Exiguobacterium Sp. HA2, isolated from the Ilam Mountains of Iran

Reza Heidari[1], Mostafa Akbariqomi[1], Gholamreza Tavoosidana[1,] Garshasb Rigi[2, 3*]

[1]Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

[2]Department of Genetics, Faculty of Basic Science, Shahrekord University, Shahrekord, Iran.

[3]Department of Industrial Biotechnology, Research Institute of Biotechnology, Shahrekord University, Shahrekord, Iran

***Corresponding author**: Dr. Garshasb Rigi, Department of Genetics, Faculty of Basic Science, Shahrekord University, Rahbar Blvd, P O Box 115, Shahrekord 881 863 4141, Iran, Email: garshasbiotech@sku.ac.ir

ABSTRACT

A motile, Gram-stain-positive, rod-shaped, non-sporing, tolerate up to 5% NaCl, grew at 0–25 °C, designated *Exiguobacterium* sp. HA2 was isolated from the soil of the Ilam Mountains of Iran during October 2016. The major isoprenoid quinone is MK-7 and in the smaller amount are MK-6 and MK-8. Polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine. Major fatty acids (>10 %) are iso$C_{13:0}$, iso$C_{15:0}$ and $C_{16:0}$. The bacterial cell wall peptidoglycan layer was lysine-glycine. The 16S rRNA sequence was analyzed at the phylogenetic levels. Also, A supplemental comparison was made between five other genes including *csp*, *gyr*B, *hsp*70, *rpo*B, and *cit*C. According to the results of genotypic and phenotypic characteristics, the strain was categorized in the genus *Exiguobacterium*. This bacterium had the closest relation with *Exiguobacterium unda*e, and thus was dubbed *Exiguobacterium* sp. HA2. The different in the Phenotypic, functional characteristics and phylogenetic indicated *Exiguobacterium* sp. HA2 can be regarded as representing considered a novel species within the genus *Exiguobacterium*.

Keywords: Psychrotolerant, *Exiguobacterium*, novel species, Polyphasic taxonomy

Author Notes: The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene, *gyr*B, *cit*C, *rpo*B, *hsp*70, and *csp* sequence of strain *Exiguobacterium* sp. HA2 are MK272780, KX574228, MH370480, MH378445, MH378775, and MH379139, respectively.

Introduction:

The genus of *Exiguobacterium* includes psychrophilic, thermophilic and mesophilic strains and species [1]. The *Exiguobacterium* genus is anaerobic, gram-positive bacteria of low G+C contents. This bacterium has been repeatedly isolated from the ancient Siberian sediments, as well as environments such as Greenland glacial, the spa springs (Yellowstone National Park), and food processing environments. Therefore, the isolation sources of this bacterium are totally different and often in extreme environments [2]. Accordingly, each of these species or strains appears to have specifically and exclusively adapted to the environments. The diversity in their genome has been responsible for their adaptation to different conditions [3].

This genus was divided into two groups according to the 16S rDNA sequence and genomic sequence; group I is the psychrophilic *Exiguobacterium*, and group II thermophilic [4]. Many strains of *Exiguobacterium* have unique properties that make them suitable for applications in biotechnology, detergent industry, sewage treatment, and agriculture [5]. Psychrophilic microorganisms have been described that are capable of growing at $<20^0$C [6].

In the present study, *Exiguobacterium* was isolated from the soil samples collected from the Ilam Mountains (one of Iran's cold provinces), and its genotypic and phenotypic characteristics were assessed and identified.

Isolation, cultivation conditions and maintenance of strain

Exiguobacterium sp. HA2 has been isolated from soil samples by cold enrichment method. The soil samples were collected from the top layer of soil (5 cm) from an area in Ilam (33° 45' 30.32" N, 46° 11' 51.33" E). The soil sample was store at cold room at 4 °C for 72h before isolation. For isolation, 10 g of soil sample was added in 50 ml sterile water, and then the suspension was incubated at 4°C for 60 min. The strain was isolated on Tryptone Soy Agar (TSA; Difco Laboratories, Detroit, MI, USA) at 4^0C for 5 days. The isolated bacterium was stored at -80^0C in TSB (Tryptone Soya Broth) supplemented with 25% glycerol for cryoprotectection.

