown by a colour change of the indicator to red.

2.2.7 *Aesculin hydrolysis* Cells were inoculated into aesculin broth (AppendixB-4) and incubated at 37°C for 5 days. Black colour formation indicated a positive test.

2.2.8 *Casein hydrolysis* Cells were inoculated on the C agar medium containing 1% (w/v) skim milk (Appendix B-5) and incubated at 37°C for 5 days. Clear zone surrounded colony indicated tyrosine hydrolysis

2.2.9 *Gelatin hydrolysis* Cells were inoculated onto gelatin medium (AppendixB-6) and incubated at 37°C for 5 days then flooded the surface with 5-10 ml of 30% (v/v) trichloroacetic acid. Clear zone surrounded colony indicated the hydrolysis.

2.2.10 *Methyl red and Voges-Proskauer* Cells were inoculated into MR-VP broth (Appendix B-17) and incubated at 37°C for 5 days. The culture broth was mixed with methyl red reagent, red colour indicated a MR positive test. After added 5% α-naphthol solution and 40% KOH solution, strong red colour indicated a VP positive test.

2.2.11 *Starch hydrolysis* Cells were inoculated onto 10% starch agar medium (AppendixB-9) and incubated at 37°C for 5 days, then flooded with Lugol's iodine solution (Appendix C-30). Clear colourless zone surrounded colony indicated starch hydrolysis.

2.2.12 *Tyrosine hydrolysis* Cells were inoculated onto the C agar medium containing 0.5% (w/v) tyrosine (Appendix B-11) and incubated at 37°C for 5 days. Clear zone surrounded colony indicated tyrosine hydrolysis.

2.2.13 *Deoxyribonuclease (DNase) activity* Cells were inoculated on DNase test agar (Appendix B-12) and incubated at 37°C for 5 days then flooded with 1 N HCI. Clear zone surrounded colony indicated a positive test.

2.2.14 *Indole test* Cells were inoculated into tryptone broth (Appendix B-13) and incubated at 37°C for 5 days. The culture broth was mixed with Kovac's reagent (Appendix C-29). Red colour indicated a positive test.

2.2.15 *Nitrate reduction* Cells were inoculated into nitrate broth (AppendixB-14) and incubated at 37°C for 5 days, then one drop each of Solution A and Solution B of nitrate reduction test reagents (AppendixC-7) were added. Red colour developed within 5 minutes indicated a positive test.

2.2.16 *Simmon Citrate test* Cells were inoculated into citrate agar slant (AppendixB-8) and incubated at 37°C for 5 days. Blue colour formation indicated a positive test.

2.2.17 *Triple Sugar Iron agar (TSI)* Cells were inoculated into TSI agar slant (AppendixB-16) and incubated at 37°C for 5 days. Black colour formation indicated a positive test.

2.2.18 *Anaerobic growth* Cells were inoculated on the C agar medium and incubated at 37°C for 5 days in an anaerobic jar, then the growth was observed.

2.2.19 *Dihydroxyacetone from glycerol* Cell were inoculated on the C agar medium contain 1% glycerol and incubated at 37°C for 5 days, then flooded with Fehling's solution (AppendixC-27). Yellow colonial appearance indicated a positive test.

2.2.20 *Urease activity* Cells were inoculated onto the C agar slant medium containing urea 2% (w/v) (AppendixB-16). A positive reaction is shown by a colour change of the indicator to pink.

2.2.21 *Acid from carbohydrates* The acid from carbon sources were performced in C broth containing 22 different kinds of carbon sources, 0.5% (w/v) including L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol,

inulin, lactose, *myo*-inositol, maltose, D-mannitol, mannose, melibiose, melezitose, raffinose, rhammose, D-ribose, salicin, sucrose, D-sorbitol, D-trehalose, D-xylose. The media were adjusted to pH 7.2 and phenol red 0.2% solution (w/v) was added as an indicator. Cells were inoculated into C broth containing each kind of carbon sources and incubated at 37^{o}C for 5 days. The colour of culture broth changed from red to yellow will be indicated as a positive result.

2.3 Chemotaxonomy

2.3.1 *Cell wall analysis* Whole-cell hydrolysis. Approximate 3 mg of dried cells were hydrolysed with 1 ml of 6 N HCI in a screw-capped tube at 100^{o}C for 18 hours. After cooling, the hydrolysate was filtered and dried to dryness by a rotary evaporator. The dried material was dissolved in 1 ml of water and repeat drying. The residue was redissolved in 0.3 ml of water and analysed by thin-layer chromatography (TLC). Each samples was applied as 3 µl on the base line of a plastic cellulose TLC plate (Merck No. 5577, E. Merck, Darmstadt, FRG). One µl of 0.01 M DL-diaminopimelic acid (DAP) was applied as reference. TLC was developed with methanol-water-6N HCI-pyridine (80:17.5:1.5:10, v/v) system which last 3 hours or more, then visualized by spraying with 0.2% (w/v) ninhydrin in water-saturated n-butanol followed by heating at 100^{o}C for 5 min. DAP isomers appeared as dark-green spots and the developed spot gradually disappeared in a few hours.

2.3.2 *Quinone analysis* Dried cells (100-500 mg) were extracted with chloroform:MeOH (2:1) for a few hours. The suspension was then filtered and dried under rotary evaporator. The dried sample was dissolved with a small amount of acetone and applied onto a silica gel TLC (Merck no.1.05744). The applied TLC was then developed with petroleum ether-diethyl ether system (85: 15, v/v) and the band of menaquinone was detected by using a UV lamp (254 nm). The menaquinone band was scraped and dissolved with HPLC acetone. The suspension was filtered and dried it up with N_2 gas. The menaquinone sample was analyzed by HPLC employing methanol-

isopropanal (4:1) with the μ-BondapakC_{18} column (Water Associates, Milford, Mass., USA).

2.3.3 *DNA base composition* DNA was isolated by the method described by Saito and Miura (1963). Briefly, log phase cells grown in the complex agar medium at 37°C for 1 day were harvested by scraping and suspended in 10 ml of saline-EDTA buffer pH 8.0 (Appendix C-10). Bacterial cell lysis was induced by 20 mg/ml lysozyme in 0.1 M Tris buffer pH 9.0 and 10% (w/v) sodium dodecyl sulfate (SDS) at 55°C for 10 min. After cell lysis, the suspension became turbid to opalescent and viscous. Protein was denatured by extracting with 4 ml of phenol-chloroform (1:1 v/v) for 30 sec, and then centrifuged at room temperature, 12,000 rpm (9,200 g) for 10 min. DNA was precipitated by adding cold 95% (v/v) ethanol into supernatant. DNA was spooled with a grass-rod, rinsed with 70% then 95% (v/v) ethanol, air dried, then dissolved in 5 ml of 0.1 x SSC. RNase A solution (0.3 ml) was added into the DNA solution, incubated at 37°C for 20 min, then extracted by 2 ml of phenol-chloroform. After centrifugation at room temperature, 12,000 rpm (9,200 g) for 10 min, the upper layer was transferred to new tube. The DNA was precipitated by adding cold 95% (v/v) ethanol and spooled with a grass-rod then rinsed with 70% then 95% (v/v) ethanol. DNA was air dried and dissolved in 5 ml of 0.1 x SSC. The purity and quality of DNA were determined from the ratio of an absorbance at 260 and 280 nm (A_{260}/A_{280}) as described by Marmur and Doty (1962).

DNA base composition was analyzed by the method described by Tamaoka and Komagate (1984), DNA was hydrolysed into nucleosides by nuclease P1 (EC 3.1.3.30) and alkaline phosphatase (EC 3.1.3.1). DNA sample (0.5-1.0 g of DNA/litre of distilled water ; OD_{260} = 10-20) was boiled in boiling water bath for 5 min and immediately cooled in ice water. Ten microliter of the DNA was mixed with 10 μl of nuclease P1 solution (Appendix C-20), incubated at 50°C for 1 hour, and then 10 μl of alkaline phosphatase solution (Appendix C-21) was added and keep at 37°C for 1 hour.

DNA base composition of DNA hydrolysate was analyzed by HPLC using conditions as shown in Table 9

Table 9. HPLC conditions for DNA base composition analysis

Detector wave length	270 nm
Column	Nakarai Cosmosil packed column $5C_{18}$ (150x4.6 mm)
Column temperature	Room temperature
Eluent	0.2 M $NH_4H_2PO_4$: acetonitrile (20:1, v/v)
Flow rate	1 ml/min
Sample	5-10 μl

2.4 16S rDNA sequence and phylogenetic analysis

The 16S rRNA gene was PCR amplified using 9F (5'GAGTTTGATCCTGGCTCAG'3, *Escherichia coli* numbering) as forward primer, and 1541R (5'AAGGAGGTGATCCAGCC'3) as reverse primer. Sequence of the amplified product was analyzed by an automated DNA sequencer, ABI PRISM 377 Genetic analyzer (Applied Biosystems) using the following primers:339F (5'CTCCTACGGGAGGCAGCAG'3),785F (5'GGATTAGATACCCTGGTAGTC'3), 1099F (5'GCAACGAGCGCAACCC'3), 357R (5'CTGCTGCCTCCCGTAG'3) and 802R (5'TACCAGGGTATCTAATCC'3). The DNA sequence was multiply aligned by CLUSTAL X program (version 1.83; Thompson *et al*., 1994), then the alignment was manually verified and edited prior to construction of a phylogenetic tree. The phylogenetic tree was constructed by neighbour-joining method (Saitou and Nei, 1987) in MEGA program version 2.1 (Kumar *et al*., 2001). The confidence values of branches of the phylogenetic tree were determined by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The values for sequence similarity among the closest strains were calculated manually after pairwise alignments obtained using the

CLUSTAL X version 1.83. Gap and ambiguous nucleotides were eliminated from the calculations.

2.5 DNA-DNA hybridization (Ezaki *et al*., 1989)

DNA-DNA hybridization ; Under optimal condition, 100 µl of heat-denatured, purified DNA solution of unknown and type strains (10 µg of DNA/1ml of phosphate buffered saline containing 0.1 M $MgCl_2$) were incubated at 37°C for 2 hours in microdilution plates (Nunc Corp., Denmark). Photobiotinylation of DNA was performed by mixing 10 µg/ ml of photobiotin with an equal volume of DNA solution (10 µg of DNA/ ml), and then irradiated by sunlamp (500 W) for 25 min. After irradiation, free photobiotin was removed by n-butanol extraction. The biotanylated DNA was used for hybridization immediately.

For quantitative detection of biotinylated DNA in microdilution wells, 200 µl of a prehybridization solution (20xSSC, 5% (v/v) Denhardt solution, 50% (v/v) formamide) containing 10 µg of denatured salmon sperm DNA/ml was added to microdilution plates and incubated at 37°C for 1 hr. Then, the prehybridization solution was discarded and replaced by 100 µl of hybridization mixture (20xSSC, 5% (v/v) Denhardt solution, 3% (w/v) dextrane sulfate, 50% (v/v) formamide, 10 µg of denatured salmon sperm DNA/ml) containing 10 µg of bitinytlated DNA. The microplates were then covered with aluminium foil, and incubated overnight (16 hours) at 52°C for Group 1 and 40°C for Group 8. After hybridization, the microdilution wells were washed three times by 200 µl of 0.2xSSC buffer, and 100 µl of streptavidin peroxidase conjugate solution (Boehringer, Germany) was added, and incubated at 37°C for 30 min. After incubation, the wells were washed three times by phosphate buffered. Then the enzyme solution was discarded and 100 µl substrate, 3.3',5.5' – tetramethyl benzidine – H_2O_2 solution (Wako, Japan), was added to each well. The plates were incubated at 37°C for 10 min. The reaction was stopped by addition of 2 M H_2SO_4 and color intensity was measured by Microplate Reader Bio-Rad, Model 3350,

(CA,USA) at wavelength of 450 nm. The homology values for the DNA-DNA hybridization were calculated. In practice, a DNA-homology above 70% indicates a relationship in the species level as reported by Wayne *et al*. (1987).

3. Optimization of crude xylanase production

Xylanase producing bacterial cultures inoculated in 10 ml of the xylan medium in 25x200 cm test tube and incubated on a rotary shaker at 200 rpm at 40^{o}C for 2 days was used as inoculum. Three milliliters of inoculum were transferred into 30 ml of the xylan medium in 250 ml Erlenmyer flask and incubated as mention aboved. The influence of medium composition, initial pH, incubation temperature on xylanase production was determined by varying each parameters. An optimum condition of prior experiment was used as the basis in the later experiment to optimize the conditions. Cell-free supernatant taken at different times were assayed for xylanase activity.

4. Characterization of crude xylanase

Xylanase activity was determined by the method described by Nakajima *et al*. (1984) as mention above. The influence of incubation temperature on xylanase activity was determined by varying each parameters. The temperature stability of crude enzyme were carried out by incubation of xylanase at various temperatures for 30 min before an analysis of xylanase activity.

CHAPTER IV

RESULTS AND DISCUSSIONS

1. Screening of thermotolerant xylanase producing bacteria

1.1 Screening of thermotolerant xylanase producing bacteria on agar plate

The thermotolerant xylanase-producing bacteria were isolated from 86 soil samples in Viengsa and Muang districts, Nan province, Thailand, using the enrichment culture method and incubated at 40^{o}C. Sixty isolates showing clear zone surrounded colonies grown on xylan complex (XC) medium(Table 11).

Table 10 Location, sample number, and the number of isolates obtained.

Location	Sample no.	No. sample
Viengsa district :	X1, X2, X3, X4, X5, X6, X7, X8, X9, X10 X11, X12, X13, X14, X15, X16, X17, X18, X19, X20	20
Muang district :	MX1, MX2, MX3, MX4, MX5, MX6, MX7,MX8, MX9, MX 10, MX 11, MX12, MX13, MX 14, MX15, MX16, MX17, MX18, MX19, MX20, MX21 MXC1-1, MXC 1-2, MXC1-3, MXC1-4, MXC1-5, MXC1-5, MXC1-6, MXC1-7 MXC2-1, MXC2-2, MXC2-3, MXC2-4, MXC2-5, MXC2-6, MXC, MXC2-7 MXC2-8, MXC 2-9, MXC2-10, MXC2-11, MXC2-11, MXC2-12, MXC2-13 MXC3-1, MXC3-2, MXC3-3, MXC3-4, MXC3-5, MXC3-6, MXC3-7, MXC3-8, MXC3-9, MXC3-10, MXC3-11 MXC4-1, MXC4-2, MXC4-3, MXC4-4, MXC4-5, MXC4-6, MXC4-7, MXC 4-8, MXC4-9 S1, ST1, ST2, SF	66
Total		**86**

1. 2 Xylanase activity assay

Twenty-five (Group1) isolates produced xylanase activity ranged 0-0.15 units/ml (Fig.3) while Group 2, 3, 4, and 5 (20 isolates) produced 0-0.2 units/ml (Fig.4) and Group 6, 7, 8, 9, 10, 11, and 12 produced 0-0.17 units/ml (Fig.5). Strain S3-4A was found to produce a maximal xylanase at 0.20 units/ml. Therefore, this isolate was selected for further study (Fig.4).

2. Identification of isolates

2.1 Cell and cultural morphological characteristics

A total of sixty isolates were divided into 12 different groups based on their morphological, cultural, physiological and biochemical characteristics. Twenty-five isolates (Group 1) were non-sporing, Gram-positive irregular rods. Colonies were circular, low convex, entire margins, opaque, moist and yellowish white. Twenty isolates (Group 2, 3, 4, 5, and 9) were Gram-positive or Gram-variable. All showed circular/oval, convex/flat/raise, entire/ undulate margins, opaque, moist, yellow and yellowish white colour colonies. Eight isolates (Group 6, 7 and 8) were Gram-positive. They showed irregular, rough, crateriform, opaque and cream or light brown colour colonies. One isolate (Group 10) was Gram-negative and showed circular, convex, entire margins, opaque, viscid, yellow or orange colour colonies. One isolate of Gram-negative (Group 11) showed circular, convex, entire margins, glistening, mucoid, off-white colour colonies. The last Gram-positive rods/cocci (Group 12, 2 isolates) showed circular, raised, undulate, opaque and light orange colour colonies (Table 11).

Table 11 Morphological, cultural characteristics and xylanolytic activity of the isolates.

Isolate no.	Colony morphology / size	Cell shape	Gram	Endospore	Clear zone diameter (cm)
Group 1 : X7-2	Circular, low convex,	Rods	+	-	0.4
X9-2	entire margins, opaque,	Rods	+	-	1.5
MX16-1	moist and yellowish	Rods	+	-	1.2
MX16-2	white pigment/ 0.1-0.3	Rods	+	-	1.2
MX17-2	cm in diameter	Rods	+	-	1.6
MX18		Rods	+	-	1.8
MXC2-3-1		Rods	+	-	1.4
MXC2-3-2		Rods	+	-	1.7
MXC3-1		Rods	+	-	1.4
MXC3-2		Rods	+	-	1.2
MXC3-4-1		Rods	+	-	1.8
MXC3-5-1		Rods	+	-	2.2
MXC3-5-2		Rods	+	-	2.4
MXC3-7-1		Rods	+	-	1.8
MXC3-7-2		Rods	+	-	1.6
MXC3-10-1		Rods	+	-	1.4
MXC3-10-2		Rods	+	-	0.8
MXC4-1-1		Rods	+	-	0.9
MXC 4-1-2		Rods	+	-	1.1
MXC4-2-1		Rods	+	-	2.5
MXC4-5-1		Rods	+	-	1.4
MXC4-5-2		Rods	+	-	1.4
MXC4-6-2		Rods	+	-	2.5
MXC4-9-3		Rods	+	-	1.9
ST1		Rods	+	-	1.8

Table 11 (Cont) Morphological, cultural and xylanolytic activity of the isolates.

Isolate no.	Colony morphology / size	Cell shape	Gram	Endospore	Clear zone diameter (cm)
Group 2 : S3-4A	Circular, convex, entire margins, opaque, moist, yellow and yellowish white colour/ 0.1-0.3 cm in diameter	Rods	+	+	3.5
MX2-3		Rods	+	+	2.1
Group 3 : S5-3	Circular or oval, flat, undulate, opaque and white colour/ 0.05-0.1 cm in diameter	Rods	+	+	1.7
X13-1		Rods	+	+	1.7
MXC2-2		Rods	+	+	2.6
MXC4-3-1		Rods	+	+	1.9
ST2		Rods	+	+	1.5
Group 4: X5-1	Circular, raise or low convex, undulate, opaque and white colour/ 0.05-0.7 cm in diameter	Rods	-	+	1.5
X8-1		Rods	-	+	0.8
X8-2		Rods	-	+	0.7
X9-1		Rods	-	+	0.6
X11-1		Rods	-	+	0.3
X11-2		Rods	-	+	0.4
X12-2		Rods	-	+	0.5
X15-1		Rods	-	+	0.5
X19-1		Rods	-	+	1.2
X19-2		Rods	-	+	1.3
MX6-2		Rods	+	+	1.0
MX8-1		Rods	+	+	1.5
Group 5 : MXC4-2-2	Circular, low convex, entire margins, moist, opaque and white colour/ 0.05-0.2 cm in diameter	Rods	-	+	1.1
Group 6 : S2-1	Irregular, rough, umbonate, opaque and cream colour/ 0.1-0.3 cm in diameter	Rods	+	+	2.3

Table 11 (Cont) Morphological, cultural and xylanolytic activity of the isolates.

Isolate no.	Colony morphology / size	Cell shape	Gram	Endospore	Clear zone diameter (cm)
Group 7: MX1-1	Irregular, rough, crateriform, opaque and cream or light brown colour / 0.2 cm in diameter	Rods	+	+	1.1
Group8 : MX1-2	Irregular, rough, raised, opaque and cream colour/ 0.1-0.7 cm in diameter	Rods	+	+	2.2
MX2-1		Rods	+	+	2.4
MX3-2		Rods	+	+	2.3
MX12-2		Rods	+	+	1.6
MXC1-3		Rods	+	+	2.8
MXC3-4-2		Rods	+	+	2.8
Group 9 : S1-3	Circular or oval, raised, opaque and yellow colour/ 0.05-0.4 cm in diameter	Rods	-	+	2.5
MX15-2		Rods	+	+	1.8
MX21-2		Rods	-	+	2.3
Group 10 : MX8-2	Circular, convex, entire margins, opaque, viscid, yellow or orange colour/ 0.3-0.5 cm in diameter	Rods	-	-	1.4
Group 11 : MXC3-9	Circular, convex, entire margins, glistening, mucoid , off-white colour/ 0.1-0.2 cm in diameter	Rods	-	-	1.3
Group 12 : SF	Circular, raised, undulate, opaque and light orange colour/ 0.05-0.35 cm in diameter	Rods/cocci	+	-	1.6
MXC4-7-1		Rods/cocci	+	-	2.1