Genomic characterization

Genomic DNA was extraction and purification using the THP(Triton/Heat/Phenol) slight modification of protocol [7]. Also, Phylogenetic comparisons were performed between the mentioned new strain and sequences of five other genes including *csp* (universal major cold shock

protein), *gyr*B (gyrase subunit B), *hsp*70 (Class I-heat shock protein-chaperonin), *rpo*B (DNA-directed RNA polymerase beta subunit), and *cit*C (isocitrate dehydrogenase). To confirm bacterial identification, we amplified the genes (16s rRNA, *csp*, *gyrB*, *hsp70*, *rpoB*, *citC*) directly by using the universal primers for *Exiguobacterium* **(Table1)**. The annealing temperatures were respectively 56, 53, for *csp*, *rpo*B, and 49^0C for *cit*C and *gyr*B, and 47^0C for *hsp*70. The PCR amplified products were purified using QIAquick Kit (PCR Purification Kit, Qiagen, Valencia, CA, USA) and then sequenced [8]. The TOPO TA Cloning Kit (Life Technologies) was used for the cloning and sequencing of the amplicons of other genes. The Qiagen Plasmid Kit (Qiagen, Valencia, CA, USA) was then used to extract the obtained clones. An ABI 373a DNA sequencer was used for the analysis of the products of cycle sequencing performed by a Perkin-Elmer 9600 thermal cycler and an ABI Dye Terminator Chemistry (PE Applied Biosystems).

Genotypic analyses were conducted to clarify the phylogenetic relationship between the new strain and the most relevant set of five reference strains. The sequence analysis of the 16S rRNA gene indicated that new strain was categorized in the genus *Exiguobacterium* **(Fig. 1)**. The sequence was deposited in GenBank with the accession number KT967971. The sequences of *gyr*B, *cit*C, *rpo*B, *hsp*70, and *csp*, also were deposited in GenBank with the accession numbers of KX574228, MH370480, MH378445, MH378775, and MH379139, respectively. Phylogeny analysis of other genes was performed to confirm identification the phylogenetic relationships of this strain within *Exiguobacterium*. Phylogenetic trees constructed based on *gyr*B **(Fig. 2)**, *cit*C **(Fig. 3)**, *rpo*B **(Fig. 4)**, *hsp*70 **(Fig. 5)**, and *csp* **(Fig. 6)** sequences of the following genes. Analysis of the 16S rRNA gene sequences indicates that HA2 had a high level of similarity to SH3 and undae (96%). Also, the alignment and comparison sequence of *gyr*B, *cit*C, *rpo*B, *hsp*70, *csp* of HA2 showed highest similarity to *Exiguobacterium* undae. Analysis of the gene sequences of HA2 with other members of the genus *Exiguobacterium* indicated that similarities with phylogenetic neighbours were in the range 60-100%.

Using the MEGA5.0 program, we constructed Phylogenetic trees based on the neighbor-joining and distance methods. The phylogenetic tree was reconstructed with generated by the maximum-likelihood method and the reliability of each node was assessed with 1000 bootstrap test replications. The scale bar represents 0.1 substitution per nucleotide position. The roots of the trees were determined using those genes from *Bacillus*.

Morphology, physiology and biochemical characterization

The optimum temperature required for the growth of *Exiguobacterium* sp. HA2 was 15^0C. A colony size desirable for scanning electron microscopy (SEM) was reached by incubating inoculated R2A plates at 4^0C for 28 days. Samples for scanning electron microscopy (SEM) studies were prepared according to the previously published method [9]. Gram-staining was performed as described previously [10]. Minimum and maximum temperatures for growth was investigated on R2A agar after incubation for 5 days at −4 to 30°C (−4, 0, 4, 10, 15, 20, 25, 30°C). Tolerance to NaCl was determined by incubating R2A broth supplemented with NaCl (0–10%, w/v, at 0.5% intervals) concentrations in 1.5 ml. The pH range was evaluated using R2A broth containing various pH 6–10 (at intervals of 0.5 pH unit) prior to autoclaving using appropriate buffers [11]. Presence of spores was evaluated by staining with malachite green. API 20NE, API ID 32GN and API ZYM (bioMerieux) were used for analyzing the production of different enzymes. Fatty acid extraction and analysis was determined by gas chromatography (GC) as described previously [12]. Antibiotic sensitivity testing was performed using standard methods [13]. The culture's ability to assimilate different carbon compounds was assessed using minimal medium [K_2HPO_4 2%(w/v); KH_2PO_4 0.5% (w/v); agar 1% (w/v)]. The analysis of chemotaxonomic markers was performed through cell wall amino acids [14], polar lipids [15], peptidoglycan structure [16] and isoprenoid quinones [17]. *E. sibiricum* DSM 17290, *E. antarcticum* DSM 14480T, *E. oxidotolerans* JCM 12280T, *E. acetylicum* DSM 20416T, *E. undae* DSM 14481T, and were used as reference strains in morphological and biochemical assessments and fatty acids identification.

Total the membrane lipoquinones and polar lipids were extracted and separated according to the previous described [18] phospholipids were visualized by staining with molybdenum blue spray (Sigma). The peptidoglycan was isolated and analyzed using a method adapted from Schleifer and Kandler [14]. after hydrolysis the cells, total-cell sugars were analysis by TLC on cellulose plates according to the previous methods [19]. The major isoprenoid quinone is MK-7 and in the smaller amount are MK-6 and MK-8. Polar lipids compositions including diphosphatidylglycerol, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine. Major fatty acids (>10 %) are iC13:0, iC15:0 and C16:0; minor components are listed in **Table 2**. The peptidoglycan type is lysine-glycine.

Cells of strain HA2 were Gram-positive, non-sporing, motile, facultatively anaerobic, rod-shaped, approximately 0.8–1µm in width and 1.5–2µm in length **(Fig.7)**. Growth occurred at between 0 and 25°C (optimally, 15°C), pH grows at pH 6–10 (optimally, 7.0) and in the presence of 0–12 (w/v) NaCl with an optimum of approximately 3 (w/v) NaCl. positive in tests for oxidase, Gelatinase, b-galactosidase, DNase, catalase, Caseinase, Phosphatase, Lysine decarboxylase, but are negative for, urease and H_2S production and for the indole test. *Exiguobacterium* sp. HA2 utilize D-Fructose, D-Galactose, D-Mannose, L-Rhamnose, Cellobiose, D-Lactose, Maltose, Starch, Amygdalin, Arbutin, Glycogen, Citrate utilization. Strain HA2 was susceptible to Amikacin (30µg), Amoxicillin (30µg), Clindamycin (25µg), Colistin (10µg), Doxycycline (25µg), Co-trimoxazole (25µg), Nalidixic acid (30µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Sulfamethoxazole (50µg), tobramycin (15µg), lomefloxacin (30µg), roxithromycin (30µg), ciprofloxacin (30µg), lincomycin (15µg), cefotaxime (30µg), cefazolin (30µg), kanamycin (30µg), novobiocin (30µg), chloramphenicol (30 µg), ampicillin (25µg), tetracycline (30µg), streptomycin (25µg), erythromycin (15µg), bacitracin (10µg), gentamicin G(30µg), polymyxin B (50 µg), oleandomycin (15µg), spectinomycin (100µg), rifampicin (25µg) and carbenicillin (100µg). but resistant to ceftriaxone (30µg) norfloxacin (10µg), gentamycin (10µg). The phenotypic features and biochemical profile of HA2 compare with other reference strains are description and illustrated in **Table 3**.