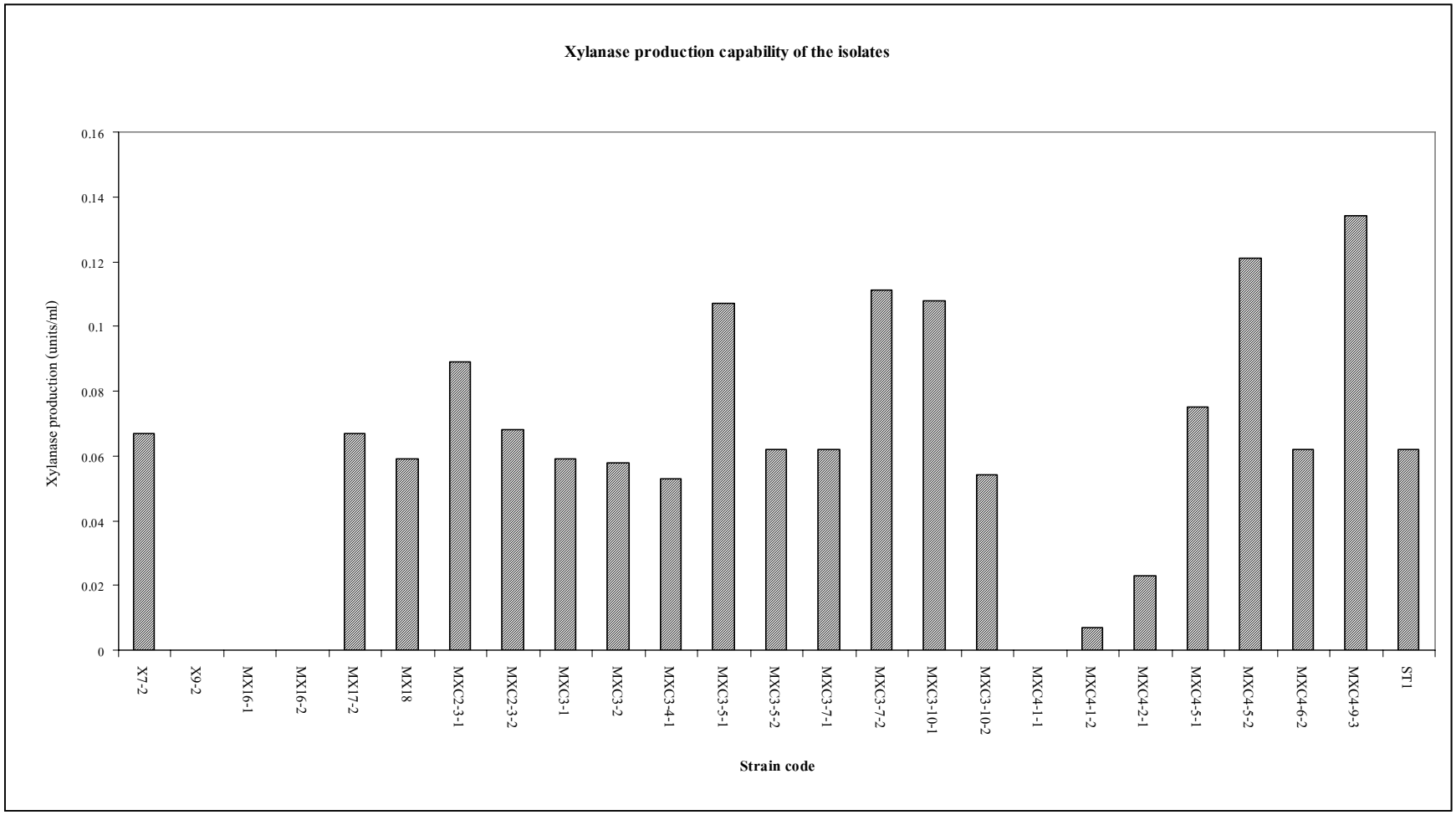

Fig. 3 Xylanase production capability of the isolates Group 1

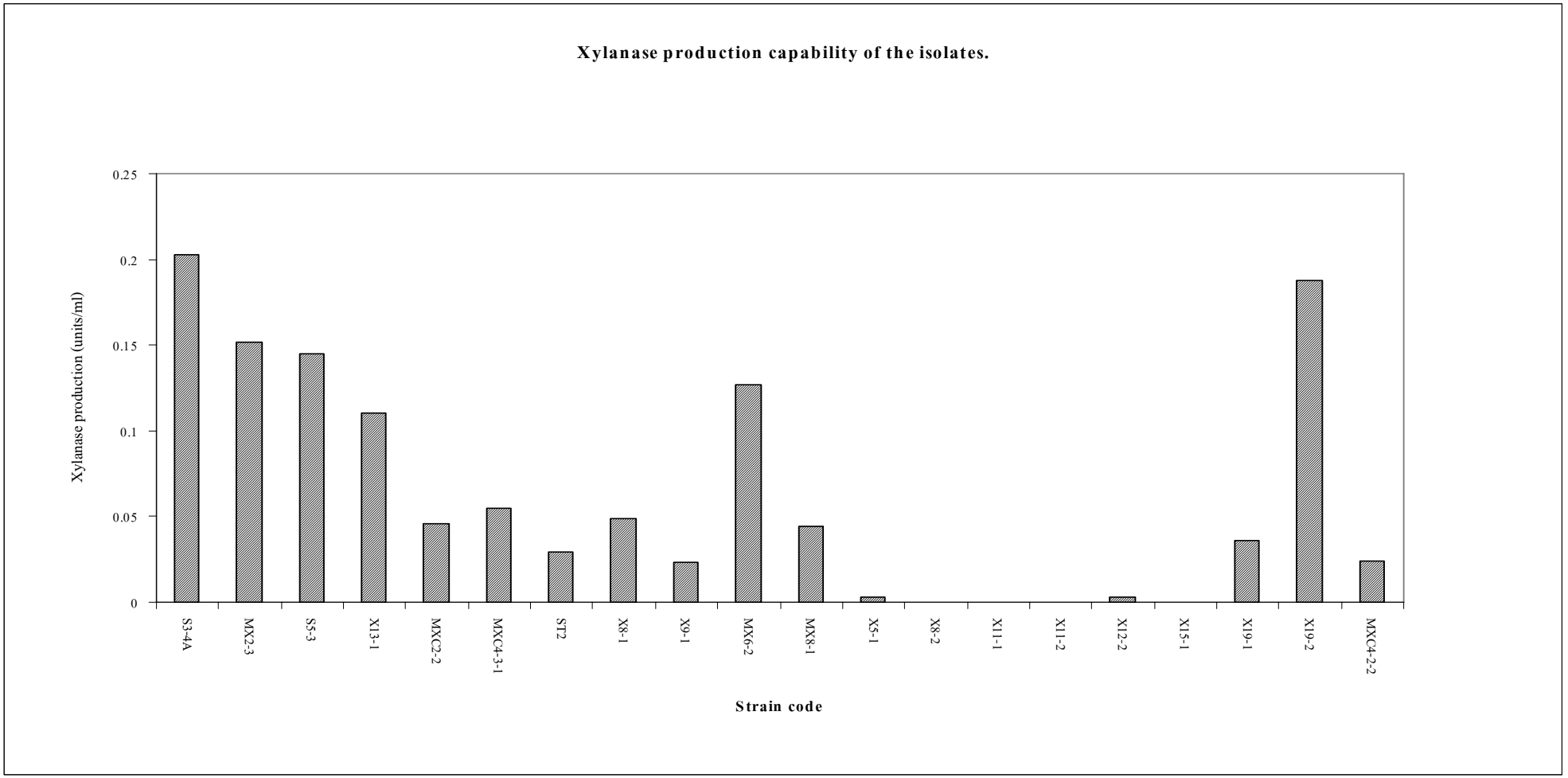

Fig. 4 Xylanase production capability of the isolates Group 2-5

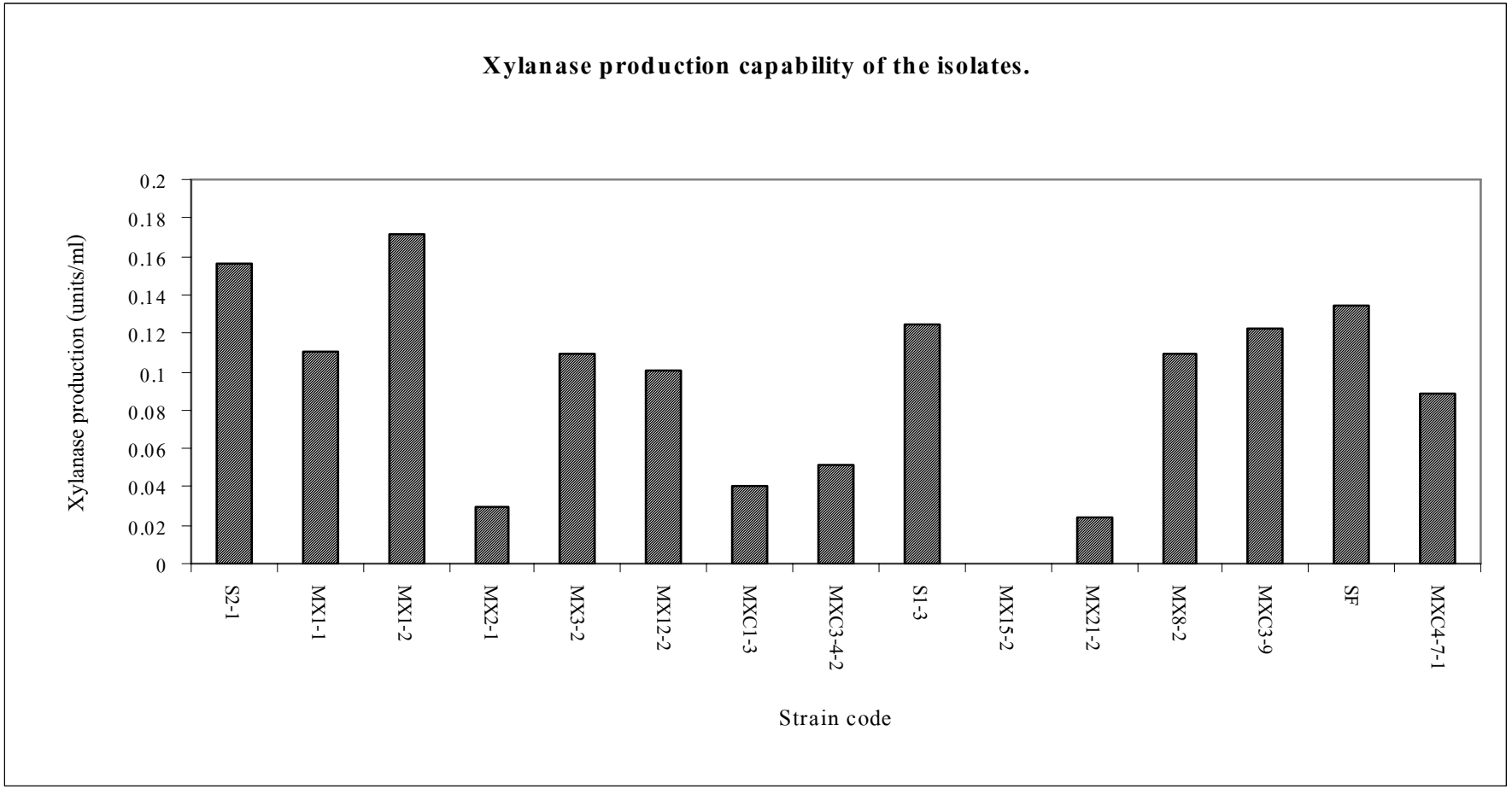

Fig. 5 Xylanase production capability of the isolates Group 6-12

2.2 Physiological and biochemical characteristics

Catalase and oxidase tests of all the isolates were shown in Table 12. Most of them grew at 15, 20, 45 and 50^{o}C. All isolates grew at pH 7-9 and at 40^{o}C, and in anaerobic condition but were negative for indole production, TSI and gelatin hydrolysis. Methyl red, DNase, urease, citrate, nitrate reduction, dihydroxyacetone, aesculin hydrolysis, hydrolysis of L-arginine, casein, L-tyrosine, starch and Tween 80 were variable characteristics as shown in Table 12. Acids were not produced from gluconate and L-sorbose. Most of the isolates produced acids from D-cellobiose, D-maltose, D-mannitol, D-melibiose, D-melezitose, raffinose, salicin and sucrose (Table 13).

Table 12 Physiological and biochemical characteristics of the isolates

Isolate no.	Growth in (%NaCl)		Growth at pH				Growth at ºC							Catalast test	Oxidast test	Anaerobic growth	Methyl red	Voges-Proskauer	DNase	Urease	Indole production	Citrate	TSI	Nitrate reduction	Aesculin	L-arginine	Dihydroxyacetone	Hydrolysis				
	3	5	5	6	8	9	10	15	20	45	50	55	60															Casein	Gelatin	Starch	L-tyrosine	Tween 80
X7-2	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	-	-	-	+	+	-	+	-	+	+	-
X9-2	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
MX16-1	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
MX16-2	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-
MX17-2	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
MX18	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
MXC2-3-1	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	-	-	-	+	+	+	+	-	+	+	-
MXC2-3-2	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	-	-	-	+	+	+	+	-	+	+	-
MXC3-1	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-
MXC3-2	+	+	-	+	+	+	W	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-
MXC3-4-1	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-
MXC3-5-1	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
MXC3-5-2	+	+	-	+	+	+	W	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
MXC3-7-1	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-
MXC3-7-2	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-	-	+	-	-

Table 12 (Cont) Physiological and biochemical characteristics of the isolates

Isolate no.	Growth in (%NaCl)		Growth at pH				Growth at ºC							Catalase test	Oxidase test	Anaerobic growth	Methyl red	Voges-Proskauer	DNase	Urease	Indole production	Citrate	TSI	Nitrate reduction	Aesculin	L-arginine	Dihydroxyacetone	Hydrolysis				
	3	5	5	6	8	9	10	15	20	45	50	55	60															Casein	Gelatin	Starch	L-tyrosine	Tween 80
MXC3-10-1	+	+	-	+	+	+	W	+	+	+	-	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	-	-	+	-	-
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	+	+	-	+	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	+	+	-	W	-	+	-	-
S3-4A	+	-	-	-	+	+	-	-	+	+	+	+	W	+	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-	+
MX2-3	+	-	-	-	+	+	-	+	+	+	-	-	-	+	+	+	-	+	-	+	-	-	-	+	+	-	-	-	-	+	-	+
S5-3	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+
X13-1	+	+	-	-	+	+	-	+	+	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+
MXC2-2	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	+

Table 12 (Cont) Physiological and biochemical characteristics of the isolates

Isolate no.	Growth in (%NaCl)		Growth at pH				Growth at ºC							Catalase test	Oxidase test	Anaerobic growth	Methyl red	Voges-Proskauer	DNase	Urease	Indole production	Citrate	TSI	Nitrate reduction	Aesculin	L-arginine	Dihydroxyacetone	Hydrolysis				
	3	5	5	6	8	9	10	15	20	45	50	55	60															Casein	Gelatin	Starch	L-tyrosine	Tween 80
MXC4-3-1	+	+	-	-	+	+	-	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+
ST2	+	+	-	-	+	+	-	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+
X8-1	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-
X9-1	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	+
MX6-2	+	+	-	-	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	-	-	-	+
MX8-1	+	+	-	-	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	+	-	-	-	+
X5-1	+	-	-	-	+	+	-	+	+	+	+	-	-	+	-	+	-	+	+	+	-	-	-	-	W	-	-	-	-	-	-	+
X8-2	+	-	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	-	-	-	-	W	-	-	-	-	-	-	+
X11-1	+	-	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	-	-	-	W	W	-	-	-	-	-	-	+
X11-2	+	-	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	-	-	-	W	-	-	-	-	-	-	-	+
X12-2	+	-	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	-	-	-	W	-	-	-	-	-	-	-	+
X15-1	+	+	-	-	+	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	+	W	-	-	-	-	-	-	+
X19-1	+	-	-	-	+	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	W	-	-	-	-	-	-	+
X19-2	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+
MXC4-2-2	+	+	-	-	+	+	-	-	-	+	+	+	-	+	-	+	-	+	-	+	-	-	-	+	+	-	-	+	-	-	-	+

Table 12 (Cont) Physiological and biochemical characteristics of the isolates

Isolate no.	Growth in (%NaCl)		Growth at pH				Growth at ºC							Catalase test	Oxidase test	Anaerobic growth	Methyl red	Voges-Proskauer	DNase	Urease	Indole production	Citrate	TSI	Nitrate reduction	Aesculin	L-arginine	Dihydroxyacetone	Hydrolysis				
	3	5	5	6	8	9	10	15	20	45	50	55	60															Casein	Gelatin	Starch	L-tyrosine	Tween 80
S2-1	+	+	-	-	+	+	-	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	-	+	+	-	-	-	-	+	-	+
MX1-1	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	W	-	+	-	-	-	+	+	-	-	+	-	+	-	-
MX1-2	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	W	-	+	-	+	-	+
MX2-1	-	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+
MX3-2	-	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	W	-	-	-	+	-	+
MX12-2	-	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-
MXC1-3	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-	-
MXC3-4-2	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-
S1-3	+	-	-	-	+	+	-	-	+	+	-	-	W	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	+
MX15-2	-	-	-	-	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	+	-	-	+	-	+	-	-
MX21-2	-	-	-	-	+	+	-	-	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-
MX8-2	+	+	-	+	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-
MXC3-9	+	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	-	-	W	+	-	-	-	-	+	+
SF	+	+	-	+	+	+	W	+	+	+	-	-	-	+	-	+	-	W	+	+	-	-	-	+	W	+	-	-	-	-	+	+
MXC4-7-1	+	+	-	+	+	+	W	+	+	+	-	-	-	+	-	+	-	W	+	+	-	-	-	+	W	+	-	-	-	-	+	+

Table 13 Acid from carbohydrates

Isolate no.	D-Amygdalin	L-Arabinose	D-Cellobiose	D-Fructose	D-Galactose	D-Glucose	Gluconate	Glycerol	Inositol	Inulin	Lactose	D-Maltose	D-Mannitol	D-Mannose	D-Melibiose	D-Melezitose	α－Methyl-D-glucoside	Raffinose	L-Rhamnose	D-Ribose	Salicin	D-Sorbitol	L-Sorbose	Sucrose	D-Trehalose	D-Xylose
X7-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
X9-2	-	-	+	-	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MX16-1	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MX16-2	-	-	+	-	-	-	-	+	+	+	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
MX17-2	-	-	+	-	-	-	-	+	-	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MX18	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC2-3-1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
MXC2-3-2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
MXC3-1	-	-	+	-	-	+	-	+	-	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC3-2	-	-	+	-	-	W	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC3-4-1	-	-	+	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC3-5-1	-	-	+	-	-	-	-	+	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-	+	-	-
MXC3-5-2	-	-	+	-	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC3-7-1	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC3-7-2	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-

Table13 (Cont) Acid from carbohydrates

Isolate no.	D-Amygdalin	L-Arabinose	D-Cellobiose	D-Fructose	D-Galactose	D-Glucose	Gluconate	Glycerol	Inositol	Inulin	Lactose	D-Maltose	D-Mannitol	D-Mannose	D-Melibiose	D-Melezitose	α – Methyl-D-glucoside	Raffinose	L-Rhamnose	D-Ribose	Salicin	D-Sorbitol	L-Sorbose	Sucrose	D-Trehalose	D-Xylose
MXC3-10-1	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
MXC3-10-2	-	-	-	-	-	-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC4-1-1	-	-	+	-	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC4-1-2	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC4-2-1	-	W	+	-	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC4-5-1	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-
MXC4-5-2	-	-	+	-	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC4-6-2	-	-	-	-	-	-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
MXC4-9-3	-	-	+	-	-	-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-
ST1	-	-	+	-	-	-	-	+	+	+	-	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-
S3-4A	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+
MX2-3	-	+	+	+	+	+	-	+	-	+	-	+	-	-	+	-	-	+	+	+	+	-	-	-	+	+
S5-3	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
X13-1	+	+	+	+	+	+	-	-	-	-	+	+	+	W	+	+	+	+	-	+	+	-	-	+	+	+
MXC2-2	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+

Table13 (Cont) Acid from carbohydrates

Isolate no.	D-Amygdalin	L-Arabinose	D-Cellobiose	D-Fructose	D-Galactose	D-Glucose	Gluconate	Glycerol	Inositol	Inulin	Lactose	D-Maltose	D-Mannitol	D-Mannose	D-Melibiose	D-Melezitose	α－Methyl-D-glucoside	Raffinose	L-Rhamnose	D-Ribose	Salicin	D-Sorbitol	L-Sorbose	Sucrose	D-Trehalose	D-Xylose
MXC4-3-1	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
ST2	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
X8-1	+	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	-	+	-	+	+	-	-	+	+	+
X9-1	+	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	-	+	-	+	+	-	-	+	+	+
MX6-2	+	+	+	+	+	+	-	+	+	-	+	+	+	+	W	-	+	+	+	+	+	+	-	+	+	+
MX8-1	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+
X5-1	-	-	-	-	-	W	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
X8-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
X11-1	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
X11-2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
X12-2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
X15-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
X19-1	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
X19-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MXC4-2-2	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	+

Table13 (Cont) Acid from carbohydrates

Isolate no.	D-Amygdalin	L-Arabinose	D-Cellobiose	D-Fructose	D-Galactose	D-Glucose	Gluconate	Glycerol	Inositol	Inulin	Lactose	D-Maltose	D-Mannitol	D-Mannose	D-Melibiose	D-Melezitose	α – Methyl-D-glucoside	Raffinose	L-Rhamnose	D-Ribose	Salicin	D-Sorbitol	L-Sorbose	Sucrose	D-Trehalose	D-Xylose
S2-1	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+
MX1-1	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	-	-	+	-	-	+	+	-
MX1-2	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
MX2-1	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
MX3-2	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
MX12-2	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
MXC1-3	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
MXC3-4-2	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S1-3	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+
MX15-2	-	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+
MX21-2	+	+	+	+	+	+	-	-	-	-	+	+	-	+	+	W	+	+	+	+	+	+	-	+	+	+
MX8-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MXC3-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MXC4-7-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: +, all strains positive ; w, weakly positive ; - , all strains negative

2.3 Chemotaxonomic characteristics and DNA base compositions

The tested strains in Group 2 (S3-4A); Group 3 (S5-3, X13-1); 4(X11-1, X15-1, MX8-1), Group 5 (MXC4-2-2), Group 6 (S2-1), Group 7 (MX1-1), 8 (MX1-2, MX2-1, MX3-2, and MXC3-4-2), Group 9 (S1-3 and MX21-2) and Group 12 (SF), contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan, except the tested strains in Group 1 (X7-2, MXC2-3-1 and MXC3-1), Group 10 (MX8-2) and Group 11 (MXC3-9). The predominant menaquinone with seven isoprene units (MK-7) was found in the tested strains of Group 2 (S3-4A), Group 8 (MXC3-4-2), and Group 9 (MX21-2). The tested strain in Group 12 (SF) contained MK-8(H_2) as major menaquinone while Group 1 strain (MXC3-1) contained MK-11 (65.6%) and MK-12 (34.4%). The ubiquinone with eight isoprene units (Q-8) was found in the tested strains of Group 10 (MX8-2) and Group 11 (MXC3-9). The DNA G+C content of the tested strain for Group 1, MXC2-3-1 was 71.0 and MXC3-1 was 71.5 mol% and Group 11 (MXC3-9) was 71.4 mol%. Group 2 (S3-4A) had 52.7 mol% and X13-1 and MXC2-2 (Group 3) 47.3 and 48.8 mol%, respectively. The strains X8-1, X11-1, X15-1 and MX8-1 (Group 4) had 54.3, 41.7, 54.0, and 56.2 mol%, respectively. Group 5 (MXC4-2-2) had 61.6 mol%. Group 6 (S2-1) had 39.3, Group 7 (MX1-1) 37.3 and Group 8 (MX1-2 and MX12-2) 36.6 and 43.9 mol%, respectively. Group 9 (S1-3) contained 53.3 and Group 12 (SF) contained 61.4 mol%.

On the basis of their phenotypic and chemotaxonomic characteristics, Twenty-five isolates in Group 1 were identified as *Microbacterium* (Sook-Lee *et al*., 2006 ; Takeuchi and Hatano, 1998). Twenty isolates (Group 2, 3, 4, 5, and 9) showed characteristics that closed to *Paenibacillus* and *Cohnella* (Elo *et al*., 2001 ; Hoon Yoon *et al*., 2003 ; Kampfer *et al*., 2006 ; Uetanabaro *et al*., 2003). Eight isolates (Group 6, 7 and 8) were identified as *Bacillus* (Heyrman *et al*., 2004 ; Venkateswaran *et al*., 2003). One isolate (Group 10) was identified as *Pseudoxanthomonas* (Yen *et al*., 2002). One isolate (Group 11) was identified as *Cupriavidus* (Vandamme and

Coenye, 2004). The last group (2 isolates in Group 12) was identified as *Rhodococcus* (Hoon Yoon *et al*., 2000).

2.4. Phylogenetic tree analysis

The representative isolates of Group 1, MXC 4-2-1(1478 bp) and MXC4-6-2 (1443 bp) showed 99.27 and 99.41 of 16S rDNA sequence similarity (%) to *Microbacterium barkeri* DSM 20145^T, respectively (Fig 6, Table 14.) The 16S rDNA sequence similarity values between Group 2, isolate S3-4A (1485 bp) and MX2-3 (1494 bp) with *Paenibacillus agaridovorans* DSM1355^T were 97.13 and 96.49%, respectively (Fig 7, Table 15). Isolates in Group 3, X13-1 (1504 bp), S5-3 (1464 bp), ST2 (1474 bp), MXC4-3-1 (1490 bp) and MXC2-2 (1466 bp) showed 96.41, 95.85, 97.84, 97.61 and 96.49% of 16S rDNA sequence similarity to *Paenibacillus granivorans* A-30^T, respectively (Fig 7, Table 15). Group 4 isolates, X8-1 (1490 bp) and MX8-1 (1518 bp) showed 99.06 and 98.95% 16S rDNA sequence similarity to *Paenibacillus favisporus* GMP01^T (Fig 8, Table 16) while X15-1(1476 bp) showed 99.69% of 16S rDNA sequence similarity to *Paenibacillus naphthalenovorans* PR-N1^T (Fig 8, Table 16). Isolate in Group 4 , X11-1 (1058 bp) and Group 5, MXC4-2-2 (1476 bp) showed 96.59 and 93.9% of 16S rDNA sequence similarity to *Paenibacillus validus* JCM9077^T (Fig 8, Table 16). Isolate in Group 6, S2-1(1321 bp) showed 99.41% of 16S rDNA sequence similarity to *Bacillus niabensis* 4T19^T (Fig 9, Table 17). Isolate in Group 7, MX1-1(1492 bp) showed 99.33% of 16S rDNA sequence similarity to *Bacillus megaterium* IAM13418^T (Fig 9, Table 17). Isolate in Group 8 , MX1-2(1483 bp), MX2-1(1476 bp), MX3-2(1491 bp), MX12-2(1475 bp), MXC1-3(1463 bp) and MXC3-4-2(1474 bp) showed 99.63, 99.33, 99.48, 99.48, 99.78 and 99.78% of 16S rDNA sequence similarity to *Bacillus funiculus* NAF001^T, respectively (Fig 9, Table 17). Isolate in Group 9, S1-3(1499 bp), MX15-2(1494 bp) and MX21-2(1081 bp) showed 95.92, 99.21 and 98.3% of 16S rDNA sequence similarity to *Cohnella*^{T}, respectively (Fig 10, Table 18). Isolate in Group 10, MX8-2

(1480 bp) showed 99.62% of 16S rDNA sequence similarity to *Pseudoxanthomonas suwonensis* 4M1^{T} (Fig 11, Table 19). Isolate in Group 11, MXC3-9(1457 bp) showed 99.04% of 16S rDNA sequence similarity to *Cupriavidus gilardiis* LMG5886^{T} (Fig 12, Table 20). Isolate in Group 12, SF(1490 bp) showed 99.55% of 16S rDNA sequence similarity to *Rhodococcus rhodochrous* DSM43241^{T} (Fig 13, Table 21).

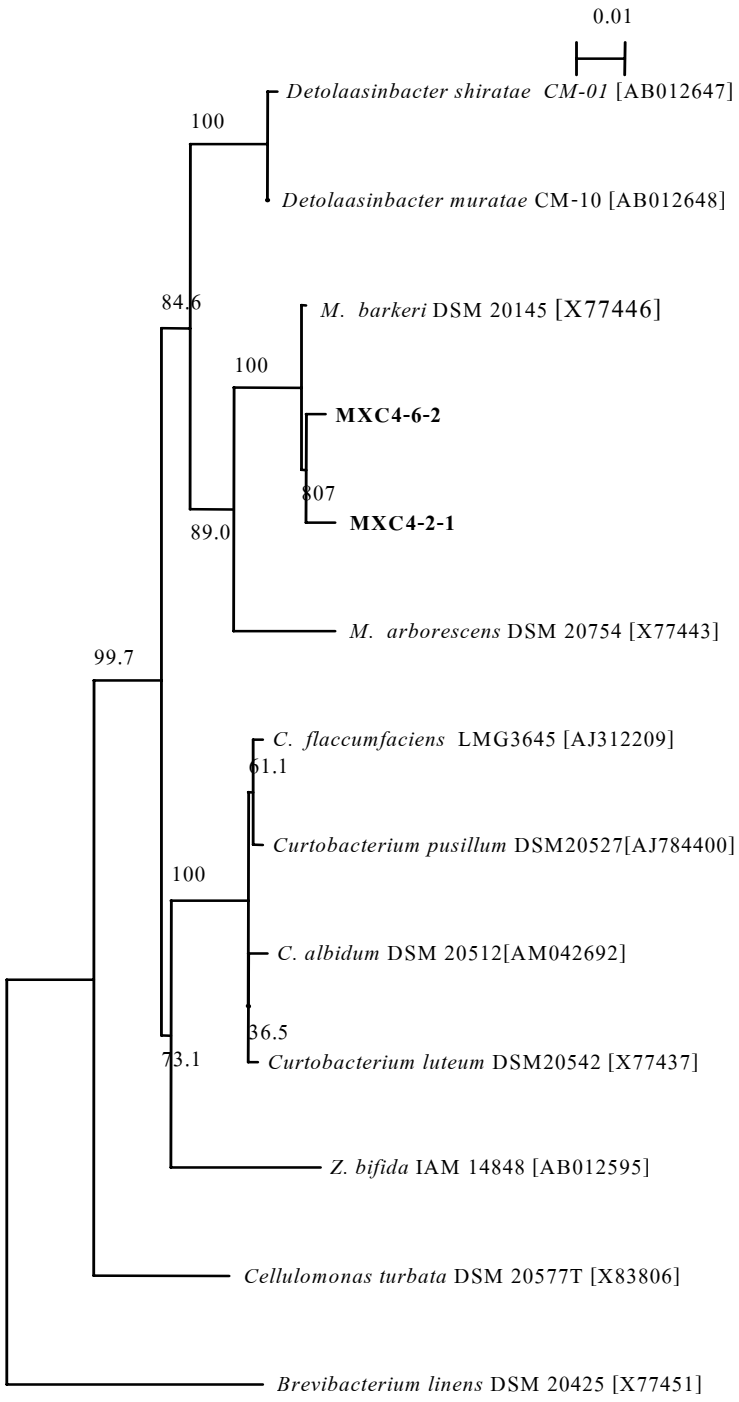

Fig. 6 Neighbour-joining-tree showing the phylogenetic positions of strain MXC4-2-1, MXC4-6-2 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.01 substitutions per nucleotide position. Bootstrap values expressed as percentages of 10 replications.

Table 14 Percentage similarities of MXC4-2-1 and MXC4-6-2 and related *Microbacterium* species.