Description of *Exiguobacterium* sp. HA2

Phenotypic evaluations were also conducted to clarify the phenotypic distinguishability of the new isolates from each other and the reference *Exiguobacterium* strains. Scanning electron microscope images of the strain are shown in Fig. 7. Cells are Gram-positive, rod-shaped (2–3mm), aerobic, non-spore-forming. As presented in Table 1, surface colonies are bright, yellowish orange, convex, entire and shiny. Growth occurs at 0 to 25^0C; optimal temperature for growth about 15^0C. Tolerate to 5 % NaCl, grow at pH 6–10, are positive in tests for oxidase, Gelatinase, b-galactosidase DNase, catalase, Caseinase, Phosphatase, amylase, lipase and protease. *Exiguobacterium*. sp. HA2 utilize D- glucose, sucrose, starch, Trehalose, Raffinose, Cellulose, Amygdalin, Arbutin, Glycogen, D-Sorbitol, Citric acid, Lactic acid. Cells are sensitive to the following antibiotics (µg): Amikacin (30) Amoxicillin (30) Clindamycin (25) Colistin (10) Doxycycline (25) Co-trimoxazole (25)

Nalidixic acid (30) Norfloxacin (10) Nitrofurantoin (300) Sulfamethoxazole (50). The peptidoglycan type is lysine-glycine. The major isoprenoid quinone is MK-7 and in the smaller amount are MK-6 and MK-8. Polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine. Major fatty acids (>10 %) are iC13:0, iC15:0 and C16:0; minor components are listed in Table 2. Isolated from a soil from Ilam, Iran.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

This article does not contain any studies with animals performed by any of the authors.

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Table 1. Primers used for PCR amplification for *Exiguobacterium* genus analysis.

Gene amplified	Sequence (5´–3´)	Fragment size (bp)
16s rRNA		1506 bP
16s-F	AGG GTT GCG CTC GTT G	
16s-R	AAG GAG GTG WTC CAR CC	
DNA Gyrase—beta subunit		930 bp
gyrB-F	AAA CGT CCG GGT ATG TAT ATC GGA TCG AC	
gyrB-R	CGG CGG CTG SGC AAT RTA SAC GTA	
ClassI-heat shock protein-chaperonin		1453 bp
hsp70-F	CCC GAA TTC GGT AHA GTA AAA TGG TTY AAC KC	
hsp70-R	CCC GGA TCC GGT TAC GTT ASC WGC TKS HGG DCC	
DNA-directed RNA polymerase beta subunit		1072 bp
rpoB-F	CGA ACA TGC AAC GTC AGG C	
rpoB-R	ACA TCY TCY TCA CGN GCA CC	
Isocitrate dehydrogenase		1163 bp
citC-F	GGD GAY GGM ACW GGW CCW GAY ATT TGG	
citC-R	AAT TCW GAA CAT TTM ACT TCT GT	
Universal major cold shock protein		201 bp
CSP-U5	CCC GAA TTC GGT AHA GTA AAA TGG TTY AAC KC	
CSP-U3	CCC GGA TCC GGT TAC GTT ASC WGC TKS HGG DCC	

N=A:T:C:G; H=A:T:C; D=T:G:A; K=T:G; Y=C:T; M=C:A; W=A:T; R=A:G; S=C:G; all 1:1

Table 2. Fatty acid composition of the Siberian permafrost isolates and the type strains of Exiguobacterium

	HA2	**7-3**[b]	**255-15**[b]	**190-11**[b]	**E. undae**[a]	**E. antarcticum**[a]	**E. aurantiacum**[a]
iC11:0							2
iC12:0	3	2	2	2	2	3	3
C12:0	1	1		1		1	2
iC13:0	11	9	13	8	9	12	18
AiC13:0	8	11	15	10	9	11	12
iC14:0	9	1	1	1	2	1	
C14:0	2	3	1	2	2	2	3
iC15:0	11	13	12	13	10	11	4
aiC15:0	4	3	4	3	3	2	
C16:1−11c	9	8	3	7	8	18	10
iC16:0	3	2	2	2	2		
C16:1−5c		1		1			
C16:1−7c		1		1	7	3	
C16:0	16	17	20	12	17	13	27
C17:1−10c	4	2	2	3	2	3	
iC17:0	5	9	12	8	7	5	6
aiC17:0	3	3	3	3	2		
C18:1−9c		2	1	2	3	6	2
C18:1−7c	2	2	1	3	3		
C18:0	5	4	5	3	6	5	5

Strains: 1, *Exiguobacterium* sp. HA2; 2, *Exiguobacterium* strains 7-33; 3, *Exiguobacterium* strains 255-15; 4, *Exiguobacterium* sp. 190-11; 5, E. undae DSM 14481T; 6, E. antarcticum DSM 14480T; 7, E. aurantiacum DSM 6208T.