	MXC4-6-2	MXC4-2-1	X77446	X77443	AB012648	AB012647	X77437	AM042692	AJ784400	AJ312209	AB012595	X83806	X77451
MXC4-6-2	100												
MXC4-2-1	99.12	100											
X77446	**99.41**	**99.27**	100										
X77443	95.76	95.61	96.23	100									
AB012648	94.82	94.5	95.13	95.94	100								
AB012647	94.66	94.34	94.98	95.79	99.85	100							
X77437	94.76	94.44	95.07	93.79	95.92	95.77	100						
AM042692	94.43	94.12	94.76	93.79	95.76	95.6	99.41	100					
AJ784400	94.52	94.2	94.84	93.71	95.69	95.53	99.56	99.27	100				
AJ312209	94.36	94.04	94.68	93.55	95.53	95.37	99.41	99.27	99.56	100			
AB012595	93.38	93.06	93.71	92.38	94.24	94.08	95.06	94.74	94.98	95.13	100		
X83806	91.41	91.08	91.75	92.41	92.59	92.42	93.47	93.55	93.48	93.63	91.8	100	
X77451	87.46	87.29	88	87.85	87.77	87.76	88.41	88.42	88.06	88.22	87.18	89.23	100

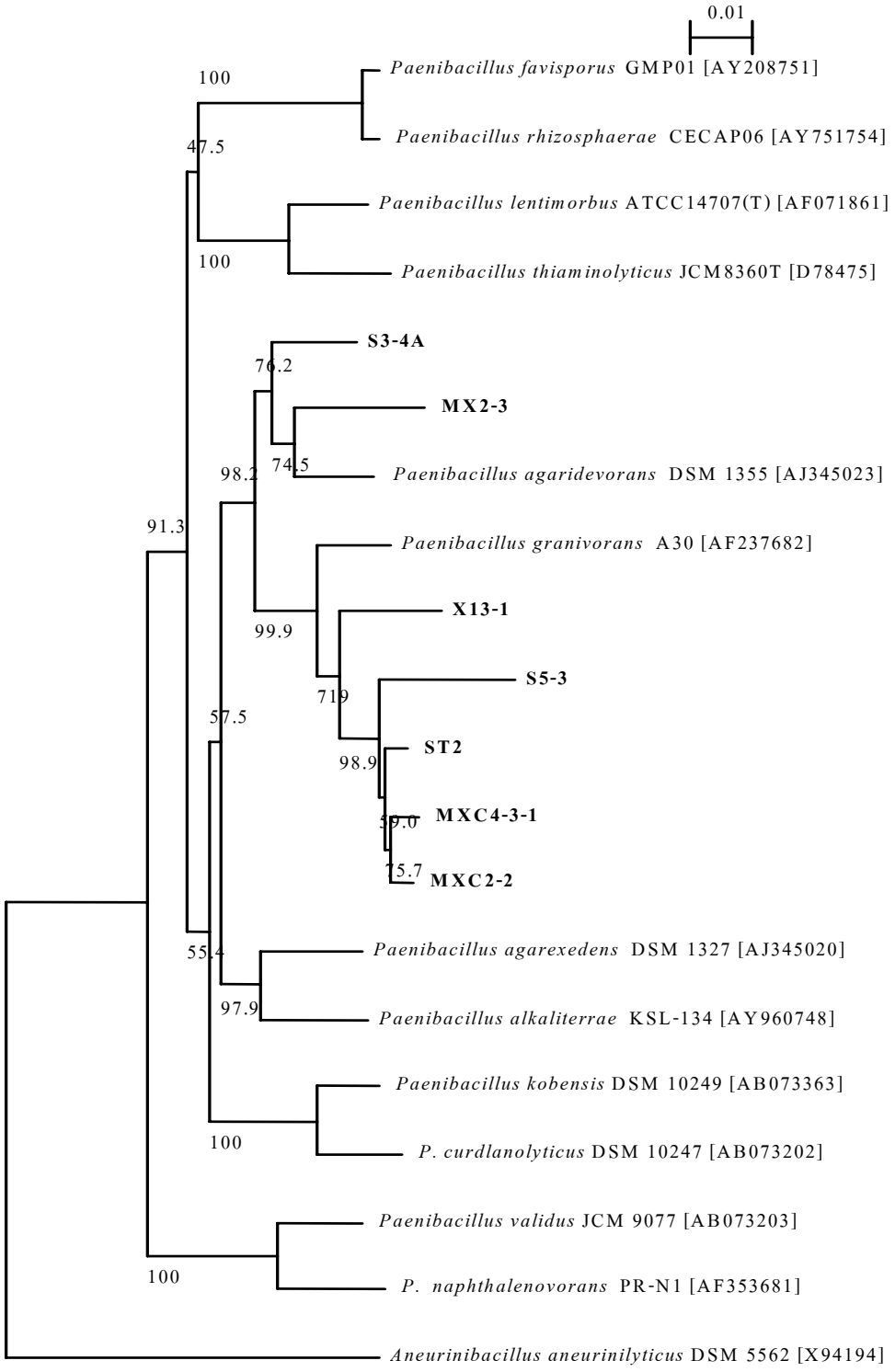

Fig. 7 Neighbour-joining-tree showing the phylogenetic positions of strain S3-4A, MX2-3, X13-1, S5-3, ST2, MXC4-3-1, MXC2-2 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.01 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 15 Percentage similarities of S3-4A, MX2-3, X13-1, S5-3, ST2, MXC4-3-1, MXC2-2 and related *Paenibacillus* species.

	AF353681	AB073203	AY208751	AY751754	D78475	AF071861	AY960748	AJ345020	AB073363	AB073202	MXC2-2	MXC4-3-1	ST2	S5-3	X13-1	AF237682	MX2-3	AJ345023	S3-4A	X94194
AF353681	100																			
AB073203	96.89	100																		
AY208751	91.89	92.85	100																	
AY751754	91.72	92.76	99.47	100																
D78475	91.77	92.38	94.46	94.3	100															
AF071861	92.87	93.05	93.88	93.88	97.22	100														
AY960748	93.6	93.1	93.45	93.45	93.12	93.96	100													
AJ345020	92.52	92.19	93.87	93.71	94.15	95.05	96.66	100												
AB073363	92.77	92.27	93.53	93.19	93.02	94.04	94.02	94.94	100											
AB073202	92.44	92.43	93.03	93.02	92.42	93.53	94.61	94.2	97.61	100										
MXC2-2	90.85	91.64	93.53	93.7	93.87	94.2	94.04	94.14	93.37	92.84	100									
MXC4-3-1	90.77	91.57	93.46	93.62	93.79	94.12	93.96	94.07	93.29	92.76	99.24	100								
ST2	91.04	91.99	93.54	93.71	93.95	94.37	93.96	94.23	93.37	92.85	99.16	99.01	100							
S5-3	88.89	89.97	91.56	91.73	92.16	92.42	92.42	92.61	91.47	91.1	97.2	97.29	97.29	100						
X13-1	90.44	90.73	93.01	93.01	92.67	93.43	94.21	93.7	92.66	92.31	97.53	97.05	97.29	95.18	100					
AF237682	91.21	92	93.82	93.81	94.2	94.29	94.88	94.97	94.3	93.63	**97.68**	**97.61**	**97.84**	**95.85**	**96.41**	100				
MX2-3	91.61	91.78	93.44	93.28	92.65	92.91	94.54	94.11	93.54	92.95	94.36	94.45	94.94	92.76	94.87	94.86	100			
AJ345023	91.51	92.31	94.69	94.52	93.6	93.76	95.6	95.51	94.2	93.61	95.26	95.27	95.27	93.59	95.35	96.08	**96.49**	100		
S3-4A	92.75	92.75	94.79	94.46	93.69	94.35	95.35	95.44	95.28	94.55	95.84	95.85	95.84	94.19	95.59	95.85	96.19	**97.13**	100	
X94194	86.75	87.07	87.4	87.04	86.9	86.21	86.77	86.95	86.87	86.87	86.12	86.04	86.23	84.54	85.08	87.01	85.56	86.48	87.14	100

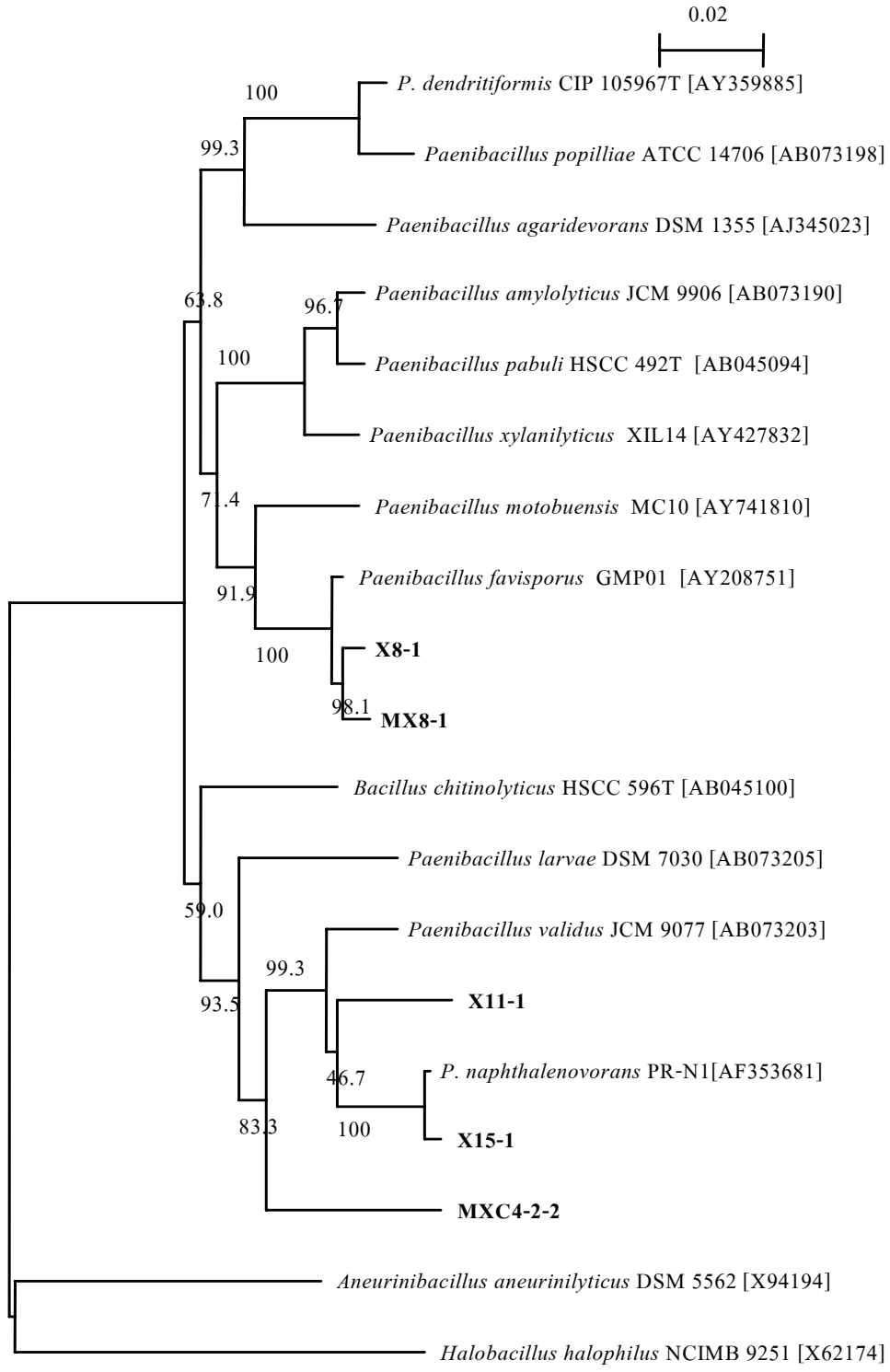

Fig. 8 Neighbour-joining-tree showing the phylogenetic positions of strain X8-1, MX8-1, X11-1, X15-1, MXC4-3-1 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.02 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 16 Percentage similarities of X8-1, MX8-1, X11-1, MX15-1, MXC4-2-2 and related *Paenibacillus* species.

	X15-1	AF353681	X11-1	MXC4-2-2	AB073203	AB073205	AB045100	MX8-1	X8-1	AY208751	AY741810	AY427832	AB045094	AB073190	AB073198	AY359885	AJ345023	X94194	X62174
X15-1	100																		
AF353681	**99.69**	100																	
X11-1	95.8	95.91	100																
MXC4-2-2	93.44	93.56	92.74	100															
AB073203	95.91	96.02	**96.59**	**93.9**	100														
AB073205	93.06	93.41	92.84	92.62	93.9	100													
AB045100	92.68	93.03	92.7	92.12	93.88	93.18	100												
MX8-1	90.05	90.42	89.37	90.42	91.64	92.15	92.94	100											
X8-1	90.16	90.53	89.47	90.53	91.75	92.26	93.05	99.05	100										
AY208751	90.06	90.42	90.09	90.78	91.65	92.74	93.75	**98.95**	**99.06**	100									
AY741810	90.94	91.31	90.61	90.59	91.1	92.28	93.44	95.82	95.93	96.04	100								
AY427832	91.28	91.64	90.47	91.98	92.35	91.51	93.63	94.78	94.89	94.78	94.24	100							
AB045094	91.98	92.22	91.06	91.51	93.39	91.06	93.19	94.45	94.55	94.45	93.8	97.68	100						
AB073190	91.51	91.87	90.47	91.51	92.7	90.94	93.87	94.22	94.33	94.34	93.47	98.1	98.95	100					
AB073198	90.39	90.76	90.99	90.48	91.45	91.73	92.21	91.18	91.28	92.12	92.48	91.88	92.46	92.34	100				
AY359885	90.24	90.61	91.09	90.58	91.08	91.34	93.42	92.51	92.61	93.43	93.44	92.39	93.19	92.85	98.41	100			
AJ345023	89.94	90.31	90.22	90.85	90.94	91.32	93.38	93.18	93.28	94.1	93.17	93.27	93.27	93.96	93.85	94.89	100		
X94194	84.88	85.28	86.35	84.92	84.93	85.77	86.03	84.86	84.84	85.77	86.41	85.51	85.03	85.16	84.57	84.91	85.32	100	
X62174	81.39	81.65	81.42	80.4	81.91	82.62	83.14	83.85	83.83	84.78	83.25	83.48	83.64	83.64	82.09	82.53	82.89	84.44	100

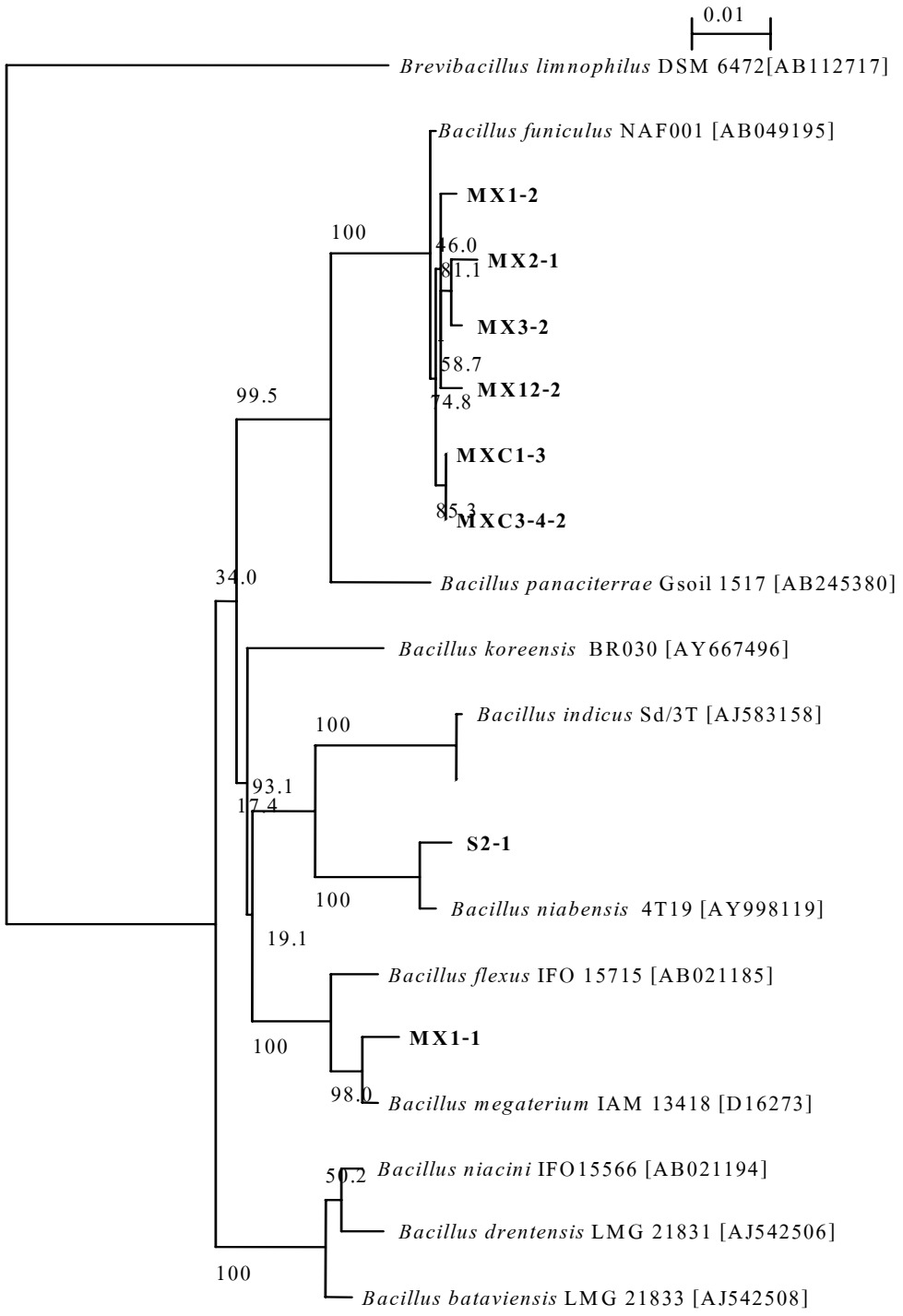

Fig 9. Neighbour-joining-tree showing the phylogenetic positions of strain MX1-2, MX2-1, MX3-2, MX12-2, MXC1-3, MXC3-4-2, S2-1, MX1-1 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.01 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 17. Percentage similarities of MX1-2, MX2-1, MX3-2, MX12-2, MXC1-3, MXC3-4-2, S2-1, MX1-1 and related *Bacillus* species.

	AJ542508	AJ542506	AB021194	AY998119	S2-1	AY550276	AJ583158	D16273	MX1-1	AB021185	MX3-2	MXC1-3	MXC3-4-2	MX12-2	MX2-1	MX1-2	AB049195	AB245380	AY667496	AB112717
AJ542508	100																			
AJ542506	99.26	100																		
AB021194	98.81	99.11	100																	
AY998119	95.42	95.25	95.49	100																
S2-1	95.25	95.08	95.32	**99.41**	100															
AY550276	95.57	95.41	95.64	96.67	96.2	100														
AJ583158	95.5	95.33	95.56	96.6	96.12	99.93	100													
D16273	96.22	95.66	96.05	95.81	95.72	95.49	95.41	100												
MX1-1	95.82	95.19	95.66	95.57	95.48	95.09	95.01	**99.33**	100											
AB021185	96.52	96.05	96.36	96.05	95.96	95.57	95.49	98.88	98.51	100										
MX3-2	94.85	94.2	94.6	94.29	94.04	93.81	93.73	95.18	94.93	95.02	100									
MXC1-3	95.01	94.37	94.76	94.46	94.21	93.97	93.89	95.34	95.1	95.19	99.56	100								
MXC3-4-2	95.01	94.37	94.76	94.46	94.21	93.97	93.89	95.34	95.1	95.19	99.56	100	100							
MX12-2	94.85	94.2	94.6	94.29	94.04	93.8	93.72	95.17	94.93	95.02	99.48	99.55	99.55	100						
MX2-1	94.68	94.03	94.43	94.12	93.87	93.72	93.63	95.09	94.93	94.94	99.55	99.41	99.41	99.25	100					
MX1-2	94.85	94.2	94.6	94.29	94.04	93.8	93.72	95.17	94.93	95.02	99.48	99.7	99.7	99.48	99.25	100				
AB049195	95.25	94.6	95	94.69	94.45	94.05	93.97	95.57	95.33	95.42	**99.48**	**99.78**	**99.78**	**99.48**	**99.33**	**99.63**	100			
AB245380	95.42	95.09	95.48	95.03	94.86	94.55	94.47	95.58	95.34	95.82	96.83	97.14	97.14	96.82	96.74	96.98	97.21	100		
AY667496	95.9	95.57	95.89	95.51	95.18	95.42	95.34	96.14	95.9	96.76	94.93	95.09	95.09	94.93	94.85	94.93	95.17	95.89	100	
AB112717	89.68	89.42	88.98	88.04	87.48	88.81	88.72	88.48	87.94	88.48	88.2	88.38	88.38	88.19	88	88.19	88.56	87.55	89.01	100

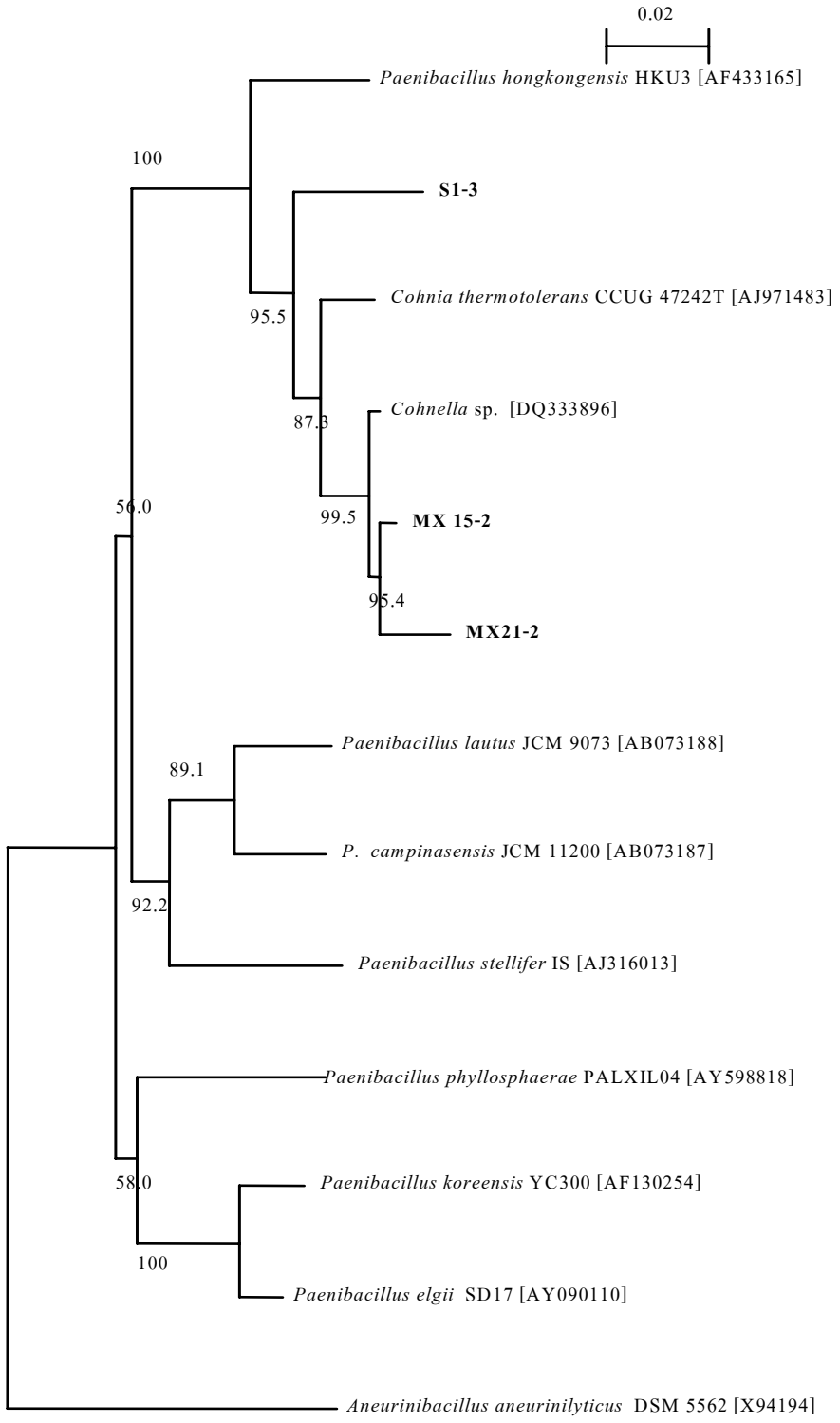

Fig 10. Neighbour-joining-tree showing the phylogenetic positions of strain S1-3, MX15-2 , MX21-2 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.02 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 18 Percentage similarities of S1-3, MX15-2, MX21-2 and related *Cohnella* species.

	AY090110	AF130254	X94194	AB073187	AB073188	AJ316013	AY598818	MX15-2	MX21-2	DQ333896	AJ971483	S1-3	AF433165
AY090110	100												
AF130254	98	100											
X94194	87.92	87.18	100										
AB073187	91.83	90.91	87.32	100									
AB073188	92.48	91.02	86.1	96.25	100								
AJ316013	92.68	91.43	85.39	92.35	94.46	100							
AY598818	93.35	92.55	85.67	91.35	91.12	91.16	100						
MX15-2	89.55	89.41	84.59	91.2	90.99	89.42	89.83	100					
MX21-2	88.26	88.12	83.09	89.96	89.74	88.61	88.44	98.41	100				
DQ333896	89.91	89.78	84.72	91.54	91.33	90.26	89.92	**99.21**	**98.3**	100			
AJ971483	90.74	90.61	85.51	91.23	91	90.27	90.05	97.28	96.35	98	100		
S1-3	89.24	89.45	85.35	90.08	89.13	88.56	89.71	95.82	94.66	**95.92**	95.61	100	
AF433165	91	90.62	84.49	91.47	91.35	90.97	91	95.28	94.22	95.71	95.59	93.74	100

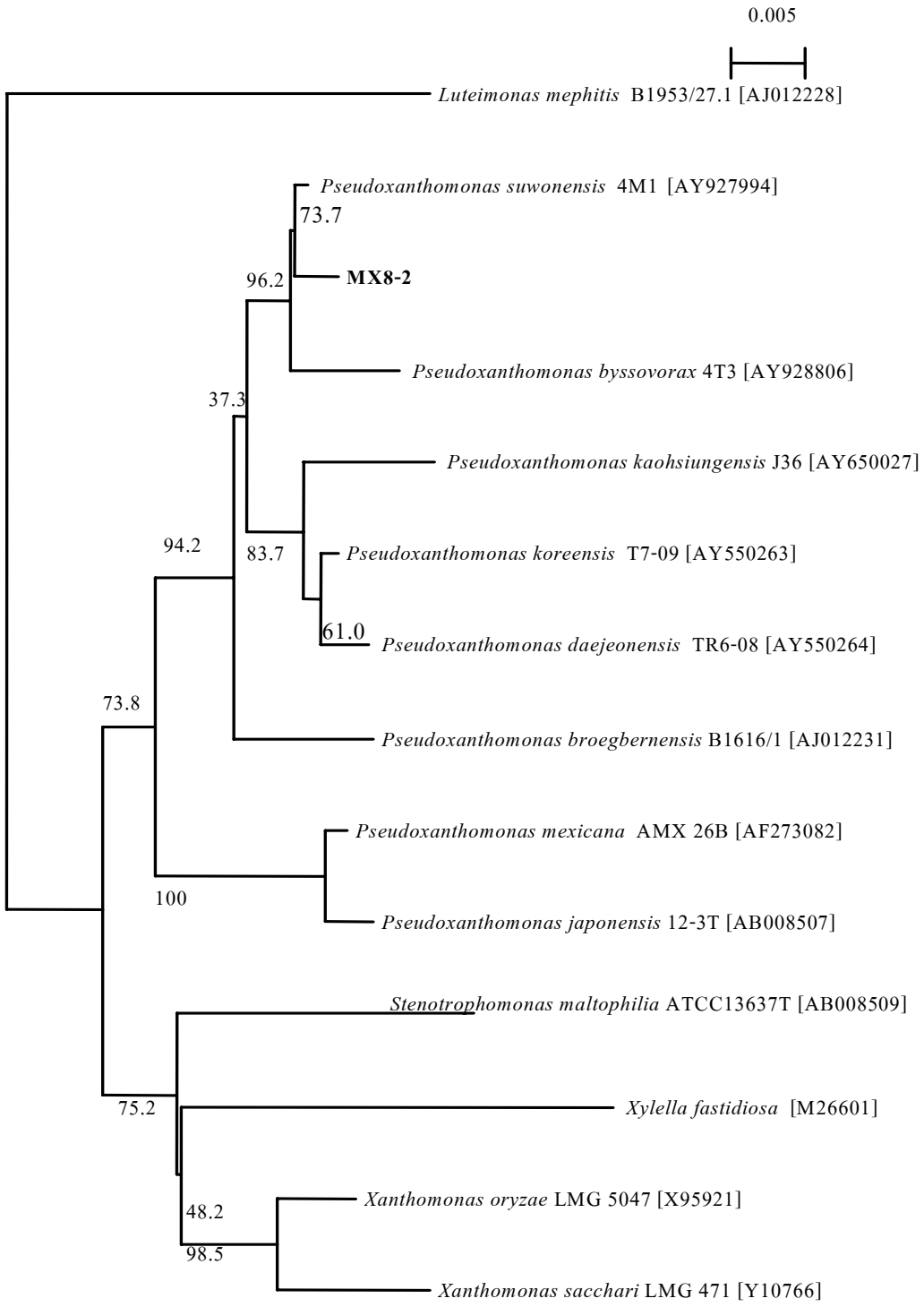

Fig 11. Neighbour-joining-tree showing the phylogenetic positions of strain MX8-2 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.005 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 19 Percentage similarities of MX8-2 and related *Pseudoxanthomonas* species.

	Y10766	X95921	AB008509	AF273082	AB008507	AY550264	AY550263	AJ012231	MX8-2	AY927994	AY928806	AY650027	AJ012228	M26601
Y10766	100													
X95921	98.48	100												
AB008509	96.36	96.76	100											
AF273082	96.43	96.59	95.8	100										
AB008507	96.43	96.59	95.8	99.55	100									
AY550264	96.03	96.68	95.8	97.54	97.07	100								
AY550263	95.8	96.44	95.49	97.31	96.84	99.55	100							
AJ012231	96.35	96.99	96.12	97.38	96.91	98.48	98.4	100						
MX8-2	96.11	96.75	95.72	97.46	97.3	98.4	98.78	98.4	100					
AY927994	96.19	96.83	95.8	97.54	97.39	98.63	99.01	98.63	**99.62**	100				
AY928806	95.47	96.12	95.07	97.15	96.99	97.94	98.32	97.93	98.94	99.17	100			
AY650027	95.55	96.27	95.07	96.59	96.59	98.55	99.01	97.39	98.24	98.32	97.62	100		
AJ012228	93.41	94.23	94.16	94.41	94.32	94.82	94.82	93.92	94.57	94.74	94.65	94.81	100	
M26601	95.48	95.73	94.83	95.24	95.23	94.68	94.36	95.08	94.67	94.76	94.68	94.1	92.58	100

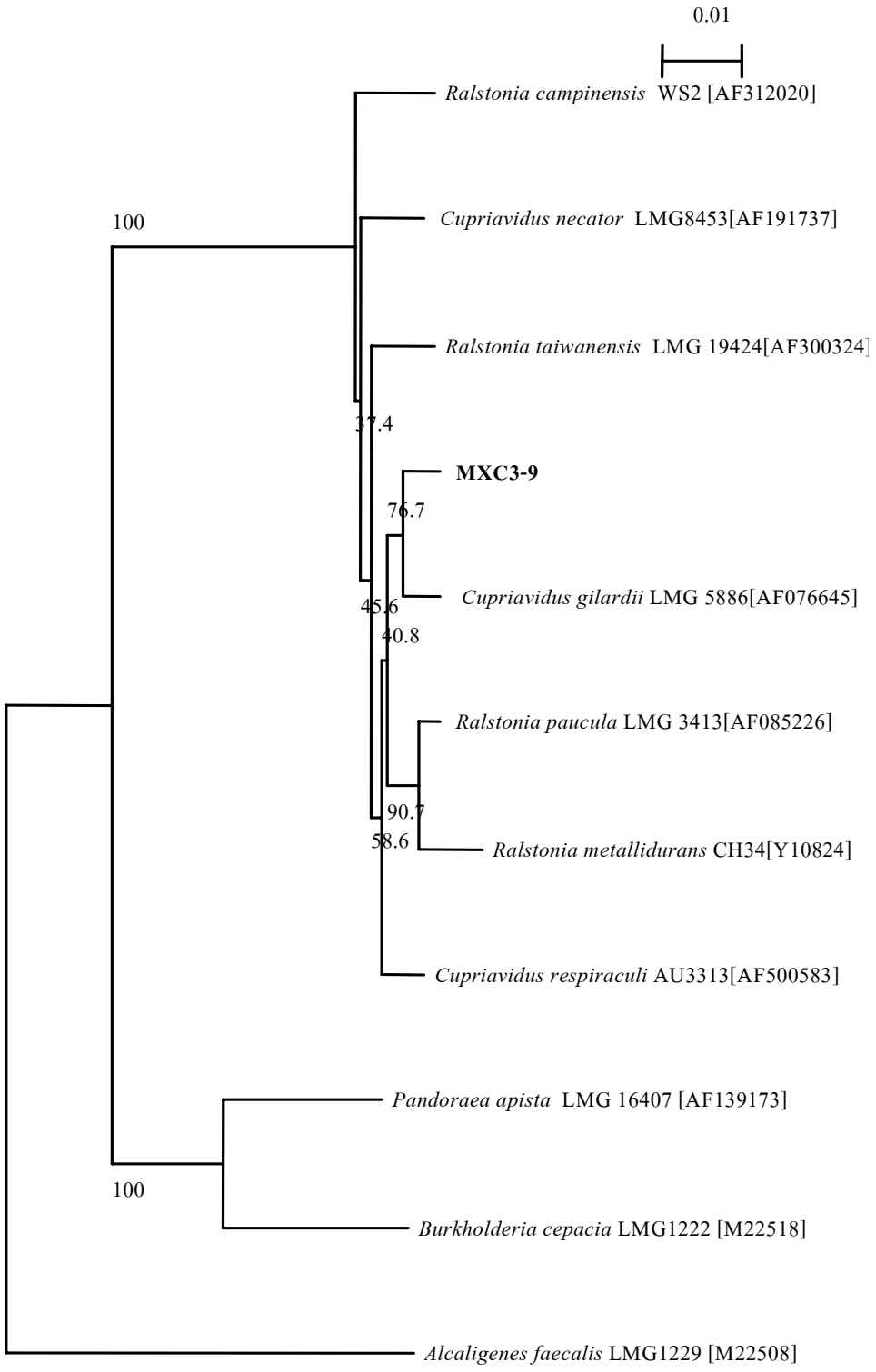

Fig. 12 Neighbour-joining-tree showing the phylogenetic positions of strain MXC3-9 and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.01 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 20 Percentage similarities of MXC3-9 and related *Cupriavidus* species.

	AF139173	M22518	AF076645	AF085226	AF312020	AF300324	Y10824	MXC3-9	AF500583	AF191737	M22508
AF139173	100										
M22518	95.48	100									
AF076645	92.08	91.67	100								
AF085226	91.91	91.41	98.56	100							
AF312020	92.06	91.3	97.99	98.16	100						
AF300324	91.44	91.76	98.32	98.4	97.99	100					
Y10824	91.72	90.59	98.32	98.88	97.26	97.5	100				
MXC3-9	91.53	91.3	**99.04**	98.48	97.66	98.4	98.16	100			
AF500583	91.8	91.48	98.56	98.64	98.08	98.48	98.32	98.88	100		
AF191737	92.26	91.68	97.99	98.07	98.16	98.24	97.42	97.99	98.56	100	
M22508	88.87	88.48	88.21	88.01	88.02	88.21	87.55	88.1	88.23	87.95	100

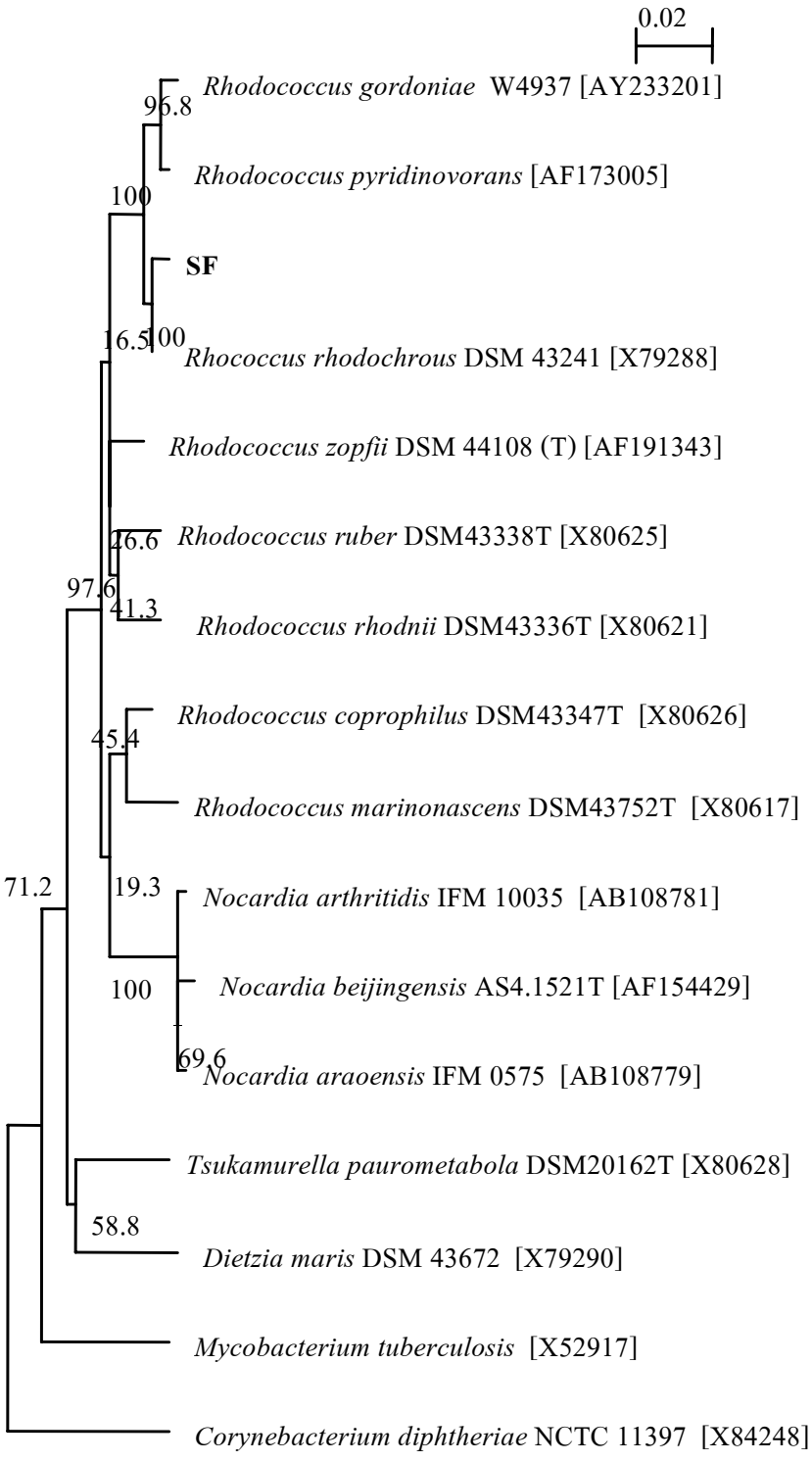

Fig. 13 Neighbour-joining-tree showing the phylogenetic positions of strain SF and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.02 substitutions per nucleotide position. Bootstrap values expressed as percentages of 1000 replications.

Table 21 Percentage similarities of SF and related *Rhodococcus* species.

	AB108779	AF154129	AB108781	X80617	X80621	AF191343	X80626	X79288	SF	AY233201	AF173005	X80625	X80628	X79290	X52917	X84248
AB108779	100															
AF154129	99.48	100														
AB108781	99.63	99.25	100													
X80617	96.97	96.58	96.97	100												
X80621	96.95	96.56	96.79	96.42	100											
AF191343	96.87	96.64	96.87	97.19	97.81	100										
X80626	96.41	96.01	96.33	97.81	96.88	98.03	100									
X79288	96.64	96.25	96.56	96.65	97.26	97.87	97.72	100								
SF	96.25	95.94	96.18	96.26	96.95	97.56	97.42	**99.55**	100							
AY233201	96.16	95.92	96.08	96.17	96.4	97.09	96.94	98.64	98.34	100						
AF173005	96.16	95.76	95.92	95.94	96.87	97.25	97.49	99.02	98.72	99.17	100					
X80625	96.48	96.16	96.39	96.25	97.72	97.87	97.1	97.49	97.18	97.1	97.57	100				
X80628	93.69	93.44	94.02	94.91	95.13	95.69	95.21	95.38	95.06	94.64	94.56	95.2	100			
X79290	93.7	93.45	93.79	94.6	94.67	94.81	95.22	95.31	94.99	94.33	94.49	94.57	95.05	100		
X52917	93.17	92.75	92.92	93.74	93.68	93.42	93.92	93.32	93	93.33	93.75	94.16	92.73	92.26	100	
X84248	90.64	90.03	90.55	91.17	91.93	91.82	91.83	91.58	91.34	90.9	91.07	91.23	92.09	91.85	91.26	100

Characteristic of the isolates

Group 1 contained 25 isolates, as shown in Table 11.

They were Gram-positive, non spore-forming, motile rods measuring from 0.5 to 1.0 by 2.0-4.0 μm. All grew in 3 and 5% NaCl, at pH 6-9 and at 15-45°C, and in anaerobic condition but not at pH 5 and at 60°C. Catalase, VP, DNase, urease, hydrolysis of aesculin, L-arginine and starch were positive but negative for oxidase test, MR, indole production, TSI, nitrate reduction, gelatin and Tween80 hydrolysis. No acid production from D-amygdalin, L-arabinose, D-fructose, D-galactose, gluconate, lactose, D-mannose, α-methyl-D-glucoside, D-ribose, D-sorbitol, L-sorbose, D-trehalose and D-xylose. The tested strains had no *meso*-diaminopimelic acid in the cell wall but contained MK-11 and MK-12. DNA G +C contents were 71.0 to 71.5 mol%. On the basis of DNA-DNA similarity, 19 isolates (Group 1A) showed the high degree of homology with MXC3-1 over 70.0% (Table 22) except for the 3 isolates that had not been done for DNA-DNA hybridization. They were included in the same species (Wayne *et al*., 1987). The isolates (X7-2) in Group 1B showed high degree of homology with MXC2-3-1 over 70.0%, while MXC2-3-2 had the same physiological and biochemical characteristics as MXC2-3-1. They were included in the same species (Table 22).

Table 22 DNA G +C contents and DNA-DNA similarity of Group 1A isolates.

Isolate	DNA G+C (%mol)	%DNA similarity with labelled strain MXC3-1
Group 1A		
MXC3-1	71.5	100
MXC3-10-2		98.3
MXC3-5-1		95.0
MXC4-9-3		92.0
MX17-2		91.3
MX18		89.8
X9-2		89.2
ST1		88.0
MXC3-2		83.0
MXC4-5-2		81.4
MX16-2		80.7
MXC3-4-1		80.0
MXC4-1-1		75.5
MXC4-6-2		73.9
MXC3-7-1		70.5
MXC3-7-2		70.3
MXC4-5-1		70.0
MXC3-5-2		70.0
MXC4-1-2		70.0
		MXC2-3-1
Group 1B		
MXC2-3-1	71.0	100
X7-2		86.0
MXC2-3-2		57.8

Isolates of Group 1A, MXC 4-2-1 (1478 bp) and MXC4-6-2 (1443 bp) showed 99.27 and 99.41%, respectively of 16S rDNA sequence similarity to *Microbacterium barkeri* DSM 20145^{T} (Fig 6, Table 14). Their differential characteristics from

Microbacterium barkeri DSM 20145^T were shown in Table 23. Therefore, the 22 isolates in Group1A and MXC4-2-1 were identified as *Microbacterium barkeri* (Lee *et al*., 2006 ; Takeuchi and Hatano, 1998), and the 3 isolates in Group 1B were identified as *Microbacterium*.

Table 23 Differential characteristics of the isolates in Group 1A, *Microbacterium barkeri* DSM20145^T and Group1B (Matsuyama *et al*., 1999 ; Takeuchi and Hatano, 1998).

Characteristics	Group 1A 22 isolates	*M. barkeri* DSM 20145^T	Group 1B 3 isolates	*M. arborescens* IFO3750^T
Colour	Y	Y	O/Y	O
Growth at 37°C	+	+	+	-
Voges-Proksauer	+	-	+	-
Motility	+	+	+	+
H_2S formation	-	+	-	+
Acid from glucose	+	-	-	+
Hydrolysis of :				
Arginine	+	+	+	-
Gelatin	-	+	-	+
Starch	+	+	+	-
Tween 80	-	-	-	-

Group 2 contained 2 isolates (S3-4A and MX2-3).

They were Gram-positive, spore-forming (Fig.14), motile rods measuring from 0.5 to 0.8 by 2.0-5.0 μm. All grew in 3%NaCl, at pH 7-9, 20-45°C, anaerobic condition but not in 5%NaCl, at pH5-6 and at 10°C. Catalase, VP, hydrolysis of aesculin, starch and Tween 80 were positive but negative for MR, indole production, citrate, TSI, dihydroxyacetone, L-arginine, casein, gelatin and L-tyrosine. Acid production from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, inulin, D-maltose, raffinose, L-rhamnose, D-ribose, salicin, D-trehalose, D-xylose. No acid production from gluconate, inositol, D-melezitose, D-sorbitol and L-sorbose. The tested strains had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. DNA G+C content was 52.7 mol%.

Fig. 14 Scanning electron micrograph of S3-4A grown on XC medium at 37°C for 1 day.

Isolates of Group 2, S3-4A (1485 bp) and MX2-3 (1494 bp) showed 97.13% and 96.49% 16S rDNA sequence similarity (%) to *Paenibacillus agaridovorans* DSM1355^T (Fig 7, Table 15). Their differential characteristics from *Paenibacillus agaridovorans*

DSM1355^T were shown in Table 24. Therefore, the 2 isolates in Group 2 were identified as a new species in the genus *Paenibacillus* (Uetanabaro *et al*., 2003).

Table 24 Differential characteristics of S3-4A and MX2-3 in Group 2 and *Paenibacillus agaridevorans* DSM1355^T (Uetanabaro *et al*., 2003).

Characteristics	S3-4A	MX2-3	*P. agaridevorans* DSM 1355^T
Catalase	+	+	+
Oxidase	-	+	+
Anaerobic growth	+	+	-
Growth at 35°C	+	+	+
at pH 5.7	-	-	-
in 5%NaCl	-	-	-
Voges-Proskauer	+	+	-
Indole production	-	-	-
Nitrate reduction	-	+	-
DNase	+	-	-
Urease	-	+	-
Acid from glucose	+	+	+
Hydrolysis of :			
Aesculin	+	+	+
Casein	-	-	-
Starch	+	+	-
Tween 80	+	+	-
Tyrosine	-	-	-

Group 3 contained 5 isolates (S5-3, X13-1, MXC2-2, MXC4-3-1 and ST2).

They were Gram-positive, spore-forming, motile rods measuring from 0.5 to 1.0 by 3.0-7.0 μm. All grew in 3 and 5%NaCl, at pH 7-9, and at 20-45°C, and in anaerobic condition but not at pH 5-6, 10 and 55-60°C. VP, hydrolysis of aesculin, starch and Tween 80 were positive but negative for oxidase, MR, DNase, indole production, citrate, TSI, nitrate reduction, dihydroxyacetone, L-arginine, casein, gelatin and L-tyrosine hydrolysis. Acid production from D-amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, sucrose and D-xylose. No acid production from gluconate, inositol and L-sorbose. The tested strain had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C contents were 47.3 to 48.8 mol%.

Isolates of Group 3, X13-1 (1504 bp), S5-3 (1464 bp), ST2 (1474 bp), MXC4-3-1 (1490 bp) and MXC2-2 (1466 bp) showed 96.41, 95.85, 97.84, 97.61 and 96.49% of 16S rDNA sequence similarity to *Paenibacillus granivorans* A-30^T, respectively (Fig 7, Table 15). Therefore, the 5 isolates in Group 3 were identified as a new species in the genus *Paenibacillus* (Van der Maarel *et al*., 2001).

Group 4 contained 12 isolates, as shown in Table 11.

They were Gram-variable, spore-forming, motile rods measuring from 0.5 to 2.0 by 2.0-7.0 μm. All grew in 3% NaCl, at pH 7-9, and at 15-50°C, and in anaerobic condition but not at pH 5-6, 10 and at 60°C. Catalase, VP and Tween 80 hydrolysis were positive but negative for MR, indole production, citrate, TSI, dihydroxyacetone, L-arginine, gelatin and L-tyrosine hydrolysis. No acid production from gluconate and L-sorbose. The tested isolates had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C contents were 41.7 to 56.2 mol%.

Group 5 contained 1 isolate (MXC4-2-2).

This isolate was Gram-negative, spore-forming, motile rod measuring from 0.5 to 1.0 by 5.0-7.0 μm. This strain grew in 3 and 5% NaCl, at pH 7-9 and at 40-55°C, and in anaerobic condition but not at pH 5-6, 10-20 and at 60°C. Catalase, VP, urease, nitrate reduction, hydrolysis of aesculin, casein and Tween 80 were positive but negative for oxidase, MR, DNase, indole production, citrate, TSI, dihydroxyacetone, L-arginine, gelatin, starch, L-tyrosine hydrolysis. Acid production from D-amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, inulin, lactose, D-maltose, D-melibiose, D-melezitose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, sucrose, D-trehalose and D-xylose. No acid production from gluconate, inositol, D-mannitol, D-mannose, D-sorbitol and L-sorbose. It had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C content was 61.6 mol%.

Isolates of Group 4A, X8-1 (1490 bp) and MX8-1 (1518 bp) showed 99.06 and 98.95% of 16S rDNA sequence similarity to *Paenibacillus favisporus* GMP01^T (Fig 8, Table 16) while a isolate of Group 4B, X15-1(1476 bp) showed 99.69% of 16S rDNA sequence similarity to *Paenibacillus naphthalenovorans* PR-N1^T (Fig 8, Table 16). Isolate in Group 4C , X11-1 (1058 bp) and in Group 5, MXC4-2-2 (1476 bp) showed 96.59 and 93.9% of 16S rDNA sequence similarity to *Paenibacillus validus* JCM9077^T (Fig 8, Table 16). Therefore, MX8-1 in Group4A , X11-1 in Group 4C and MXC4-2-2 in Group 5 were identified as a new species in the genus *Paenibacillus* (Daane *et al*., 2002 ; Velazquez *et al*., 2004) while X8-1 was identified as *Paenibacillus favisporus* GMP01^T (Velazquez *et al*., 2004) and X15-1 was *Paenibacillus naphthalenovorans* PR-N1^T (Daane *et al*., 2002).

Table 25 Differential characteristics of the isolates in Group 4A (X8-1, X9-1, MX6-2 and MX8-1), *P. favisporus* GMP01^T (Velazquez *et al*., 2004), Group 4B(X5-1, X15-1 and X19-1) and *P. napthalenovorans* PR-N1^T (Daane *et al*., 2002)

Characteristics	Group4A 4 isolates	*P. favisporus* GMP 01^T	Group4B 3 isolates	*P. naphthalenovorans* PR-N1^T
Anaerobic growth	+	+	+	-
Catalase	+	+	+	+
Growth in 5%NaCl	+	w	-	-
Nitrate reduction	-	+	+	V
Citrate	-	-	-	V
Indole production	-	-	-	-
Hydrolysis of :				
Casein	+(-2)	-	-	-
Starch	+(-2)	+	-	V

Table 26 Differential characteristics of Group 4C(X8-2, X11-1, X11-2, X12-2 and X19-2), Group 5 (MXC4-2-2) and *Paenibacillus validus* JCM 9077^{T} (Daane *et al*., 2002).

Characteristics	Group 4C 5 isolates	Group 5 1 isolate	*P. validus* JCM 9077^{T}
Anaerobic growth	+	+	-
Growth with 3%NaCl	+	+	-
Growth at 10°C	-	-	-
Hydrolysis of :			
Aesculin	W	+	+
Casein	-	+	-
Gelatin	-	-	-
Starch	-	-	+
Citrate	-	-	-
Urease	+	+	+
Indole production	-	-	-
Voges-Proskauer	+	+	-
Nitrate reduction	W	+	-
Acid production from :			
Glycerol	-	+	+
L-Arabinose	-	+	-
Ribose	-	+	+
D-Xylose	-	+	+
Galactose	-	+	+
D-Glucose	-	+	+
D-Fructose	-	+	+
Rhamnose	-	+	-
Mannitol	+	-	+
Amygdalin	-	+	-
Salicin	-	+	-
Cellobiose	-	+	-

Group 6 contained 1 isolate, S2-1.

This isolate was Gram-positive, spore-forming, motile rod measuring from 1.0 to 2.0 by 2.0-4.0 μm. This strain grew in 3 and 5% NaCl, at pH 7-9, and at 15-55°C, and in anaerobic condition but not at pH 5-6, 10 and at 60°C. VP, DNase, urease, nitrate reduction, hydrolysis of aesculin, starch and Tween 80 were positive but negative for catalase, oxidase, MR, indole production, citrate, TSI, dihydroxyacetone, L-arginine, casein, gelatin, L-tyrosine hydrolysis. Acid production from D-amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, inositol, lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, D-trehalose and D-xylose. No acid production from gluconate , inulin, D-melezitose, L-sorbose and sucrose. It had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C content was 39.3 mol%. This isolate showed 99.41% of 16S rDNA sequence similarity to *Bacillus niabensis* 4T19^{T} as shown in (Fig 9 and Table 17), therefore it was identified as *Bacillus niabensis*.

Group 7 contained 1 isolate, MX1-1.

This isolate was Gram-positive, spore-forming, motile rod measuring from 0.8 to 2.0 by 5.0-11.0 μm. This strain grew in 3 and 5% NaCl, at pH 7-9, and at 15-55°C, and anaerobic condition but not at pH 5-6, 10 and at 60°C. Catalase, oxidase, VP, urease, nitrate reduction, hydrolysis of aesculin and starch were positive but negative for MR, DNase, indole production, citrate, TSI, dihydroxyacetone, L-arginine, gelatin, L-tyrosine and Tween 80 hydrolysis. Acid production from D-amygdalin, D-cellobiose, glycerol, inulin, lactose, D-maltose, D-mannitol, D-melibiose, D-melezitose, α-methyl-D-glucoside, raffinose, salicin, sucrose and D-trehalose. No acid production from L-arabinose, D-fructose, D-galactose, D-glucose, gluconate, inositol, D-mannose, L-rhamnose, D-ribose,

D-sorbitol, L-sorbose and D-xylose. . It had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C content was 37.3 mol%. This strain showed 99.33% of 16S rDNA sequence similarity to *Bacillus megaterium* IAM13418^{T} as shown in (Fig 9 and Table 17) therefore, it was identified as *Bacillus megaterium*.