Only values >1% are indicated; values ≥5% are given in bold

aData obtained from Rodrigues et al. (2006) [20]

bData obtained from Fruhling et al. (2002) [21]

Table 3. Phenotypic features distinguishing strain *Exiguobacterium* sp. HA2 from the six most closely related species of the genus *Exiguobacterium*

Characteristic	1	2	3	4	5	6	7
Source	Soil	Microbial mat	Siberian	Garden pond	Glacial water	Drainage from	Creamery waste
Colony size (mm)	2–3	2–3	2–3	2–4	2–4	1–5	2–5
Colony shape	Round	Round	Round	Round	Round	Round	Irregular
Colony colour	Yellowish orange	Orange	Orange	Orange	Yellowish orange	Orange	Yellowish orange
Growth temperature (uC)							
2.5	+	-	+	-	w	w	-
5	+	-	+	-	+	+	-
37	–	+	+	+	-	+	+
Maximum growth temperature (ºC)	25	41	40	41	30	40	37
Gelatinase	+	+	+	+	-	+	+
DNase	+	+	-	+	-	+	+
Caseinase	+	+	+	+	-	+	+
Phosphatase	+	+	+	-	+	+	+
Lysine decarboxylase	+	-	+	+	+	-	-
Arginine decarboxylase	+	+	+	+	+	-	-
Tryptophan eamination	+	+	+	-	-	+	+
Aesculin hydrolysis	+	+	+	+	-	+	+
Starch hydrolysis	+	+	+	+	-	+	+
Citrate utilization	-	-	+	-	+	+	+
Malonate utilization	+	-	-	-	+	-	-
Reduction of nitrate to nitrite	-	-	-	-	+	-	-
Methyl red test	+	+	-	+	2	+	+
Acid production from:							
Melibiose	-	-	+	-	-	-	-
D-Adonitol	-	-	-	-	-	-	+
Cellobiose	+	+	-	-	-	-	+
D-Fructose	+	+	+	+	-	+	+
D-Glucose	+	+	+	+	-	+	+
D-Mannose	+	-	+	+	-	+	+
Maltose	+	+	+	+	-	+	+
D-Ribose	+	+	+	+	-	+	+
L-Rhamnose	+	+	-	+	-	+	+
Sucrose	+	+	+	+	-	+	+
Trehalose	+	+	+	+	-	+	+
L-Xylose	+	-	+	-	-	-	-
Inulin	-	-	+	-	-	-	-
Carbon source utilization							

L-Arabinose	-	-	+	-	-	-	-
D-Arabinose	-	-	+	-	-	-	-
D-Ribose	+	+	+	+	-	+	+
D-Xylose	-	-	-	-	+	-	-
L-Xylose	-	-	-	-	+	-	-
D-Fructose	+	+	+	+	-	+	+
D-Galactose	+	-	-	+	+	-	-
D-Mannose	+	+	-	+	-	+	+
L-Rhamnose	+	-	-	-	+	-	-
Cellobiose	+	+	-	+	+	-	+
D-Lactose	+	-	+	-	-	-	-
Maltose	+	+	-	+	-	+	+
L-Fucose	-	-	-	-	-	-	+
Melibiose	-	-	+	-	+	-	-
Trehalose	+	+	+	+	-	+	+
Melezitose	-	-	-	-	-	-	+
Raffinose	+	+	-	+	+	-	-
Cellulose	+	-	-	+	-	-	-
Inulin	-	-	-	-	+	-	-
Starch	+	+	+	+	-	+	+
Amygdalin	+	+	+	+	-	+	-
Arbutin	+	+	-	+	+	+	-
Salicin	-	-	ND	+	-	-	+
Glycogen	+	+	-	+	+	-	-
myo-Inositol	-	-	-	-	+	-	-
Dulcitol	-	-	-	-	+	-	-
Erythritol	+	-	ND	+	+	+	-
D-Mannitol	-	-	-	+	-	+	+
D-Sorbitol	+	-	+	-	+	-	-
Xylitol	-	-	-	-	-	-	-
Methyl a-D-mannoside	+	+	-	-	-	-	+
Methyl a-D-glucoside	+	-	-	+	-	+	+
Methyl a-D-galactoside	-	-	-	-	+	-	-
Methyl b-D-galactoside	-	-	-	-	+	-	-
a-Ketoglutaric acid	-	-	-	-	+	-	-
Citric acid	+	+	-	+	+	-	-
Fumaric acid	-	-	-	-	+	-	-
c-Glucuronic acid	+	-	-	+	+	-	-
Lactic acid	+	-	-	-	+	-	-
L-Malic acid	-	-	-	-	+	-	-
Valeric acid	-	+	ND	-	+	-	-
Sodium acetate	+	+	-	+	+	-	-
5-Ketogluconate	-	-	-	-	+	-	-