Group 8 contained 6 isolates, (MX1-2, MX2-1, MX3-2, MX12-2, MXC1-3 and MXC3-4-2).

They were Gram-positive, spore-forming, motile rods measuring from 0.8 to 2.0 by 4.0-6.0 μm. All grew at pH 7-9, 20-40°C, and in anaerobic condition but not in 3 and 5%NaCl, pH5-6, 10 and at 50-60°C. Catalase, and VP were positive but negative for urease, indole production, citrate, TSI, dihydroxyacetone, gelatin, L-tyrosine and Tween 80 hydrolysis. Acid production from D-galactose and D-glucose. No acid production from D-amygdalin, D-cellobiose, gluconate, glycerol, inositol, inulin, lactose, D-maltose, D-mannitol, D-mannose, D-melezitose, α-methyl-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, sucrose, D-trehalose and D-xylose. The tested strain had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C contents were 36.6 to 43.9 mol%. On the basis of DNA-DNA similarity, 6 isolates showed the high degree of homology with MX12-2 over 70.0% (Table 27). They were included in the same species (Wayne *et al*., 1987).

Table 27 DNA G +C contents and DNA-DNA similarity of Group 8 isolates.

Isolate	DNA G+C (%mol)	%DNA similarity with labelled strains MX12-2
MX12-2	43.9	100
MXC1-3		100
MX1-2	36.6	80.07
MX2-1		78.03
MX3-2		70.42
MXC3-4-2		70.42

Isolates of Group 8, MX1-2(1483 bp), MX2-1(1476 bp), MX3-2(1491 bp), MX12-2(1475 bp), MXC1-3(1463 bp) and MXC3-4-2(1474 bp) showed 99.63, 99.33, 99.48, 99.48, 99.78 and 99.78% of 16S rDNA sequence similarity to *Bacillus funiculus* NAF001^T, respectively (Fig 9, Table 17). Therefore, the 6 isolates in Group 8 were identified as *Bacillus funiculus* NAF001^T (Ajithkumar *et al*., 2002)

Table 28 Differential characteristics of the 6 isolates in Group 8 and *Bacillus funiculus* NAF001^T.

Characteristics	Group 8 6 isolates	*Bacilus funiculus* NAF001^T
Catalase	-	+
Oxidase	+ (-2)	-
Indole production	-	-
Anaerobic growth	+	-
Nitrate reduction	- (+1)	+
Hydrolysis of :		
Aesculin	+(-3)	+
Casein	+(-1)	-
Gelatin	-	-
Starch	+	+
Tween 80	+(-3)	-

Group 9 contained 3 isolates, (S1-3, MX15-2 and MX21-2).

They were Gram-variable, spore-forming, non-motile rods measuring from 0.5 to 1.0 by 2.0-6.0 μm. All grew at pH7-9, 20-45°C, and in anaerobic condition but not in 5%NaCl, pH 5-6, 10-15 and at 60°C. VP, hydrolysis of aesculin and starch were positive but negative for MR, indole production, citrate, TSI, nitrate reduction, dihroxyacetone, L-arginine, gelatin, L-tyrosine hydrolysis. Acid production from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannose, α-methyl-D-glucoside, raffinose, D-ribose, salicin, sucrose, D-trehalose and D-xylose. No acid production from gluconate, inositol, inulin, D-melezitose and L-sorbose. The tested

isolates had *meso*-diaminopimelic acid in the cell wall and contained MK-7 as a major menaquinone. The DNA G+C content was 53.3 mol%.

The representative strains of Group 9, S1-3(1499 bp), MX15-2(1494 bp) and MX21-2(1081 bp) showed 95.92, 99.21 and 98.3% of 16S rDNA sequence similarity to *Cohnella*^{T} (Fig 10, Table 18). Therefore, the 3 isolates in Group 9 were identified as a new species in the genus *Cohnella* (Kampfer *et al*., 2003).

Group 10 contained 1 isolate, MX8-2.

This isolate was Gram-negative, non spore-forming, motile rod measuring from 0.5 to 0.8 by 0.9-1.4 μm. This isolate grew in 3 and 5% NaCl, at pH 6-9, 15-50°C, and in anaerobic condition but not at pH 5, 10 and at 55-60°C. Oxidase, VP, urease, hydrolysis of aesculin and L-arginine were positive but negative for catalase, MR, DNase, indole production, citrate, TSI, nitrate reduction, dihydroxyacetone, caseine, gelatin, starch, L-tyrosine and Tween 80 hydrolysis. No acid production from D-amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, gluconate, glycerol, inositol, inulin, lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-melezitose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, D-trehalose and D-xylose. It had no *meso*-diaminopimelic acid in the cell wall and contained Q-8 as a major ubiquinone. This isolate showed 99.62% of 16S rDNA sequence similarity to *Pseudoxanthobacterium suwonensis* 4M1^{T} as shown in (Fig 11 and Table 19) therefore, it was identified as *Pseudoxanthomonas suwonensis* (Weon *et al*., 2006).

Group 11 contained 1 isolate, MXC3-9.

This isolate was Gram-negative, non spore-forming, motile rod measuring from 0.5 to 1.0 by 1.0-2.0 μm. This strain grew in 3% NaCl, pH 5-9, 15-50°C, and in anaerobic condition but not in 5%NaCl, at pH 10 and at 55-60°C. Catalase, oxidase, VP, DNase,

urease, citrate, hydrolysis of L-arginine, L-tyrosine and Tween 80 were positive but negative for MR, indole production, TSI, nitrate reduction, dihydroxyacetone, casein, gelatin and starch hydrolysis. No acid production from D-amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, gluconate, glycerol, inositol, inulin, lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-melezitose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, D-trehalose and D-xylose. . It had no *meso*-diaminopimelic acid in the cell wall and contained Q-8 as a major ubiquinone. This isolate showed 99.04% of 16S rDNA sequence similarity to *Cupriavidus gilardii* LMG5886^T as shown in (Fig 12 and Table 20) therefore, it was identified as *Cupriavidus gilardii* (Coenye *et al*., 1999).

Group 12 contained 2 isolates, (SF and MXC4-7-1).

They were Gram-positive, non spore-forming, non motile rods/cocci measuring from 0.2 to 0.5 by 1.5-3.0 μm. All grew in 3 and 5% NaCl, pH 6-9, and at 15-45°C, and in anaerobic condition but not at pH 5, and at 50-60°C. 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, casein, gelatin and starch hydrolysis. No acid production from D-amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, gluconate, glycerol, inositol, inulin, lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-melezitose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, D-trehalose and D-xylose. The tested isolates had *meso*-diaminopimelic acid in the cell wall and contained MK-8(H_4) as a major menaquinone. The DNA G+C content was 61.4 mol%.

The representative isolates of Group 12, SF(1490 bp) showed 99.55% of 16S rDNA sequence similarity to *Rhodococcus rhodochrous* DSM43241^T (Fig 13, Table 21).

Therefore, the isolate SF and MXC4-7-1 were identified as *Rhodococcus rhodochrous* (Yoon *et al*., 2000).

Table 29 Differential characteristics of SF and MXC4-7-1 in Group 12 and *Rhodococcus rhodochrous* DSM 43241^T.

Characteristics	Group 12 2 isolates	*R. rhodochrous* DSM 43241^T
Catalase	+	+
Oxidase	-	-
DNase	+	-
Voges-Proskauer	W	-
Methyl Red	-	-
Nitrate reduction	+	+
Production H_2S	-	-
Hydrolysis:		
Aesculin	W	+
Casein	-	-
Starch	-	-
Tyrosine	+	+

As described above, the xylanase producing bacteria were isolated and found to be diverse species in soil samples collected in Nan province. Nine known species and thirteen novel species were isolated. The isolates, *Microbacterium barkeri*, *P. favisporus, P. naphthalenovorans*, new species of *Paenibacillus* that closed to *P. granivorans* (1 new species) and *P. validus* (1 new species) were isolated from soils collected in Viengsa districts while *Microbacterium barkeri* ; novel species of *Paenibacillus* that closed to *P.*

agaridevorans (2 new species), *P. granivorans* (4 new species), *P. favisporus* (1 new species) and *P. validus* (1 new species); *Bacillus niabensis; B. funiculus;* a new species of *Bacillus* that was closed to *B. megaterium;* 3 new species of *Cohnella; Pseudoxanthomonas suwonensis; Cupriavidus gilardii,* and *Rhodococcus rhodochrous*, were distributed in soils samples collected in Muang district (Table 30).

Table 30 Biodiversity of xylanase producing bacteria in NAN.

Location (District)	Isolate no.	Closest Species	%Similarity of 16S rDNA	Group	Identification
Viengsa	X7-2	*M. barkeri*		1A	*M. barkeri*
	X13-1	*P. granivorans*	96.41	3	*Paenibacillus*
	X8-1	*P. favisporus*	99.06	4A	*P. favisporus*
	X15-1	*P. napthalenovorans*	99.69	4B	*P. napthalenovorans*
	X11-1	*P. validus*	96.59	4C	*Paenibacillus*
Muang	MXC4-2-1	*M. barkeri*	99.27	1A	*M. barkeri*
	MXC4-6-2	*M. barkeri*	99.41	1A	*M. barkeri*
	S3-4A	*P. agaridovorans*	97.13	2	*Paenibacillus*
	MX2-3	*P. agaridovorans*	96.49	2	*Paenibacillus*
	S5-3	*P. granivorans*	95.85	3	*Paenibacillus*
	MXC2-2	*P. granivorans*	96.49	3	*Paenibacillus*
	MXC4-3-1	*P. granivorans*	97.61	3	*Paenibacillus*
	ST2	*P. granivorans*	97.84	3	*Paenibacillus*
	MX8-1	*P. favisporus*	98.95	4A	*Paenibacillus*
	MXC4-2-2	*P. validus*	93.90	5	*Paenibacillus*
	S2-1	*B. niabensis*	99.41	6	*B. niabensis*
	MX1-1	*B. megaterium*	99.33	7	*B. megaterium*

Location (District)	Isolate no.	Closest Species	%Similarity of 16S rDNA	Group	Identification
Muang	MX1-2	*B. funiculus*	99.63	8	*B. funiculus*
	MX2-1	*B. funiculus*	99.33	8	*B. funiculus*
	MX3-2	*B. funiculus*	99.48	8	*B. funiculus*
	MX12-2	*B. funiculus*	99.48	8	*B. funiculus*
	MXC1-3	*B. funiculus*	99.78	8	*B. funiculus*
	MXC3-4-2	*B. funiculus*	99.78	8	*B. funiculus*
	S1-3	*Cohnella*	95.92	9	*Cohnella*
	MX15-2	*Cohnella*	99.21	9	*Cohnella*
	MX21-2	*Cohnella*	98.30	9	*Cohnella*
	MX8-2	*Px .suwonensis*	99.62	10	*Px. suwonensis*
	MXC3-9	*Cu. gilardiis*	99.04	11	*Cu. gilardiis*
	SF	*R. rhodochrous*	99.55	12	*R. rhodochrous*

3. Optimization of crude xylanase production

Optimization of crude xylanase production of the best xylanase producing strain, S3-4A, was carried out in oat spelt xylan medium. The influence of several factors *e.g.* medium composition, initial pH, incubation temperature *etc.* on xylanase production were studied. Cell-free supernatants were analysed for xylanase activity. An effective prior condition was used as the basis for the latter experiment until the optimum condition was obtained.

3.1 Effect of medium composition on xylanase production

The strain S3-4A was cultivated in modified oat spelt xylan (XC) medium, pH 7.0 and incubated with shaking (200 rpm) at 40^{o}C for 2 days. The XC medium was modified by using peptone or tryptone at 0.5% (w/v) instead of polypeptone. The result was shown in Fig. 15. Maximum xylanase production (0.24 U/ml) was obtained in the modified XC medium containing peptone. Optimum concentration for xylanase production of peptone was 0.5% (w/v) (Fig. 16).

Optimum concentrations of other ingredients (w/v) were 1.0% oat spelt xylan (Fig. 17), 0.1% yeast extract (Fig. 18), 0.4% K_2HPO_4 (Fig. 19), and 0.05% $MgSO_4.7H_2O$ (Fig. 20) which exhibited 0.24 unit/ml xylanase activity.

3.2 Effect of initial pH on xylanase production

The strain S3-4A was cultivated in XC medium which was adjusted to pH 5.0, 6.0, 7.0, 7.2, 8.0, 9.0, 10.0 or 11.0; and incubated at the same above conditions for 2 days. As shown in figure 21, the optimum pH for xylanase production (0.30 U/ml) was 7.5. The strain S3-4A produced xylanase at pH 7.0 to 8.5 but there was no xylanase production at pH 6.5 or lower and pH 9.0 or above.

3.3 Effect of incubation temperature on xylanase production

The strain S3-4A was cultivated in XC medium (pH 7.5) with shaking (200 rpm) and incubated at 30, 35, 40, 45, 55, 60 and 65^{o}C for 2 days. The optimum temperature for xylanase production (0.43U/ml) was 55^{o}C as shown in Fig. 22.

To optimize the xylanase production, the strain S3-4A was cultivated in XC medium, pH 7.5, 55^{o}C for 7 days. Every day, xylanase activity in cell-free supernatant was analysed. Maximum xylanase (0.43 U/ml) was produced obtained after 5 days of incubation (Figure 23).

Characterization of crude xylanase

Some characteristics of crude xylanase produced by strain S3-4A were determined using oat splet xylan as substrate.

Optimal temperature : Xylanase activity at pH 7.0 was assayed at various temperature. Optimal temperature for xylanase activity was 50^{o}C (Figure 24).

Optimal pH : Xylanase activity assay was carried out over the pH range of 4 to 9 at 50^{o}C. Optimal pH for xylanase activity was 7.0 (Figure 25).

Temperature stability : After incubation in 50 mM phosphate buffer pH 7.0 at various temperatures for 30 min, xylanase activity was assayed at 50^{o}C pH 7.0. Xylanase activity of non-treated enzyme was set as 100%. The residual xylanase activity after incubated at 40 and 65^{o}C for 30 min was 95 and 35%, respectively (Figure 26) .

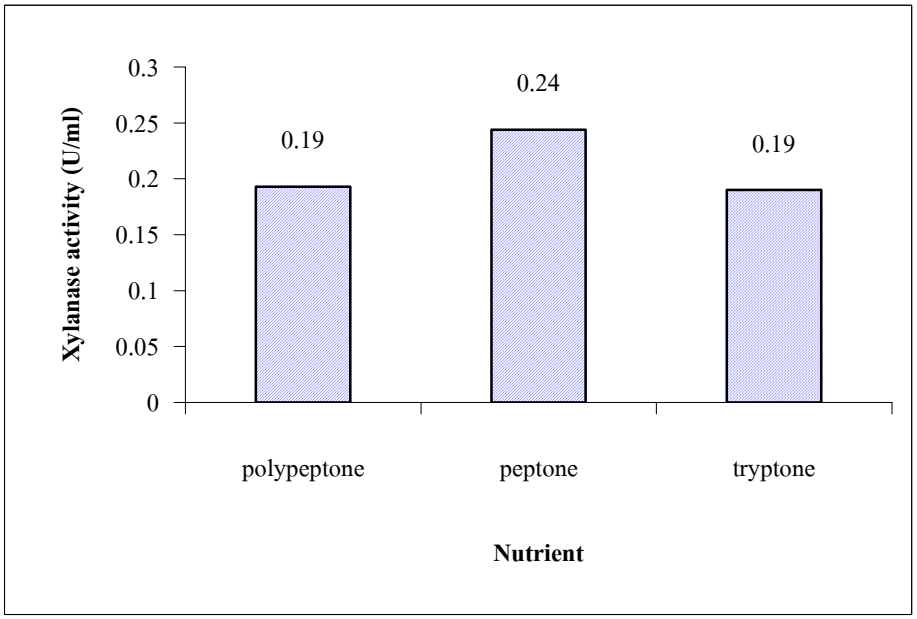

Fig. 15 Comparison of xylanase production in modified XC medium containing polypeptone, peptone or trytone at 0.5% (w/v).

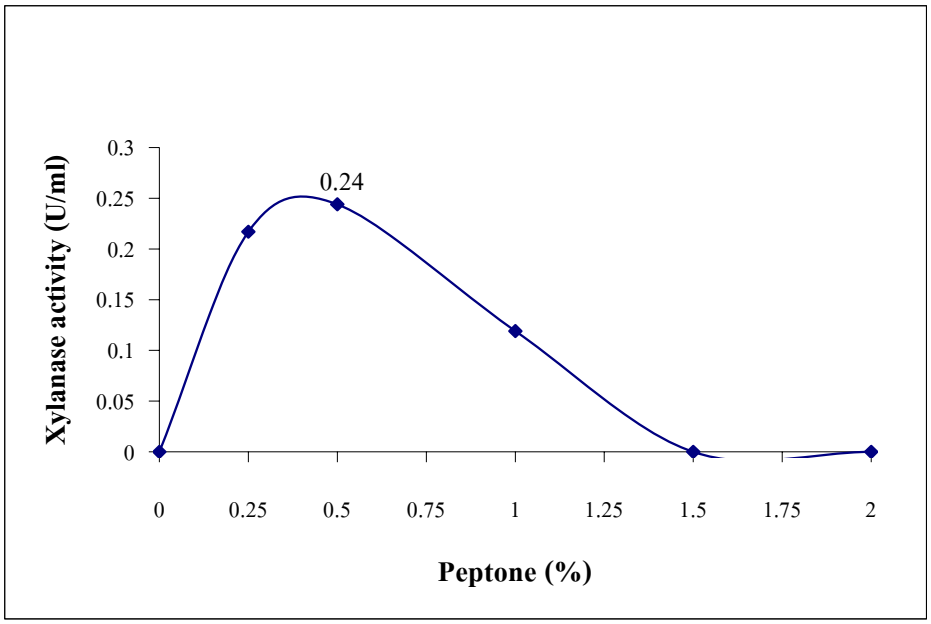

Fig. 16 Effect of peptone concentration on xylanase production.

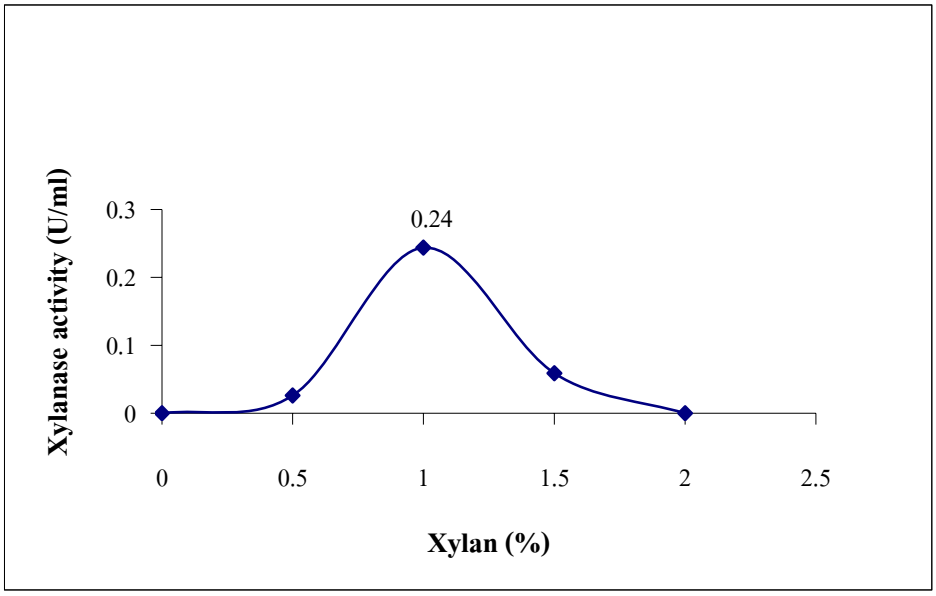

Fig. 17 Effect of oat spelt xylan concentration on xylanase production.

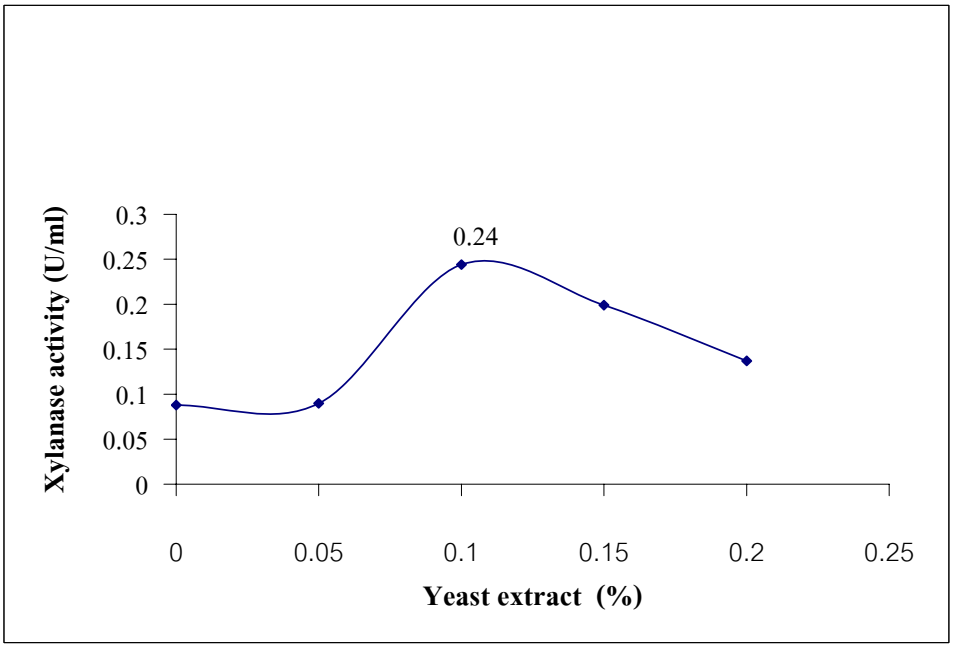

Fig. 18 Effect of yeast extract concentration on xylanase production.

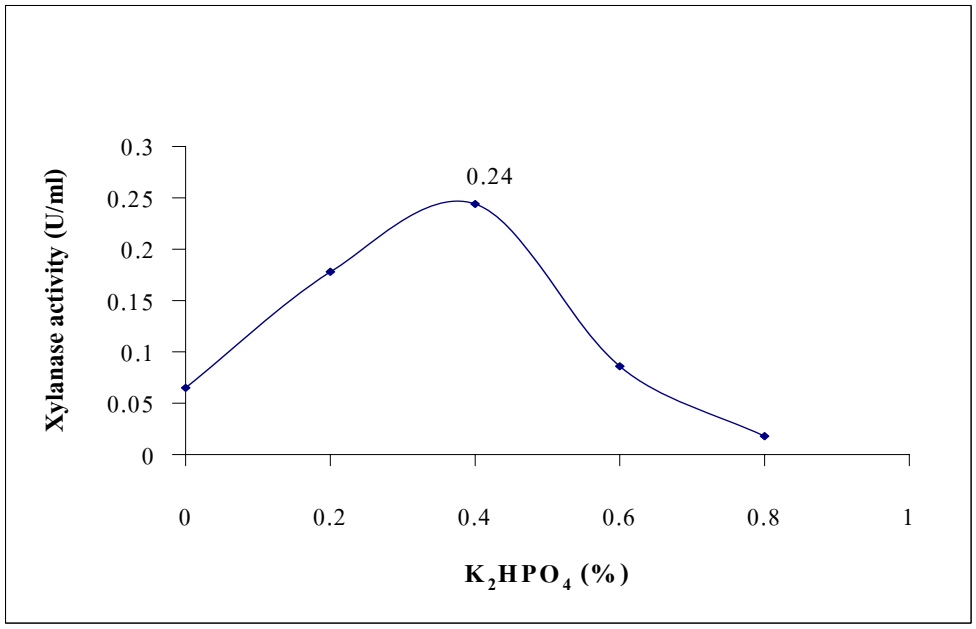

Fig. 19 Effect of K_2HPO_4 concentration on xylanase production.

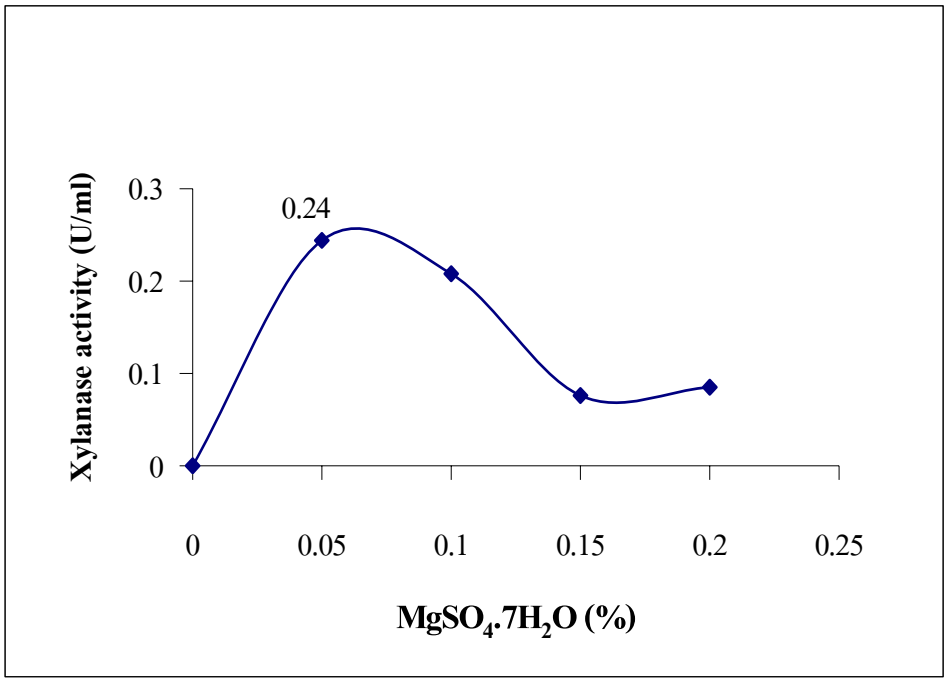

Fig. 20 Effect of $MgSO_4.7H_2O$ concentration on xylanase production.

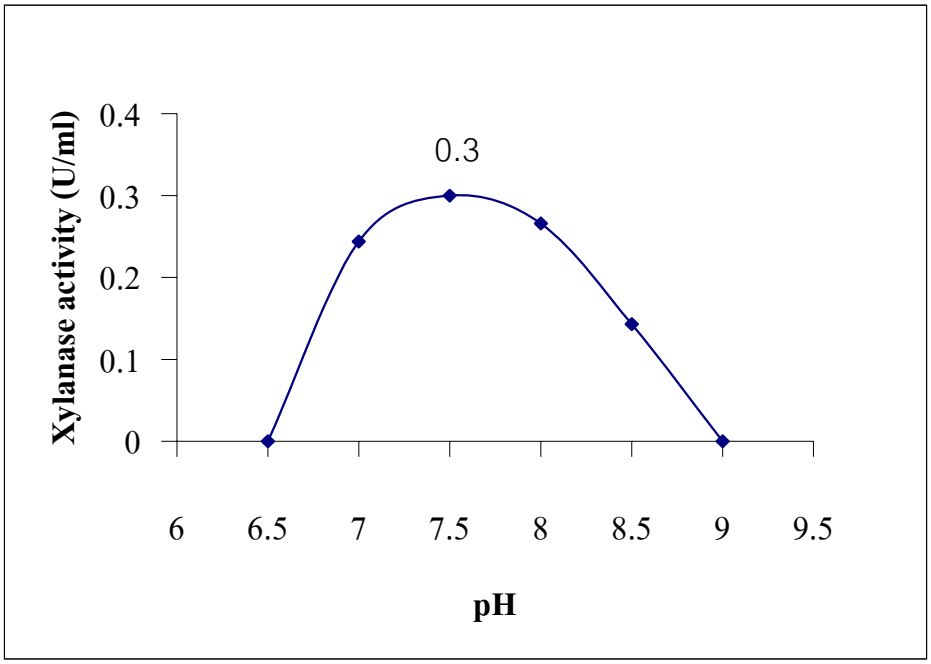

Fig. 21 Effect of initial pH on xylanase production.

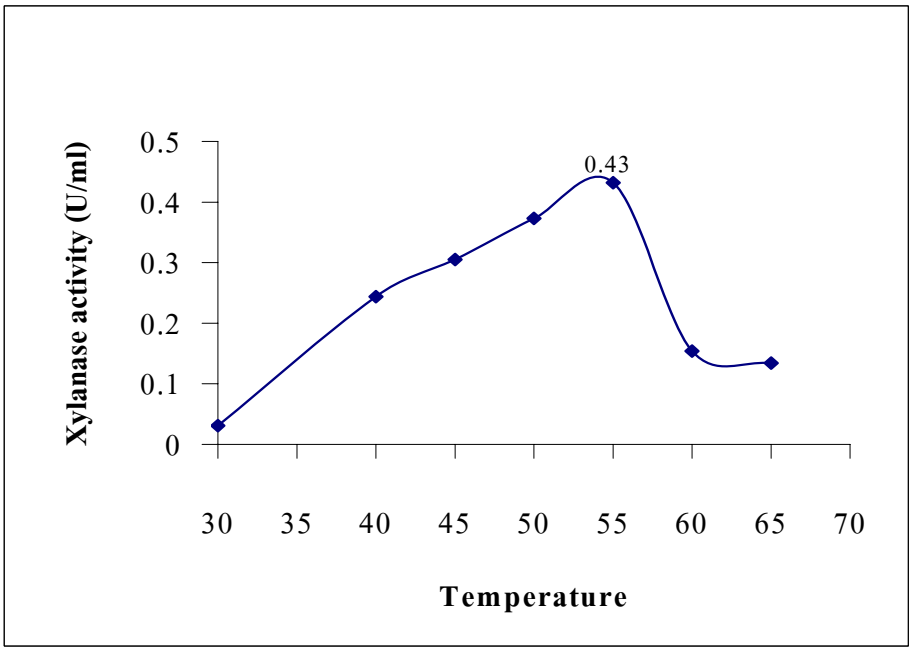

Fig. 22 Effect of incubation temperature on xylanase production

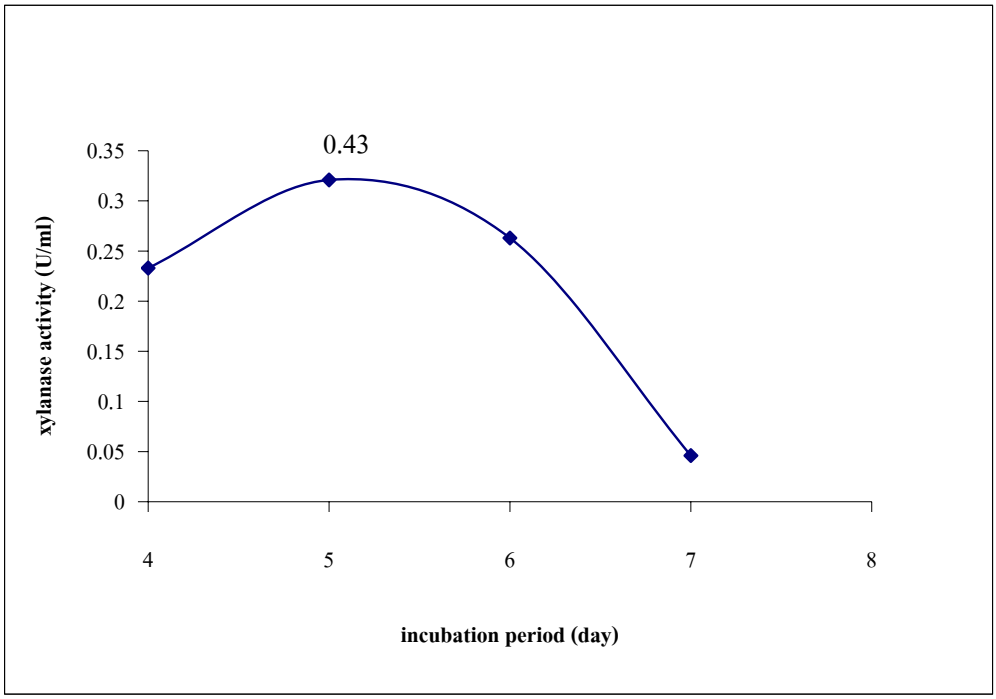

Fig. 23 Effect of incubation period on xylanase production.

Characterization of crude xylanase

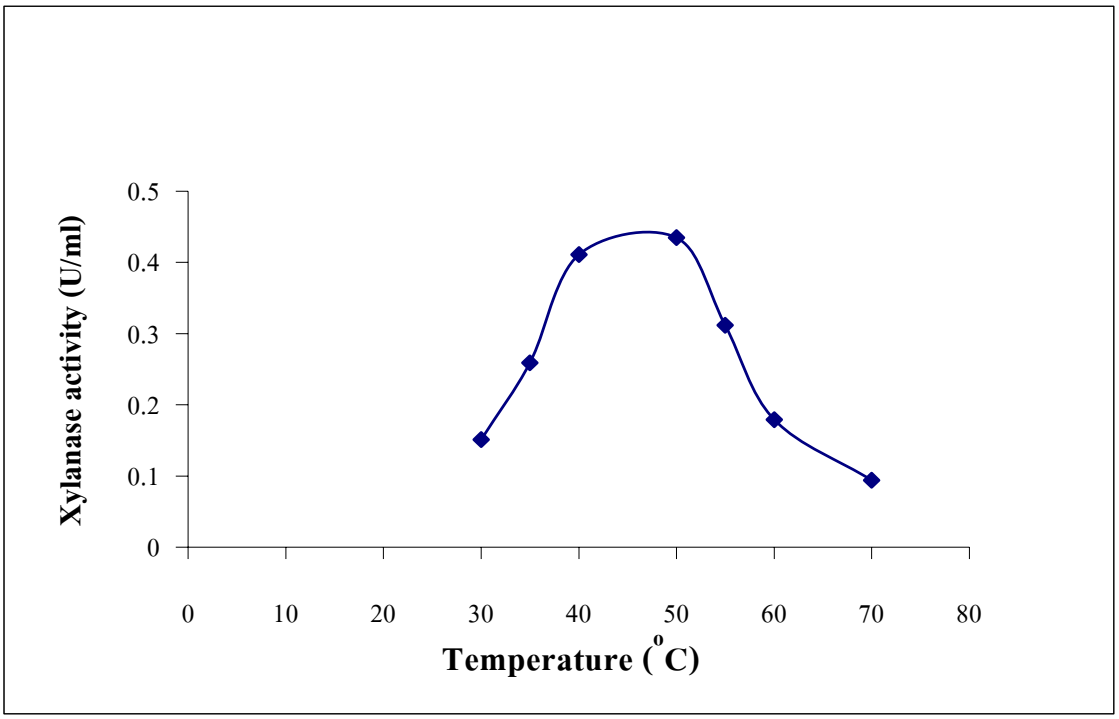

Fig. 24 Optimum temperature for xylanase activity.

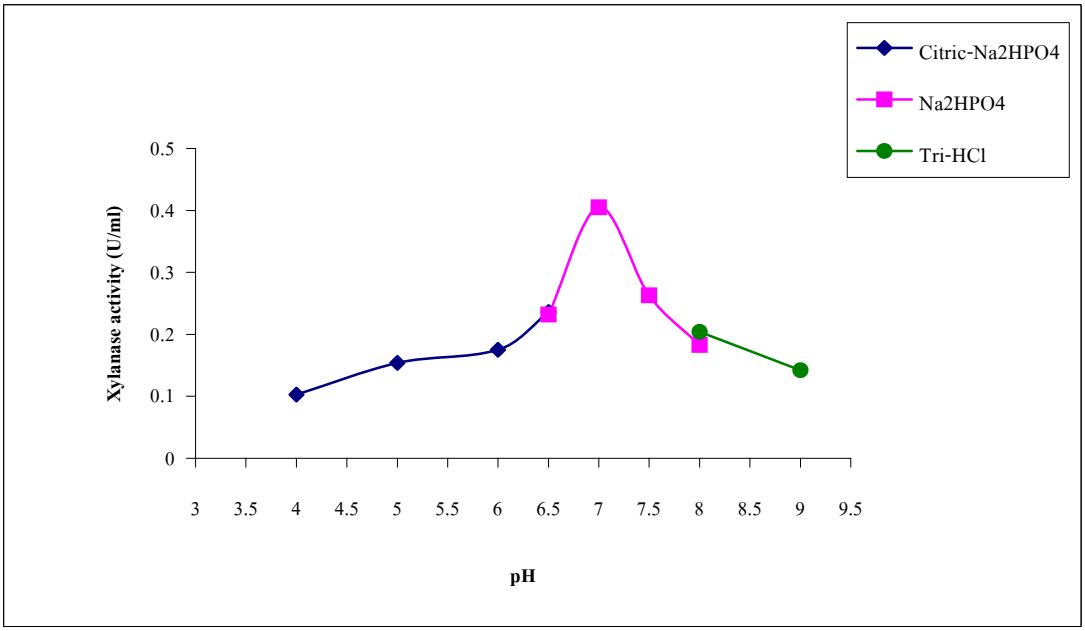

Fig. 25 Optimum pH for xylanase activity.

Stability of crude xylanase

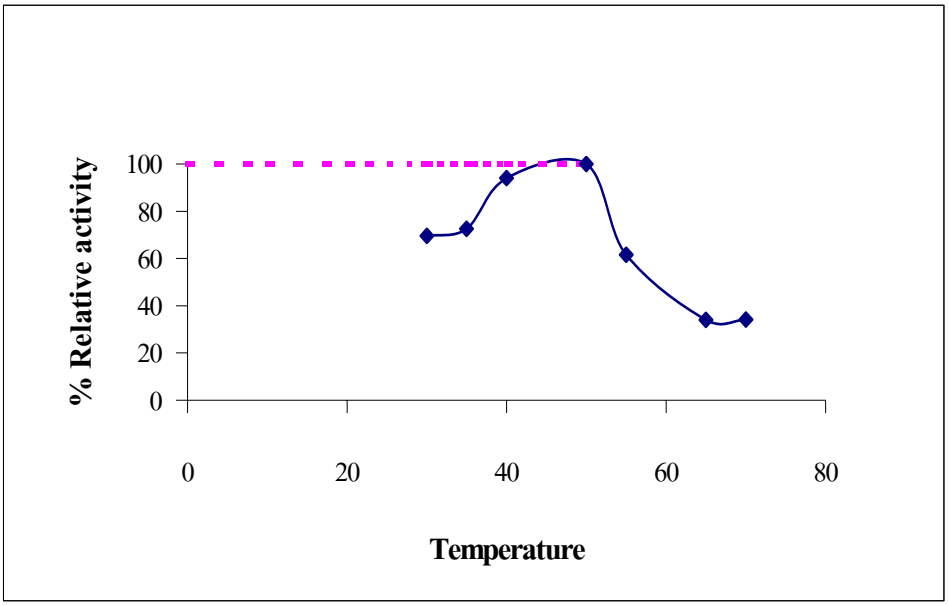

Fig. 26 Temperature stability of xylanase. Activity of non-treated xylanase was set as 100%.

CHAPTER V

CONCLUSION

In the course of investigation of thermotolerant bacteria presented in soils from NAN province, 60 bacterial isolates were screened and characterized them taxonomically. There were diversity of xylanase producing bacterial isolates. Fifty-eight isolates of rods and two isolates of rods-cocci were divided into twelve groups based on their phenotypic and chemotaxonomic characteristics, DNA-DNA similarity and 16S rDNA sequencing. Twenty-five Gram-positive, non spore-forming, rod-shaped isolates of Group 1 were closed to *Microbacterium barkeri* DSM20145^T with 99.27 and 99.41% similarity of 16S rDNA sequence, respectively. The predominant menaquinone with eleven and twelve isoprene units (MK-11 and MK-12) and high DNA G+C contents (71.4 to 71.5 mol%) were presented. They were identified as *Microbacterium barkeri*.

A Group 2 (2 isolates) of Gram-positive, spore-forming rods contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan and had 52.7 mol% of G+C contents, were closed to *Paenibacillus agaridevorans* DSM1355^T with 97.13 and 96.49% similarity of 16S rDNA sequence. They were the novel species of the genus *Paenibacillus.* Five isolates in Group 3 were Gram-positive, spore-forming rods that contained *meso*-diaminopimelic acid in the cell wall peptidoglycan. They had MK-7 as a major menaquinone. DNA G+C contents were 47.3 to 48.8 mol%. They were closed to *Paenibacillus granivorans* A30^T with 96.41, 95.85, 97.84, 97.61 and 96.49% similarity of 16S rDNA sequence and were identified as a new species of the genus *Paenibacillus.* Group 4 (12 isolates) was Gram-positive or Gram-variable, spore-forming rods contained MK-7 in membrane and *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C contents were 41.7 to 56.2 mol%. They were closed to *Paenibacillus faviporus* GMP01^T, *Paenibacillus naphthalenovorans* PR-N1^T and *Paenibacillus validus*

JCM9077^T with 98.95 and 99.66%, 99.69% , and 96.59% similarity of 16S rDNA sequence, respectively and were identified as the novel species of the genus *Paenibacillus* while 1 isolate(99.66%) was identified as *Paenibacillus faviporus* and 1 isolate (99.69%) was *Paenibacillus validus.* Group 5(1 isolate) Gram-positive or Gram-variable, spore-forming rods contained MK-7 as a major menaquinone and *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C content was 61.6 mol%. This isolate was closed to *Paenibacillus validus* JCM9077^T with 93.9% similarity of 16S rDNA sequence and was identified as a new species of the genus *Paenibacillus.*

Group 6 (1 isolate) was Gram-positive, spore-forming rods contained MK-7 as a major menaquinone and *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C content was 39.3% mol. This isolate was closed to *Bacillus niabensis* 4T19^{T}with 99.41% similarity of 16S rDNA sequence that was identified as *Bacillus niabensis.* Group 7 (1 isolate) was Gram-positive, spore-forming rods. This isolate contained MK-7 as a major menaquinone and *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C content was 37.3 mol%. It was closed to *Bacillus megaterium* IAM13418^T with 99.33% similarity of 16S rDNA sequence that was identified as *Bacillus megaterium*. Eight isolates of Group 8 were Gram-positive, spore-forming rods. They contained MK-7 as a major menaquinone and contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C contents were 36.6 to 43.9 mol%. They were closed to *Bacillus funiculus* NAF001^T with 99.33, 99.48, 99.48, 99.63, 99.78 and 99.78% similarity of 16S rDNA sequence that was identified as *Bacillus funiculus*.

Three isolates of Group 9 were Gram-variable, spore-forming rods. The predominant menaquinone with seven isoprene units (MK-7) was foun. They contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C content was 53.3 mol%. They were closed to

Cohnella ^{T} with 95.92, 98.30 and 99.21% similarity of 16S rDNA sequence that were identified as a novel species of the genus *Cohnella.* One isolate of Group 10 was Gram-negative, non spore-forming rods. The predominant ubiquinone with eight (Q-8) was found. Its phenotypic characteristics agreed with *Pseudoxanthobacterium suwonensis* 4M1^{T}. This strain was closed to *Pseudoxanthomonas suwonensis* 4M1^{T} with 99.62% similarity of 16S rDNA sequence that was identified as a known species of the genus *Pseudoxanthomonas.* An isolate of Group 11 was Gram-negative, non spore-forming rods. The predominant of Q-8 was found. All phenotypic characteristics of isolate agreed with *Cupriavidus gilardii* LMG5886^{T}. This strain was closed to *Cupriavidus gilardii* LMG5886^{T} with 99.04% similarity of 16S rDNA sequence that was identified as a known species of the genus *Cupriavidus.* Two isolates of Group 12 were Gram-positive, non spore-forming rods/cocci. The predominant menaquinone with eight isoprene units, MK-8(H_4) was found and they contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. The DNA G+C content was 61.4 mol%. The representative strain was closed to *Rhodococcus rhodochrous* DSM43241^{T} with 99.55% similarity of 16S rDNA sequence that was identified as a known species of the genus *Rhodococcus.*

The 60 isolates exhibited a xylanolytic clear zone ranged from 0.4-3.5 cm in diameter and produced a maximum enzyme of 0.20 units/ml. Strain S3-4A was found to produce a maximal xylanase at 0.20 units/ml. The best xylanase producing strain, S3-4A identified as *Paenibacillus* was selected to optimize xylanase production in XC medium. Maximum xylanase production was obtained in the modified XC medium containing peptone. Optimum concentration for xylanase production of peptone was 0.5% (w/v). Optimum concentration of other ingradients were as following : 1.0% (w/v) Oat splet xylan, 0.1%(w/v) yeast extract, 0.4%(w/v) K_2HPO_4, and 0.05%(w/v) $MgSO_4.7H_2O$. The strain S3-4A produced xylanase at pH 7.0 to 8.5 but there was no xylanase production at pH 6.5 or lower and pH 9.0 or above. The optimal condition for

xylanase production was at pH 7.5, 55°C and for 5 days of incubation. The thermal stability of crude xylanase of S3-4A was 35-50°C.

In this study, a lot of novel species of xylanase producing bacteria were found in soils samples collected from Nan province. The 16S rDNA sequencing results were useful to indicate the taxonomic position of the isolates however the DNA-DNA hybridization of the isolates with the closed type strains of each species are required for further studies in order to propose them as the new species.

As mentioned above, to obtain the divere xylanase-producing bacteria, a number of soil samples should be collected; to obtain the high activity of xylanase, the basal medium for growth should be suitable; to apply the xylanase-producing bacteria, compost and bioethanol.

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APPENDICES

APPENDIX A

Instruments, materials, chemical reagents and glassware

1. Instruments and materials

- Analytical balance: Mettler Toledo model AG204, Switzerland.
- Autoclave: Tomy model SS-325, Japan.
- Centrifuges: Beckman model Avanti J25, U.S.A; Eppendorf model 5430, Germany; Sorvall model RC-5C Plus and Sorvall tabletop centrifuge model RC-5C Plus, USA.
- Circulating Water Bath: Techre model TE8 A, UK.
- Freeze Dryer: Savant model Super Modulya 233, USA.
- Hot plate and stirrer: Thermolyne model Crimarec2, USA.
- Incubator: Memmert model BE500(30ºC, 37ºC, 45ºC, 50ºC, and 55ºC), Germany.
- Incubator shaker: New Brunswick Scientific model innova4300, U.S.A
- Magnetic stirrer: Ika model RO-10, Malaysia.
- Microwave: Sanyo model EM-815FW, Japan.
- Oven: Memmert UE 600, Germany.
- pH Meter: Mettler Toledo model CH-8603, Switzerland.
- Pipetteman: Gilson, Villiers-Le-Bel, France.
- Precision balance: Mettler Toledo model PB3002, Switzerland.
- Freezer : Sharp model FC27 (-20°C), Japan and Deep Freezer REVCO model ULT1790-7-V12 (-80°C), USA.
- Shaking Water Bath: Memmert, model WB22, Germany.
- Spectrophotometer: Sherwood Scientific model259, Cambridge, UK.
- Vortex mixer: Barnstead/Thermolyne model M37610-26, Iowa, USA.

2. Chemicals

Chemical	Company	Grade
Acetone	Merck	Analytical
L-arginine monohydrochloride	Fluka	Analytical
Bovine serum albumin	Sigma	Analytical
Chloroform	Mallinckrodt	Analytical
Copper (II) sulfate pentahydrate	Sigma	Analytical
Ethanol	Carlo Erba	Analytical
Ethylene diamine tetraacetic acid (EDTA)	Merck	Analytical
Ferric sulfate sevenhydrate	Carlo Erba	Analytical
Folin-Ciocalteu's phenol	Merck	Analytical
Hydrochloric acid	Merck	Analytical
Magnesium sulfate heptahydrate	Sigma	Analytical
Methanol	Merck	Analytical
Phenol	Carlo Erba	Analytical
Potassium hydrogen sulfate	Merck	Analytical
Di-potassium tartate	Carlo Erba	Analytical
Sodium chloride	Carlo Erba	Analytical
Tri-sodium citrate dihydrate	Merck	Analytical
Sodium dodecyl sulfate	Fluka	Analytical
Sodium hydroxide	Merck	Analytical
Sodium potassium tartate	Merck	Analytical
Trichloroacetic acid	Merck	Analytical
Trisma base	Merck	Analytical
Tyrosine	Sigma	Analytical
Xylose	Merck	Analytical

3. Glassware

- Culture tube 16x150 mm : Pyrex, U.S.A.
- Culture tube 25x250 mm : Pyrex, U.S.A.
- Petri-dish 90 mm: Millionant, SA.54, France.

APPENDIX B

Culture Media

All media were dispensed and steriled in autoclave for 15 min at 15 pounds/inch pressure (121 ºC) except the medium for acid from carbon sources testing which was sterilized at 10 pounds/inch pressure for (110 ºC) 10 min.

1. C medium

Peptone	5	g
Yeast extract	1	g
K_2HPO_4	4	g
$MgSO_4.7H_2O$	1	g
KCl	0.2	g
$FeSO_2.7H_2O$	0.02	g
Agar	15	g
Distilled water	1000	ml
Dissolve and adjust pH 7.0		

2. XC medium

Xylan (Oat spelt xylan)	10	g
Peptone	5	g
Yeast extract	1	g
K_2HPO_4	4	g
$MgSO_4.7H_2O$	1	g
KCl	0.2	g
$FeSO_2.7H_2O$	0.02	g
Agar	15	g
Distilled water	1000	ml
Dissolve and adjust pH 7.0		

3. L-arginine agar medium

Phenol red, 1.0% aq.solution	1.0	ml
L(+)arginine monohydrochloride	10.0	g
Agar	3.0	g
C medium	1000	ml

Dissolve the solids in the C medium, adjust to pH 7.2

4. Aesculin broth

Aesculin	1	g
Ferric citrate	0.5	g
C medium	1000	ml

Adjust pH 7.4

Dissolve the aesculin and iron salt in the C medium and sterilized at 110 ºC for 10 min.

5. Casein agar

Skim milk	10	g
C medium	1000	ml
Agar	15	g

Dissolve and adjust pH 7.2.

6. Gelatin agar

Gelatin	10	g
C medium	1000	ml
Agar	15	g

Dissolve and adjust pH 7.2.

7. Motility test medium

Motility medium (Difco)	20	g
Distilled water	1000	ml

Dissolve and adjust pH 7.2 ± 0.2.

8. Simmon Citrate agar

Simon citrate agar (Difco)	24.2	g
Distilled water	1000	ml

Dissolve the solids in the water, adjust to pH 6.8 ± 0.2

9. Starch agar

Starch	10	g
C medium	1000	ml
Agar	15	g

Dissolve and adjust pH 7.2.

10. Triple sugar iron agar

Triple sugar iron agar (Difco)	60	g
Distilled water	1000	ml

Dissolve and adjust pH 7.4± 0.2.

11. Tyrosine agar

Tyrosine	50	g
C medium	1000	ml
Agar	15	g

Dissolve and adjust pH 7.2.

12. Deoxyribonuclease (DNase) media

DNase test agar (Difco)	42	g
Distilled water	1000	ml

Adjust pH 7.3 ± 0.2 and heat to boiling to dissolve completely

13. Indole test

Tryptone	10	g
Meat extract	3	g
Distilled water	1000	ml

Dissolve and adjusted pH to 7.4.

14. Nitrate broth

Meat extract	3	g
Peptone	10	g
KNO_3	1	g
Distilled water	1000	ml

Dissolve and adjusted pH to 7.2

15. Tween 80 agar medium

Tween 80	2	ml
C medium	1000	ml
Agar	15	g

Dissolve and adjusted pH to 7.2

16. Urea agar medium

Urea	20	g
C medium	1000	ml
Agar	15	g

Dissolve and adjusted pH to 7.2

17. MR-VP broth

MR-VP medium (Merck)	17	g
Distilled water	1000	ml

Dissolve and adjusted pH 6.9

APPENDIX C

Reagents and Buffers

1. Determination of protein

The protein content was measured by the method of Lowry *et al*. (1951) with bovine serum albumin as standard.

1.1 Reagents

A: 2% sodium carbonate in 0.1N NaOH

B: 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate

C: 1 N Folin-Ciocalteu's phenol reagent

(2N Folin Phenol was diluted with distilled water to the final concentration in 1N, the solution should be freshly prepared before use.)

D: 1 ml Reagent B + 50 ml Reagent A (or similar ratio), Make up immediately before use.

1.2 Procedure

1. Place 0.1 ml of proper dilution of culture broth (for protein determination) or clear supernatant of reaction mixture (for soluble peptide determination)

2. Add 1 ml of Reagent D into the tube and vortex immediately. Incubate at room temperature for 10 min.

3. After the 10 min incubation, add 0.1 ml of Reagent C to sample and vortex immediately. Incubate 30 min at room temperature.

4. Absorbance (OD) of samples was measured at 750 nm. Concentrations of the samples were compared to the standard curve for determination of values. Distilled water was used instead of sample as a blank.

1.4 Preparation of standard curve of protein

Standards of 0, 0.1, 0.2, 0.3, 0.5, 0.7 and 1.0 mg/ml were prepared from bovine serum albumin. The reactions were carried out with the same procedure as described previously. Absorbances were plotted against concentrations of standards.

2. Reducing sugar

Standards of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 μg/ml were prepared from xylose. The reaction were carried out with the same procedure as described by Somogyi and Nelson method (1952).

3. 6 N HCl

Conc. HCl	60	ml
Distiller water	60	ml

Add conc. HCl into the distilled water.

4. 2 N H_2SO_4

Conc. H_2SO_4	2	ml
Distilled water	34	ml

Add conc. H_2SO_4 into the distilled water.

5. Ninhydrin solution

Ninhydrin	0.3	g
1-Butanol	100	ml
Glacial acetic acid	3	ml

6. 5% trichloro-acetic acid

Trichloro acetic acid	5	g
Distilled water	100	ml

Add conc. trichloro acetic acid into the distilled water.

7. Nitrate reduction test reagent

Sulphanilic acid solution

Sulphanilic acid	0.8	g
5 N Acetic acid	100	ml

Dissolve by gentle heating in a fume hood.

***N,N*-dimethyl-1-naphthylamine solution**

N,N-dimethyl-1-naphthylamine	0.5	g
5 N Acetic acid	100	ml

Dissolve by gentle heating in a fume hood.

Add two drops of sulphanilic acid solution and three drops of *N,N*-dimethyl-1-naphthylamine into peptone nitrate broth inoculing with the test microorganisms.

8. Phenol:Chloroform (1:1 v/v)

Crystalline phenol was liquidified in water bath at 65°C and mixed with chloroform in the ratio of 1:1 (v/v). The solution was stored in a light tight bottle.

9. 100xDenhardt solution

Bovine serum albumin	2%
Polyvinylpyrrolidone	2%
Ficoll 400	2%

10. 0.5M EDTA (pH 8.0)

800 ml of distilled water, 186.1 g of disodium ethylenediaminetetraacetate.$2H_2O$ was added and stirred vigorously on a magnetic stirrer. The pH was adjusted to 8.0 with NaOH (20 g of NaOH pellets). The volume was adjusted to 1 litre. The solution was dispensed into aliquots and sterilized by autoclaving for 15 minutes at 15 lb/in^2.

11. 2xPBS

8 mM Na_2HPO_4

1.5 mM KH_2PO_4

137 mM NaCl

2.7 mM KCl

The 2xPBS was adjusted the pH to 7.0 with 1N NaOH or 1N HCL. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

12. 10 mg/ml Salmon sperm DNA

A 10 mg of Salmon sperm DNA was dissolved in 1 ml of 10 mM TE buffer pH 7.6. Boiling for 10 minutes, immediately cooling in ice and sonication for 3 minutes.

13. 3 M Sodium acetate pH 5.2

To 800 ml of distilled water, 408.1 g of sodium acetate was added and adjusted the pH to 5.2 with glacial acetic acid. The volume was adjusted to 1 litre. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

14. 10% Sodium dodecyl sulphate (SDS)

The stock solution of 10% SDS was prepared by dissolved 10 g of sodium dodecyl sulphate in 100 ml sterilized distilled water. Streilization is not required for the preparation of this stock solution.

15. 20xSSC

3 M NaCl

0.1 M Tri-sodiumcitrate

The 20xSSC was adjusted the pH to 7.0 with 1N NaOH. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

16. 1 M Tris-HCl pH 8.0

The 1M Tris was prepared by dissolving 121.1 g of Tris base in 800 ml of distilled water. The pH was adjusted to the desired value by adding conc. HCL (pH 8.0, 42 ml of HCl). The solution was cooled to room temperature before making final adjustment to the desired pH. The volume of the solution was adjusted to 1 litter with with distilled water and sterilized by autoclaving.

17. RNase A solution

RNase A	20	mg
0.15 M NaCl	10	ml

Dissolve 20 mg of RNase A in 10 ml 0.15 M NaCl and heat at 95° C for 5-10 minutes. Keep RNase A solution in -20°C.

18. RNase T_1 solution

RNase T_1	80	µl
0.1 M Tris-HCl (pH 7.5)	10	ml

Mix 80 µl of RNase T_1 in 10 ml of 0.1 M Tris-HCl (pH 7.5) and heat at 95°C for 5 minutes. Keep RNase T_1 solution in -20°C.

19. Proteinase K

Proteinase K (Sigma)	4	mg
50 mM Tris-HCl (pH 7.5)	1	ml

Use freshly prepared solution.

20. Nuclease P_1 solution

Nuclease P1	0.1	mg
40 mM CH_3COONa+12 mM $ZnSO_4$ (pH5.3)	1	ml

Store at 4°C.

21. Alkaline phosphatase solution

Alkaline phosphatase	2.4 units
0.1 M Tris-HCl (pH 8.1)	1 ml

22. 0.1 M Tris-HCl buffer, pH 9

Tris	1.21 mg
Distilled water	100 ml

Adjust the pH to 9 with HCl.

23. TE buffer

10 mM Tris HCl (pH 8.0)

1 m M Na_2-EDTA (pH 8.0)

24. TE buffer + RNase A

TE buffer	960 ml
RNase A (2 mg/ml)	100 µl

25. Saline-Na_2 EDTA

0.1 M NaCl

50 mM EDTA.2Na (pH 8.0)

26. Reagents and buffers for DNA-DNA hybridization

26.1 Prehybridization solution

100xDenhardt solution	5	ml
10 mg/ml Salmon sperm DNA	1	ml
20xSSC	10	ml
Formamide	50	ml
Distilled water	34	ml

26.2 Hybridization solution

Prehybridization solution	100	ml
Dextran sulfate	5	g

26.3 Solution I

Bovine serum albumin (Fraction V)	0.25	g
Titron X-100	50	µl
PBS	50	ml

26.4 Solution II

Streptavidin-POD	1	µl
Solution I	4	ml

26.5 Solution III

3,3',5,5'-Tetramethylbenzidine (TMB) (10 mg/ml in DMFO)	100 µl
0.3% H_2O_2	100 µl
0.4 M Citric acid + o.2 M Na_2HPO_4 buffer pH 6.2 in 10% DMFO	100 µl

26.6 2 M H_2SO_4

H_2SO_4	22 ml
Distilled water	178 ml

The solution was sterilized by autoclaving.

27. Fehling's solution

Coppersulfate	34.64 g
Sodiumpotassiumtartate	173 g
Sodiumhydroxide	50 g

Solvent was composed of a mixture 500 ml of coppersulfate and 500 ml of mixture sodiumtatare and sodiumhydroxide.

28. Flagella staining

Basic fuchisin	0.5 g
Tannic acid	0.2 g
Aluminium sulfate	0.5 g

Solvent was composed of a mixture of 2.0 of 95% ethanol, 0.5 ml of glycerol, and 7.5 ml of Tris(hydroxymethyl)aminomethane(tris)buffer.

29. Kovacs' reagent

ρ-dimethylaminobenzaldehyde	5 g
Amyl alcohol	75 g
Conc. HCl	25 ml

Dissolve the aldehyde in the alcohol by gently warming in a water bath (about 50-55 ºC). Cool and add the acid with care. Protect from light and store at 4 ºC.

30. Lugol' s iodine

Iodine	5 g
Potassium iodide	10 g
Distilled water	100 ml

Dissolve the iodide and potassium iodine in some of the water, and adjust to 100 ml with distilled water.

APPENDIX D

Primers, 16S rDNA nucleotide sequences and DNA G+C contents

1. Primers for 16S rDNA amplification and sequencing

9F	5'-GAGTTTGATCCTGGCTCAG-3’
1541R	5'-AAGGAGGTGATCCAGCC-3'
357R	5’-CTGCTGCCTCCCGTAG-3’
802R	5’-TACCAGGGTATCTAATCCC-3’
530F	5'-GTGCCAGCAGCCGCGG-3’

2. 16S rDNA nucleotide sequences

2.1 The 16S rDNA nucleotide sequence of MXC4-2-1

TTGAGTTTGATCCTGGCTCGGCTCAGGATGAACGCTGGCGGCGTGCTCTAACACATGCAAGTTCGAACGATGAAGCCCAGCTTGCTGGGTGG
ATTAGTGGCGAACGGGTGAGTAACACGAGAGCAACCTGCCCCTGACTCTGGGATAACAGCCGGAAAACGGTTGCTAATACCGGATATGCAT
CATGGCCGCATGGTCTGTGGTGGGAAAGATTTTTACGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTACACCAA
GGCGTCAACGGGTAGCCGGCCTGAGAGGGTGACAAAGCCACACCTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGCGGAAGCCTGATGCAGCAACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAG
CGTAAGTGACGGTACCTGCAAAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTAT
TGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAAACCCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGA
GTGCGGTAGGGGAGATTGGAATTCCCGGGTGTAGCGGGGGGAATGCCCAGATATCAGGGAGGAACACCGATGGCGAAGGCAGATCTCTGG
GCCGTTACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCCACCCCGTAAACGTTGGGAACTAGTTG
TGGGGGCCTTTCCACGGTCTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTG
ACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCTG
GAAACAGTCGCCCCTTTTTGGTCGGTGTACAGGTGGTGCATGGTTGTCGACAGCATCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGTTCTATGTTGCCAGCACGTTATGGTGGGAACTCATGGGATACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAA
ATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAA
GCCGGTCCCAGTTCGGATTGAGGTCTGCAACTCGACCTCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGT
TCCCGGGTCTTGTACACACCGCCCGTCAAGTCATGAAAGTCGGTAACACCTGAAGCCGGTGGCCTAACCCCTTGTGGGAGGGAGCTGTCGAA
GGTGGGATCGGTAATT

2.2 The 16S rDNA nucleotide sequence of MXC4-6-2

TTTGATCCTGGCTCAGGATGAACGCTGGCGGCGATGCTTAACACTTATGCAAGTCGAACGATGAAGCCCAGCTTGCTGGGTGCATTAGTGGC
GAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCTGGGATAACAGCCGGAAACGGTTGCTAATACCGGTATATGCATCATGGCCGC
ATGGTCTGTGGTGGGAAAGATTTTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGTCAACGG
GTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACCGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG
GGCGGAAGCCTGATGCAGCAACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCGTAAGTGACGGTA
CCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGC
TCGTAGGCGGTTTGTCGCGTCTGCTGTGAAAACCCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAAAGTGCGGTAGGGGAA
TTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTTACTGACGCTGAGG
AGCGAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGGCCTTTCCACGGTCTC
CGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGG
CGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCTGGAGACAGTCGCCCCTTTTTGG
TCGGTGTACAGGTGGTGCATGGTTGTCGTAACAGCTCGTCTCGTGAGATGTTGCGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTT
GCCAGCACGTTATGGTGGGAACTCATGGGATACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTC
TTGGGCTTCACGCATGCTACAATGGCCGGTACAAAGGGCTGCAATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCGGATT
GAGGTCTGCAACTCGACCTCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTGTACACAC
CCCCGTCAAGTCATGAAAGTCGGTAACACCTGAAGCCGGTGGCCTAACCCCTTGTGGGAGGGAGGCTGT

2.3 The 16S rDNA nucleotide sequence of S3-4A

CCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGAGTTGATGGAGGTGCTTGCACTTCTGANGGTTAGCGGCG
GACGGGTGAGTAACACGTAGGTAACCTGCCCATAAGACCGGGATAACATTCGGAAACGGATGCTAATACCGGATACGCAATTCTCTCGCAT
GAGGGGATTGGGAAAGGCGGAGCAATCTGTCACTTATGGATGGACCTGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCC
GCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTTGGGA
GAGTAACTGCTCTCAAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTT
GTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTCATTAAGTCTGGTGTTTAAGGCTGGGGCTCAACCCCGGTTCGCACTGGAAACTG
GTGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGAC
TTTCTGGGCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCT
AGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGA
ATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCCCTGACCGGT
CTAGAGATAGGCCTTTCCTTCGGGACAGGGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGATCTTAGTTGCCAGCACTTTGGGTGGGCACTCTAGGATGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG
TCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCGGTACAACGGGAAGCGAAGGAGCGATCCGGAGCCAATCCT
ATAAAGCCGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAAT
ACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACCGCAAGGAGCCAGCCGTCG
AAGGTGGGGTAGATGATTGGGGTG

2.4 The 16S rDNA nucleotide sequence of MX2-3

CCGGACCTACACGCAGGCCGGCGTGCCTTAACCTGCAAAGTCCGACGGAGCTAAGTGAAAGCTCGTTTTCACAATGCTTAGCGGCGGACGG
GTGAGTAACACGTAGGTAACCTGCCTGTAAGACTGGGATAACATTCGGAAACGAATGCTAATACCGGATACGCGAGTTGGTCGCATGGCCG
ACTCGGGAAAGACGGAGCAATCTGTCGCTTACAGATGGACCTGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATG
CGTAGCCGACCTGAGAGGGTGATCGACCACACTGGGACTGAGACACGGCCCAGACTCCTTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGGCGAAAGCCTGACGGAGCAACGCCGCCTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTTGGGAGAGT
AACTGCTCCCAAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGTCC
GGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTCATTAAGTCTGGTGTTTAAGGCTGGGGCTCAACCCCGGTTCGCACTGGAAACTGGTGA
ACTTGAGTGCAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGAGACTTTCT
GGGCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGT
GTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTG
ACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACCGGTCCTG
GAGACAGGCCTTTCCTTCGGGACAGAGGAGACAGGTGGTGCATGGTGGTCGTCGCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCCTATTTTTAGTTGCCACACTTTGGGTGGGCACTCTAAAGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA
AATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCAGTACAACGGGAAGCGAAGGAGCGATCTGGAGCCAATCCTATCA
AAGCTGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGGGGTAACCCGCAAGGGAGCCAGCCGCCGA
AGGTGGGGTAGATGATTGGGGTGAAGTCGTAA

2.5 The 16S rDNA nucleotide sequence of S5-3

TCGAGGGGAGCTAGAGTTTTATTAATCCCGGAAATCCACTGANACTTTAGCGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCATAAG
ACCGGGATAACATTCGGAAACGGATGCTAATACCCGGATACGCGATTCTCTCGCATGAGAGAAGTTGGGAAAGGCGGAGCAATCTGTCACT
TATGGATGGACATGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGAGATGATGTGTAGCCCACGTGACAGGGTGATCGGCCA
CACTGGGNCAGAGACACGGCCCAGACTCTGACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGAAGAAAATCTGACGGAGCAACGCCGC
CTGAGTGATGAAGGTTTTCGGATCGAAAAGCTCTGTTGCCAGGGAAGAACGCTAGAGAGAGTAACTGCTCTTTAGGTGACGGTACCTGAGA
AGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGG
CGGTTGATTAAGTCTGGTGTTTAAGGCTATGGCTCAACCATAGTTCGCACTGGAAACTGGTTGAATTGAGTGCAGAAGAGGAAAGTGGAATT
CCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCGGTAACTGACGCTGAGGCACGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAA
GTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATG
TGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATGCCTCTGACCGCTCTAGAGATAGAGCTTCTCTTCGGAGCAGGGGAA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCTGCAACGAGCGCAACCCCTAATGTTAGTTGCCAGCAGG
AGCTGGGCACTCTAACGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACAA
CGTACTACAATGGCCAGTACAACGGGAAGCGAAGTCGCGAGATGGAGCCAATCCTCAAAAGCTGGTCTCAGTTCGGATTGCAGGCTGCAAC
TCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACC
ACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACCCGCAAGGGAGCCAGCCGTCGAAGGTGGGGTAGATGATTGGGGTGAAGTCGTAT

2.6 The 16S rDNA nucleotide sequence of X13-1

TTTGTCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTTAATACATGCCAAGTCGAAGCCGGAGTTGATAGAAAGCTTGCTTCCCTGAGAGAT
TAAGCGGCGGACGGGTGAGTTAACACGTAGGTAACCTGCCCCATTAAGACTGGGATAAACATTCGGAAACGAATGCTAATACCGGATACGC
AAATGGATCGCATGATTCGTTTGGGAAAGGCGGAGCAATCTGTCACTTATGGATGGACCTGCGGTGCATTAGCTAGTTGGAGAGGTAACGGC
TCCCCAAGGCGACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT
AGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTCCAGGGA
AGAACGCTAAAGAGAGTAACTGCTCTTTAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTGATTAAGTCTGGTGTTTAAGGCTGTGGCTCAACCACAGTTC
GCACTGGAAACTGGTTGACTTGACAATGCAGAAGAGGAAAGTGGAATTCTCACGTGTCAGCGGTGAAATGCGTAGAGATGTGGAGGAACAC
CAGTGGCGAAGGCGACTTTCTGGGCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGAATGCTAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAA
GACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT
GACATGCCTCTGACCGCTCTAGAGATAGAGCTTTCCTTCGGGACAGGGGACACAGGTGGTGGCATGGTTGTAGTCAGCTCCTGTCGAGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTAATGTTAGTTGCCAGCACCTTGGGTGGGCACTCTAACGTGACTGCCGGTGACAAACCGG
AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCAGTACAACGGGAAGCGAAGTCG
CGAGATGGAGCCAATCCTAGCAAAGCTGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGA
TCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACC
CGCAAGGGGCCAGCCGTCGAAGGTGGGGTAGATGATTGGG

2.7 The 16S rDNA nucleotide sequence of MXC2-2

GGCACCCCGGGGGGCCTTAAACCCCCATCGAGGGAAGTAGATTTTATTGGACCCTGAGACTTAGCGGCGGACGGGTGAGTAACACGTAGGT
AACCTGCCCATAAGACCGGGATAACATTCGGAAACGGATGCTAATACCGGATACGCGATTCTCTCGCATGAGGGAGTTGGGAAAGGCGGAG
CAATCTGTCACTTATGGATGGACCTGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGG
GTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA
GCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTAGAGAGAGTAACTGCTCTTTAGGTGACG
GTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAG
CGCGCGCAGGCGGTTGATTAAGTCTGGTGTTTAAGGCTATGGCTCAACCATAGTTCGCACTGGAAACTGGTTGACTTGAGTGCAGAAGAGGA
AAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTAACTGACGCTGA
GGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTTAGGGGTTTCGATACCT
TGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCA
GTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATGCCTCTGACCGCTCTAGAGATAGAGCTTCTCTTCGG
AGGCAGGGGACACAGGTGGTGGCATGGTTGTCGTCAGCTCCTGTCGAGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTAATGTTA
GTTGCCAGCAGGTAGAGCTGGGCACTCTAACGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA
TGACCTGGGCTACACACGTACTACAATGGCCAGTACAACGGGAAGCGAAGTCGCGAGATGGAGCCAATCCTCAAAAGCTGGTCTCAGTTCG
GATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACA
CACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACCCGCAAGGGAGCCAGCCGTACGAAGGTGGGGTAGATGAT
TGGG

2.8 The 16S rDNA nucleotide sequence of MXC4-3-1

GGATCCGAGCCCCGCGAAACCTGACGCCGTGCCTTAACCGGAATTAAGCGAGGTTGATTCGTAGCTTGGTACCCTGAGACTTAGCGGCGGAC
GGGTGAGTAACACGTAGGTAACCTGCCCATAAGACCGGGATAACATTCGGAAACGGATGCTAATACCGGATACGCGATTCTCTCGCATGAG
GGAGTTGGGAAAGGCGGAGCAATCTGTCACTTATGGATGGACCTGCGGCGCATTAGCTAGTTGGTGAGGTAACGGGCTTCACCCAAGGCGA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACCACTGGGACTGAGACACGGCCCAGATTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATTCGTAAAGCTCTGTTGCCAGGGAAGAACGCTAGA
GAGAGTAACTGCTCTTTAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCG
TTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTGATTAAGTCTGGTGTTTAAGGCTATGGCTCAACCATAGTTCGCACTGGAAAC
TGGTTGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCG
ACTTTCTGGGCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCCACGCCGTAAACGATGAA
TGCTAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAA
AGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATGCCTCTGA
CCGCTCTAGAGATAGAGCTTCTCTTCGGAGCAGGGGACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCCTAATGTTAGTTGCCAGCAGGTAGAGCTGGGCACTCTAACGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
ATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCAGTACAACGGGAAGCGAAGTCGCGAGATGGAGCC
AATCCTCAAAAGCTGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCG
GTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACCCGCAAGGGAGCC
AGCCGTACGAAGGTGGGGTAGATGATTGGG

2.9 The 16S rDNA nucleotide sequence of ST2

TCCGGACGAACCCGGGGGGGCCTTAACCAGCCAATTCGGGCGAAGTAGATAGTTATTTGGATTCCTCGAGACTTAGCGGCGGACGGGTGAG
TAACACGTAGGTAACCTGCCCATAAGACCGGGATAACATTCGGAAACGGATGCTAATACCGGATACGCGATTCTCTCGCATGAGGGAGTTG
GGAAAGGCGGAGCAATCTGTCACTTATGGATGGACCTGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAG
CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACG
AAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTAGAGAGAGTAACTGC
TCTTTAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGTCCGGAATT
ATTGGGCGTAAAGCGCGCGCAGGCGGTTGATTAAGTCTGGTGTTTAAGGCTATGGCTCAACCATAGTTCGCACTGGAAACTGGTTGACTTGA
GTGCAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTG
TAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTTAGG
GGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATGCCTCTGACCGCTCTAGAGATAG
AGCTTCTCTTCGGAGCAGGGGACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCCTAATGTTAGTTGCCAGCAGGTAGAGCTGGGCACTCTAACGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCAT
CATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCAGTACAACGGGAAGCGAAGTCGCGAGATGGAGCCAATCCTCAAAAGCTG
GTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCG
GGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACCCGCAAGGGGCCAGCCGTACGAAGGTGG
GGTAGATGATTGG

2.10 The 16S rDNA nucleotide sequence of X8-1

CTGGCTCAGGACGAACGCTGGCGGCGTGCCTTAATACATGCAAGTCGAGCGGACTTGATGGAGAGCTTGCTCTCCTGATGGTTAGCGGCGGA
CGGGTGAGTAACACGTAGGCAACCTGCCTGCAAGACCGGGATAACCCACGGAAACGTGAGCTAATACCGGATATCTCATTTCCTCTCCTGAG
GGGATGATGAAAGACGGAGCAATCTGTCACTTGCGGATGGGCATGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGA
TGCGTAGCCGACCTGAGAGGGTGAACGGCCACACTGGGACTGAGACACGGCCCAGACTCCTTACGGGAGGCAGCCAGTAGGGAATCTTCCG
CAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGTCCGGTAG
AGTAACTGCTATCGGAGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTG
TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCATTTAAGTCTGGTGTTTAAGGCCAAGGCTCAACCTTGGTTCGCACTGGAAACTGG
GTGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGATATGTGGAGGAACACCAGTGGCGAAGGCGACTC
TCTGGGCTGTAACTGACGCTGAGGCGCCAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCCACGCCGTTAAACGATGAATGC
TAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTCGCCAAGACTGAAACTCAAAG
GAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAAGAACCTTACCAGGTCTTGACATCCCTCTGACC
GGTACAGAGATGACCTTTCCTTCGGGACAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGATTTTAGTTGCCAGCACTTCGGGTGGGCACTCTAGAATGACTGCCGGTGACAAACCGGAGGAAGGCGGGGATGA
CGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCAGTACAACGGGAAGCGAAGCCGCGAGGTGGAGCCAATC
CTATCAAAGCTGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTG
AATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGAGGTAACCGCAAGGAGCCAGCCG
CCGAAGGTGGGGTAGATGATTGGGGTG

2.11 The 16S rDNA nucleotide sequence of MX8-1

CTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACTTGATGGAGAGCTTGCTCTCCTGATGGTTAGCGGCGGAC
GGGTAAGGTTTTAACATCGTAAGACAACCTGTCCTGCAAGACCGGGATAACCCACGGAAACGTGAGCTAATTACCGGATATCTCATTTTCCT
CTCCCTGAGGGGATGATGAAAGACGGAGCAATCTGTCACTTGCGGATGGGCCTGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA
AGGCGACGATGCGTAGCCGACCTGAGAGGGTGAACGGCCACCACTGGGACTTGAGACACGGCCCAGACTCCTTACGGGAGGCAGCAGTAG
GGAATCTTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCCTGAGTGATGAAGGTTTTCGGATCGTAAAGATCTGTTGCCAGGGAAG
AACGTCCCGGTAGAGTAACTGCTATCGGAGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCATTTAAGTCTGGTGTTTAAGGCCAAGGCTCAACCTTGGTTCG
CACTGGAAACTGGGTGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACCGTGTAGCGGTGAAATGCCGTAGATATGTGGATGCAACACCA
GTGGCGAAGGCGACTCTCTGGGCTGTAACTGACCGCTGAGGCCGCGAAAGCCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACC
GCCGTAAACCGATGAATGCTAGGTGTTAGGGGTTTCGATACCCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTACGGTC
GCAAGACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGGAACCTTACCAG
GTCTTGACATCCCTCTGACCGGTACAGAGATGTACCTTTCCTTCGGGACAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATTTTAGTTGCCAGCACTTCGGGTGGGCACTCTAGAATGACTGCCGGTGACAAAC
CGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCAGTCAACGGGAAGCGAAGC
CGCGAGGTGGAGCCAATCCTATCAAAGCTGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCG
GATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGAGGTAA
CCGCAAGGAGCCAGCCGCCGAAGGTGGGGTAGATGATTGGGGTGAAGTCGTAA

2.12 The 16S rDNA nucleotide sequence of X11-1

AGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATTTACCCTTCGGGGTAAGTTAGCGGCGGACG
GGTGAGTAACACGTAGGCAACCTGCCTGTAAGATCGGGATAACTACCGGAAACGGTAGCTAAAGACCGGATAGGTGGTTTCTTCGCATGAA
GAGATCCAAGAAACACGGGGCAACCTGTGGCTTACAGATGGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCCCACCAAGGCGACG
ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGC
AATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAAGGAAGAACGCCTCGGAGA
GTAACTGCTCCGGGGGTGACGGTACTTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGT
CCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCCGCTTAAGTTTGGTGTTTAAGCCCGGGGCTCAACCCCGGTTCGCACCGAAAACTGGG
CGGCTTGAGTGCAGGAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTT
CTGGACTGTAACTGACGCTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAG
GTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTAAACACAATAAGCACTCCGCCTGGGGAGTACGCTCGCAAGAGTGAAACTCAAAGGAA
TTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGAAAGCC
ATAGAGATATGGCCCCTCTTCGGAGCATTGGAGACAGGTGGTGCATGGTTTCGTC

2.13 The 16S rDNA nucleotide sequence of X15-1

TCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCTAGGGGTTCTCCCTTAGGGGAGACCTCCTGGAGCGGCGG
ACGGGTGAGTAACACGTAGGCAACCTGCCTGTAAGACCGGGATAACTACCGGAAACGGTAGCTAAGACCGGATAGGTGGTTTCTCCGCATG
GAGGGATCAAGAAACACGGTGCAAGCTGTGGCTTACAGATGGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGAC
GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCG
CAATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAAGGAAGAACGCCTTGGGG
AGTCACTGCCCTGAGGGTGACGGTACTTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTG
TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCCGCTTAAGTTTGGTGTATAAGCCCGGGGCTCAACCCCGGATCGCACCGAAAACTG
GGTGGCTTGAGTGCAGGAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGC
TTTCTGGACTGTAACTGACGATGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGC
TAGGTGTTAGGGGTTTCGATACCCTTGGTGCCGAAGTCAACACAATAAGCACTCCGCCTGGGGAGTACGCTCGCAAGAGTGAAACTCAAAG
GAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACCG
GTACAGAGATGTACCTTTCCTTCGGGACAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGAGCTTAGTTGCCAGCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGAC
GTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCGGTACAACGGGAAGCGAAGGAGCGATCCGGAGCGAATCC
TTATAAGCCGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAA
TACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGGGGTAACCGCAAGGAGCCAGCCGCCG
AAGGTGGGGTAGATGA

2.14 The 16S rDNA nucleotide sequence of MXC4-2-2

TCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCTGGATGATTTCCTTCGGGGAATTATCCGGAGCGGCGGAC
GGGTGAGTAACACGTAGGCAACCTGCCCGGAAGACCGGGATAACTACCGGAAACGGTAGCTAATACCGGATAGGTGGCTTCTTCGCATGGG
GGAGCCAAGAAACGCGGAGCAATCTGCGGCTTACGGATGGGCCTGCGGCGCATTAGCTAGTTGGCAGGGTAAACGGCCTACCAAGGCGACG
ATGCGTAGCCGACCTGAGAGGATGATCGGCCACACTGAAACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGC
AATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGAGGAAGGTCTTCGGATCGTAAAGCTCTGTTGCCAAGGAAGAACGGCCCGGAG
AGTCACTGCTCCGGGAGTGACGGTACTTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTG
TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCTGTTAAGTCTTGTGTTTAAGCCCGGGGCTCAACCCCGGTTCGCATGGGAAACTGG
CAGTACTTGAGTGCAGGAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGC
TTTCTGGACTGTAACTGAACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTG
CTAGGTGTCGGGGGTTTCGATACCCGCGGTGCCGAAGTTAACGCAATAAGCACTCCGCCTGGGGAGTACGCTCGCAAGAGTGAAACTCAAG
GAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACCG
GTGCAGAGATGTGCCTTTCCTTCGGGACAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGA
ACGAGCGCAACCCTTAGCTTTAGTTGCCAGCATGAGAGATGGGCACTCTGGAGCGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGAG
TCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCGGTACAGAGGGAAGCGAAGGAGCGATCTGGAGCGAATCCA
AAAAAGCGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA
CGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGAGGTAACCGTAAGGAGCCAGCCGCCGA
AGGTGGGGTAGAT

2.15 The 16S rDNA nucleotide sequence of S2-1

ACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATCTGAGGGAGCTTGCTCCCAAAGATTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGATTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAACATATTGAACCGCATGGTTCAATATTGAAAG
ATGGTTTCGGCTATCACTTACAGATGGACCCGCGCCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCT
GACGGAGCAACGCCGCGTGAGCGAAGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACC
TTGACGGTACCTAACCAGAAAGCCACGGCTAATTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCG
TAAAGCGCGCGCAGGCGGTTTCTTAAGTNTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACACGATGGTCTGTAACG
ACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTT
CCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTCGCTACTTCTAGAGATAGAAGGTT
CCCCTTCGGGGGACGGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT
TGACCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACTGCGAAGTCAAGCCAATCCCATAAAACCATTCTCAG
TTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCGTAAGGAGCCAGCCGCCTAAGGTGGGACAGATGA
TTGGGGCGNAGNC

2.16 The 16S rDNA nucleotide sequence of MX1-1

ATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGA
CGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGG
AGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCACGATG
CATAGCCGACCTGATAGGGTGATCGGCCACACTGGGATTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAAT
GGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTA
ACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGG
AATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGA
ACTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTT
TGGTCTGTAACTGACGCTGTGGCGCGAAAGCGTGCGTGGACGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCT
AAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTC
TAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGAC
GTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAATCCC
ATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAAGGAGCTAGCCGCCTA
AGGTGGGACAGATGATTGGGGAAAT

2.17 The 16S rDNA nucleotide sequence of MX1-2

GATCCTGGGCTCAGGATGAACCCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGGTCCTTCGGGGCCTAGCGGCGGACGGGTGAGTAA
CACGTGGGTAACCTGCCTGTAAGACCGGGATAACTTCGGGAAACCGAAGCTAATACCGGATACTTTCGAGCATCGCATGATGCTTGATGGAA
AGACGGCTTCGGCTGTCACTTACAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGAC
CTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGCGAAAG
CCTGACCGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAAGGAAGAACAAGTACGAGAGTAACTGCTCGT
ACCTTGACGGTACTTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATTATTGG
GCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTG
CAGAAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAA
CGGAAGCTGAGGCGGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGT
TTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTT
TCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
GATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC
CTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGT
TCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTACGGGAGCCAGCCGCCTAAGGTGGGACAGATG
ATTGGGGTGAAGTCGTAAC

2.18 The 16S rDNA nucleotide sequence of MX2-1

ATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGGTTCTTCGGAACCTAGCGGCGGACGGGTGAGTAACA
CGTGGGTAACCTGCCTGTAAGACCGGGATAACTTCGGGAAACCGAAGCTAATACCGGATACTTTCGAGCATCGCATGATGCTTGATGGAAAG
ACGGTTTCGGCTGTCACTTACAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGATCGTCCAGACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGCGAAAGCCT
GACCGAGCAACGCCGCGTGAGCGATGAAGGCTTCGGGTCGTAAAGCTCTGTTGTTAAGGAAGAACAAGTACGAGAGTAACTGCTCGTACCT
TGACGGTACTTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATTATTGGGCG
TAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAG
AAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACTG
ACGCTCGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTT
TCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTT
CCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTG
ATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCC
TTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTT
CGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTACGGGAGCCAGCCGCCTAAGGTGGGACAGATG
ATGAGGTGAAGT

2.19 The 16S rDNA nucleotide sequence of strain MX3-2

AGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGGTTCTTCGGAACCTAGCGGCGGACGGGTGA
GTAACACGTGGGTAACCTGCCTGTAAGACCGGGATAACTTCGGGAAACCGAAGCTAATACCGGATACTTTCGAGCATCGCATGATGCTTGAT
GGAAAGACGGTTTCGGCTGTCCACTTACAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAG
CCGACCTGAGAGGGTGATCGTCCAGACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGCG
AAAGCCTGACCGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAAGGAAGAACAAGTACGAGAGTAACTGC
TCGTACCTTGACGGTACTTAACGAGAAAGCCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATT
ATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTT
GAGTGCAGAAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGT
CTGATAACCCTCGAACGCTGAGGCTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTA
AGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACAACCC
TAGAGATAGGGCTTTCCCTTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGAATGAGATGTTGGGTTAACCGTCC
CGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAA
TCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTACGGGAGCCAGC
CGCCTAAGGTGGGACAGATGAGTAAAGT

2.20 The 16S rDNA nucleotide sequence of MX12-2

GCTGGCTCCAGGATGAACGCCGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGGTTCTTCGGGACCTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACCGGGATAACTTCGGGAAACCGAAGCTAATACCGGATACTTTCGAGCATCGCATGATGCTTGATGGAAAG
ACGGTTTCGGCTGTCACTTACAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCCAGTAGGGAATCTTCGGCAATGGGCGAAAGC
CTGACCGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAAGGAAGAACAAGTACGAGAGTAACTGCTCGTA
CCTTGACGGTACTTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATTATTGGG
CGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGC
AGAAGAGGAAAGCGGAATTCCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGATC
GGAACTGACGCTGAGGCGCGAAAGCCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA
GAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATA
GGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAGAGCGCAA
CCCTTGATCTTAGTTGCCAGCATTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCAT
GCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCT
CAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCC
TTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTACGGGAGCCAGCCGCCTAAGGTGGGACA
GATGATTGGGG

2.21 The 16S rDNA nucleotide sequence of MXC1-3

TCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGGTTCTTCGGAACCTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACCGGGATAACTTCGGGAAACCGAAGCTAATACCGGATACTTTCGAGCATCGCATGATGCTTGATGGAAAG
ACGGCTTCGGCTGTCACTTACAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGAACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGCGAAAGCC
TGACCGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAAGGAAGAACAAGTACGAGAGTAACTGCTCGTAC
CTTGACGGTACTTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATTATTGGGC
GTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCA
GAAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACT
GACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTT
TCCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTT
TCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
GATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC
CTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGT
TCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTACGGGAGCCAGCCGCCTAAGGTGGGACAGATG

2.22 The 16S rDNA nucleotide sequence of MXC3-4-2

TCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGGTTCTTCGGAACCTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACCGGGATAACTTCGGGAAACCGAAGCTAATACCGGATACTTTCGAGCATCGCATGATGCTTGATGGAAAG
ACGGCTTCGGCTGTCACTTACAGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGAACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGCGAAAGCC
TGACCGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAAGGAAGAACAAGTACGAGAGTAACTGCTCGTAC
CTTGACGGTACTTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATTATTGGGC
GTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCA
GAAGAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACT
GACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTT
TCCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTT
TCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
GATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC
CTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGT
TCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTACGGGAGCCAGCCGCCTAAGGTGGGACAGATG

2.23 The 16S rDNA nucleotide sequence of S1-3

GGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATCTTTCCTTAAGTAGCTTGCTACTTTAAGAAGGTAAGCGGC
GGGACGGGTGAGTAACACGTAGGCAACCTCCCCATAAGACCGGGATAACATTCGGAAACGAATGCTAAGACCGGATACGCAAAAGGAGGG
CATCATCCTTTTTGGGGAAACACGGTGCAAGCTGTGGCTTATGGATGGGCATGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGG
CGACGATGCGTAGCCGACCTGAGAGGGTGAACGGCCACACTGGGAAACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTTAGGG
AATTCTTCCACAATGGGCGCAAGCCTGATGGAGCAACGCCGCGTGAGTGAGGAAGGCTTTCGGGTCGTAAAAGCTCTGTTGCCAGGGAAGA
ATAAGGGCGAGGTAACTACTCGTCCGATGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGG
GCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGGTGTTTAAGTGCGGGGCTCAACCCCGTGTCGCAT
CGGAAACTGGGAGAATTGAGTGCAGAAGAGGAGAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGC
GAAGGCGGCTCTCTGGACTGTAACTGACGCTGAGGCCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTTAAAC
GATGAGTGTTCTAGGTGTTTGGGGGGGTCCACCCCTCGGTGCCGAAGTTAACACACTTAAGCACTCTCGCCCTGGGGATGTACGGTCGCAAG
ACTGAAACTCAAAGGAATTGACCGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG
ACATCCCTCTGACCGTTCCAAGAGATAGGGCTTCCCTTCGGGGCAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAAATG
TTGGTTGAATTCCGGCAACAAGCCCACCCTGGAATTTATTTCCCACCACTTTGGGTGGGCACTCTAGATTGACTGCCGGTGACAAACCGGAG
GAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGCCGGTACAACGGGTTGCGAAGGAGCG
ATCCGGAGCCAATCCTATAAAGCCGGTCTCAGTTCGGATTGGAGGCTGCAACTCGCCTCCATGAAGTCGGAATTGCTAGTAATCGCGGATCA
GCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGCCGGTGGGGTAACCGCA
AGGAGCCAGCCGTCGAAGGTGGGGTAGATGATTGGGGT

2.24 The 16S rDNA nucleotide sequence of MX15-2

CTGGCTCAGG ACCAACGCCG GCGGCGTGCC TAATACATGC AAGTCGAGCG GATCTTCAAGGGAGCTTGCT CCTGAGAAGG
TTAGCGGCGG ACGGGTGAGT AACACGTAGG CAACCTGCCCTCAAGACCGG GATAACATTC GGAAACGAAT GCTAAGACCG
GATACGCAAG AAGGAGGCATCTTCTTCTTG GGAAACACGG CGCAAGCTGT GGCTTGAGGA TGGGCCTGCG GCGCATTAGC
TAGTTGGCGG GGTAACGGCC CACCAAGGCG ACGATGCGTA GCCGACCTGA GAGGGTGAACGGCCACACTG GGACTGAGAC
ACGGCCCAGA CTCCTTACGG GAGGCAGCAG TAGGGAATCTTCCACAATGG GCGCAAGCCT GATGGAGCAA CGCCGCGTGA
GTGAGGAAGG CCTTCGGGTCGTAAAGCTCT GTTGCCAGGG AAGAATAAGA GCCAGTTAAC TGCTGGTTCG ATGACGGTAC
CTGAGAAGAA AGCCCCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGGGGCAAGCGTTGTCCGG AATTATTGGG
CGTAAAGCGC GCGCAGGCGG TTTCTTAAGT CTGGTGTTTAAGTGCGGGGC TCAACCCCGT GACGCACTGG AAACTGGGAG
ACTTGAGTGC AGAAGAGGAGAGCGGAATTC CACGTGTAGC GGTGAAATGC GTAGAGATGT GGAGGAACAC CAGTGGCGAA
GGCGGCTCTC TGGACTGTAA CTGACCGCTG AGGCCGCGAA AGCGTGGGGA GCAAACAGGATTAGATACCC TGGTAGTCCA
CGCCGTAAAC GATGAGTGCT AGGTGTTGGG GGGGTCCACCCCTCGGTGCC GAAGTTAACA CATTAAGCAC TCCGCCTGGG
GAGTACGGTC GCAAGACTGAAACTCAAAGG AATTGACGGG GACCCGCACA AGCAGTGGAG TATGTGGTTT AATTCGAAGC
AACGCGAAGA ACCTTACCAG GTCTTGACAT CCCTCTGAAT CGTCTAGAGA TAGGCGCGGCCTTCGGGACA GAGGAGACAG
GTGGTGCATG GTTGTCGTCA GCTCGTGTCG TGAGATGTTGGGTTAAGTCC CGCAACGAGC GCAACCCTTG ATCTTAGTTG C
CAGCACTTC GGGTGGGCACTCTAAGGTGA CTGCCGGTGA CAAACCGGAG GAAGGTGGGG ATGACGTCAA ATCATCATGCC
CCTTATGAC CTGGGCTACA CACGTACTAC AATGGCCGGT ACAACGGGCA GCGAAGGAGCGATCCGGAGC CAATCCTTTA A
AGCCGGTCT CAGTTCGGAT TGCAGGCTGC AACTCGCCTGCATGAAGTCG GAATTGCTAG TAATCGCGGA TCAGCATGCC GC
GGTGAATA CGTTACCCGGGTCTTGTACA CACCGCCCGT CACACCACGA GAGTTTACAA CACCCGAAGC CGGTGGGGTA ACC
GCAAGGA GCCAGCCGTC GAAGGTGGGG TAGATGATTG GGGTGAAATC GTAA

2.25 The 16S rDNA nucleotide sequence of MX21-2

CCTGGCTCAG GACGAACGCT GGCGGCGTGC CTAGGATACA TGCAACTAGA GCGGATCTTCAAGGGAGCTT GCTCCTGAGA
AGGTTAGCGG CGGACGGGTG AGTAACACGT AGGCAACCTGCCCTCAAGAC CGGGATAACA TTCGGAAACG AATGCTAAGA
CCGGATACGC AAGGAGGAGGCATCTTCTTC TTGGGAAACA CGGCGCAAGC TGTGGCTTGA GGATGGGCCT GCGGCGCATT
AGCTAGTTGG CGGGGTAACG GCCCACCAAG GCGACGATGC GTAGCCGACC TGAGAGGGTGAACGGCCACC ACTGGGACTG
AGACACGGCC CCAGACTCCT TACCGGGGAG GCCAGCAGTAGGGAATCTTC CACCAATGGG CGCAAGCCTT GATGGAGCAA
CCCCCGCGTG AGTGAGGAAGGCCTTCGGGT CGTAAAGCTC TGTTGCCAGG GAAGAATAAG AGCCAGTTAA CTGCTGGTTC
GATGACGGTA CCTGAGAAAG AAAAGCCCCC GGCTAACTAC GTGCCAGCAG CCGCGGTAATACGTAAGGGG CAAGCGTTGT
CCGGAATTAT TGGGCGTAAA GCGCGCGCAG GCGGTTTCTTAAATCTGGTG TTTAAGTGCG GGGCTCAACC CCGTGACGCA
CTGGAAACTG GGAGACTTGAGTGCAGAAGA GGAGAGCGGA ATTCCACGTG TAGCGGTGAA ATGCGTAGAG ATGTGGAGGA
ACACCAGTGG CGAAGGCGGC TCTCTGGACT GTAACTGACG CTGAAGCCCC AAAGCGTGGGGAGCAAACAG GATTAGATAC
CCTGGTAGTC CACGCCGTAA ACGATGAGTG CTAGGTGTTGGGGGGGTCCA CCCCTCGGTG CCGAAGTTAA CACATTAAGC
ACTCCGCCTG GGGAGTACGGTCGCAAGACT GAAACTCAAA GGAATTGACG GGGACCCGCA CAAGCAGTGG AGTATGTGGT
TTAATTCGAA GCAACGCGAA GAACCTTACC AGGTCTTGAC ATCCCTCTGA ATGTTTAGAGATAGCCAGGC CTTCGGGACA
GAGGAGACAG GTGGTGCATG GTTGTTCGTG CAACTCGTGTC

2.26 The 16S rDNA nucleotide sequence of MX8-2

GATCCTGGCT CAGAGTGAAC GCTGGCGGTA GGCCTAACAC ATGCAAGTCG AACGGCAGCACAGGAGAGCT TGCTCTCTGG
GTGGCGAGTG GCGGACGGGT GAGGAATACA TCGGAATCTACTCTGTCGTG GGGGATAACG TAGGGAAACT TACGCTAAAT
AACCGCATAC GACCTACGGGTGAAAGTGGG GGACCGCAAG GCCTCACGCG ATAGAATGAG CCGATGTCGG ATTAGCTAGT
TGGCGGGGTA AAGGCCCACC AAGGCGACGA TCCGTAGCTG GTCTGAGAGG ATGATACAGCCACTCTGGAA CTGAGACCAC
GGTCCAGACT CCTACGGGAG GCAGCAGTGG GGAATATTGGACAATGGGGC AAGCCTGATC CAGCCATACC GCGTGAGTGA
AGAAGGCCCT CGGGTTGTAAAGCTCTTTTG TTGGGAAAGA AATACCTGTT GGCTAATACC CGGCAGGGAT GACGGTACCC
AAAGAATAAG CACCGGCTAA CTTCGTGCCA GCAGCCGCGG TAATACGAAG GGTGCAAGCGTTACTCGGAA TTACTGGGCG
TAAAGCGTGC GTAGGTGGTG GTTTAAGTCT GTTGTGAAAGCCCTGGGCTC AACCTGGGAA TTGCAGTGGA TACTGGATCA
CTAGAGTGTG GTAGAGGGTGGCGGAATTCC CGGTGTAGCA GTGAAATGCG TAGAGATCGG GAGGAACATC CGTGGCGAAG
GCGGCCACCT TGCGGCCAAC ACTGACACTT GAGGCACGAA AGCGTGGGGA GCAAACAGGATTAGATACCC TGGTAGTCCA
CGCCCTAAAC GATGCGAACT GGATGTTGGG TTCAACTTGGAACCCAGTAT CGAAGCTAAC GCGTTAAGTT CGCCGCCTGG
GGAGTACGGT CGCAAGACTGAAACTCAAAG GAATTGACGG GGGCCCGCAC AAGCGGTGGA GTATGTGGTT TAATTCGATG
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GGTGCTGCAT GGCTGTCGTC AGCTCGTGTC GTGAGATGTTGGGTTAAGTC CCGCAACGAG CGCAACCCTT GTCCTTAGTT G
CCAGCACGT AATGGTGGGA ACTCTAAGGA GACCGCCGGT GACAAACCGG AGGAAGGTGG GGATGACGTCAAGTCATCAT
GGCCCTTACG ACCAGGGCTA CACACGTACT ACAATGGTGG GGACAGAGGG CTGCAATCCCGCGAGGGTGA GCCAATCCCA
GAAACCCTAT CTCAGTCCGG ATTGGAGTCT GCAACTCGACTCCATGAAGT CGGAATCGCT AGTAATCGCA GATCAGCATT
GCTGCGGTGA ATACGTTCCCGGGCCTTGTA CACACCGCCC GTCACACCAT GGGAGTTTGT TGCACCAGAA GCAGGTAGCTT
AACCTTCGG GAGGGCGCTT GCCACGGTGT GGCCGATGAC

2.27 The 16S rDNA nucleotide sequence of MXC3-9

TCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCGCGGGCTTCGGCCTGGCGGCGAGTGGCGAACGGG
TGAGTAATACATCGGAACGTGCCCTGTTGTGGGGGATAACTAGTCGAAAGATTAGCCTAAATACCGCATACGACCTGAGGGTGAAAGCGGG
GGACCGCAAGGCCTCGCGCAATAGGAGCGGCCCGATGTCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTG
GTCTGAGAGGAAGATCAGCCACACTGGGAATGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCA
ACCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATCGCGCTGGCTAATACCTGGC
GTGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAATCGGAATTACT
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TAGTCCGGATCGTAGTCTGCAACTCGACTACGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTC
TTGTACACACCGCCCGTCACACCATGGGAGTGGGTTTTGCCAGAAGTAGTTAGCCTAACCGCAAGGAGGGCGATTACCACGGCAGGGTT

2.28 The 16S rDNA nucleotide sequence of SF

ATGAAATAAAAATAAGAGAGAAGTTTTAGTTTGATCCCTGGCTCCAGGACGGAACGCTGGCGGCGTGACTTAACACATGCAAGTTCGAAAC
GATGAAGCCCCAGCTTCCCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGTTGATCTGCCCTGCACTCTGGGATAAGCCTGGGAA
ACTGGGTCTAATACCGGATATGACCTCTTGCTGCATGGTGAGGGGTGGAAAGTTTTTCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTT
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ACCGGACGACTGCAGAGATGTGGTTTCCCTTGTGGCCGGTAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCACGTGATGGTGGGGACTCGCAGGAGACTGCCGGGGTCAACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGTCGGTACAGAGGGCTGCGATACCGTGAGGTGGA
GCGAATCCCTTAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGC
TGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCCTCGTGG
GAGGGAGCCGTCGAAGGTGGGATTCGG

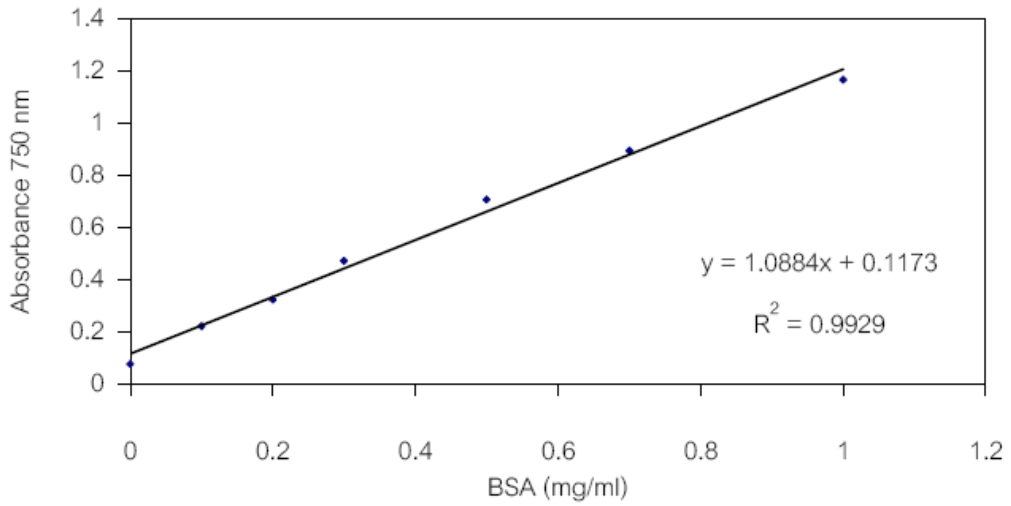
3. DNA G+C contents of the type strains

Type strain	DNA G+C content (mol%)
Microbacterium barkeri 15036^T	68.7
Paenibacillus agaridevorans DSM 1355^T	52.0
Paenibacillus favisporus GMP01^T	53.0
Paenibacillus naphthalenovorans PR-N1^T	49.0
Paenibacillus validus LMG 11161^T	50.9
Bacillus funiculus NAF001^T	37.2
Cohnella thermotolerans CCUG 47242^T	59.0
Pseudoxanthomonas suwonensis 4M1^T	68·4

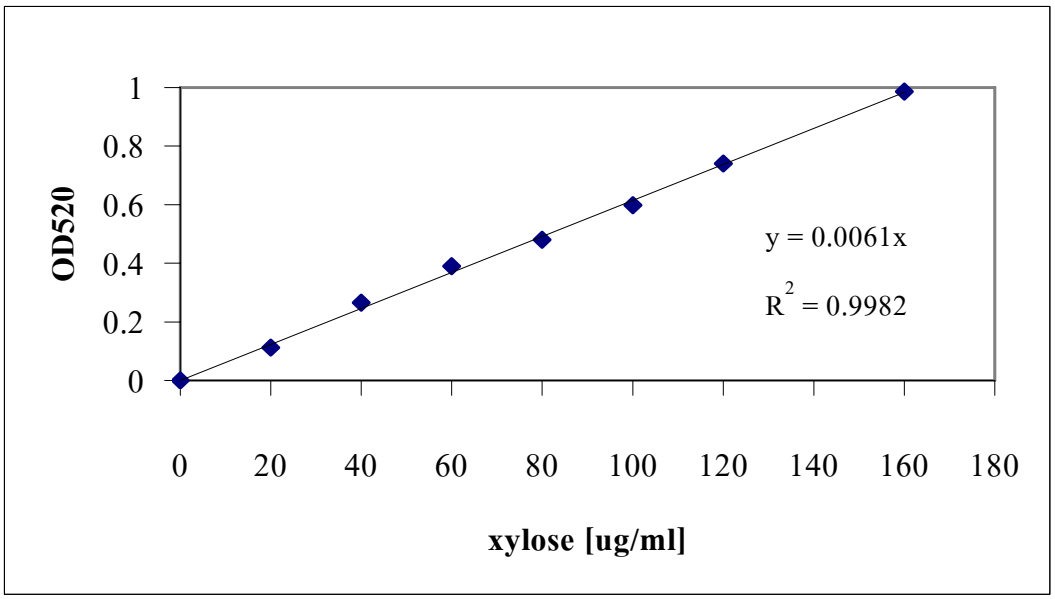
APPENDIX E

Standard curve of Bovine serum albumin(BSA) and xylose

1. Standard curve of Bovine serum albumin(BSA)



2. Standard curve of xylose



BIOGRAPHY

Miss Saowapar Kinegam was born on February 4, 1982 in Petchaburi, Thailand. She obtained a Bachelor Degree of Science in Microbiology from Prince of Songkla University, Songkla, Thailand in 2004.