Sodium gluconate	-	-	ND	-	+	-	-
Sodium fumarate	+	+	ND	-	+	-	-
Sodium formate	+	+	ND	-	+	-	-
Sodium succinate	-	-	ND	+	+	-	-
Potassium acetate	-	-	-	+	+	-	-
Sodium pyruvate	+	+	-	+	-	+	+
Amino acid utilization							
L-Alanine	+	+	+	+	+	-	-
L-Arginine	+	+	+	+	+	-	-
L-Aspartic acid	-	-	-	+	+	+	-
L-Asparagine	+	+	-	+	+	-	-
L-Cysteine	+	+	-	+	-	-	-
L-Creatinine	+	-	-	-	+	-	-
L-Glycine	+	-	-	+	+	-	-
L-Glutamic acid	+	+	+	-	+	-	-
L-Histidine	+	+	+	+	+	-	-
L-Isoleucine	+	+	-	+	+	+	-
L-Leucine	+	+	-	+	+	-	+
L-Lysine	+	+	+	+	+	+	-
L-Methionine	+	+	+	+	+	+	-
L-Ornithine	+	+	-	+	+	-	-
L-Serine	+	-	+	+	+	+	-
L-Threonine	+	+	+	+	+	-	-
L-Proline	+	+	+	+	+	-	-
L-Tryptophan	+	+	ND	-	+	-	-
L-Tyrosine	+	+	ND	+	+	-	+
L-Valine	+	+	ND	+	+	-	-
Antibiotic tolerance (μg)							
Amikacin (30)	S	S	S	S	R	S	S
Amoxicillin (30)	S	S	S	S	R	S	S
Clindamycin (25)	S	S	S	S	R	S	R
Colistin (10)	S	R	R	S	R	R	S
Doxycycline (25)	S	S	R	S	R	S	S
Co-trimoxazole (25)	S	S	S	S	R	S	S
Nalidixic acid (30)	S	S	S	S	R	S	S
Norfloxacin (10)	S	S	R	S	R	S	S
Nitrofurantoin (300)	S	S	S	S	R	S	S
Sulfamethoxazole (50)	S	S	R	R	R	S	S
PeptidoglycanD	Lys–Gly	ND	Lys–Gly	Lys–Asp	Lys–Gly	Lys–Gly	Lys–Gly
Quinone(s)	MK-6, MK-7, MK-8	MK-6, MK-7, MK-8	MK-6, MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7
Polar lipids	PG, DPG, PE, PS, PI,	PG, DPG, PE, PS, PI,	PG, DPG, PE	PG, DPG, PE, PS, PI,	PG, DPG, PE (tr)	PG, DPG, PE, PS, PI,	DPG, PS

Strains: 1, Exiguobacterium sp. HA2; 2, E. antarcticum DSM 14480T; 3, E. sibiricum DSM 17290T; 4, E. undae DSM 14481T; 5, E. indicum IAM 15368T; 6, E. oxidotolerans JCM 12280T; 7, E. acetylicum DSM 20416T. +, Positive; 2, negative; ND, not determined; W, weakly positive; R, resistant; S, sensitive; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine
Data belonging to strains 2 to 6 obtained from Rodrigues et al. (2006) [20]

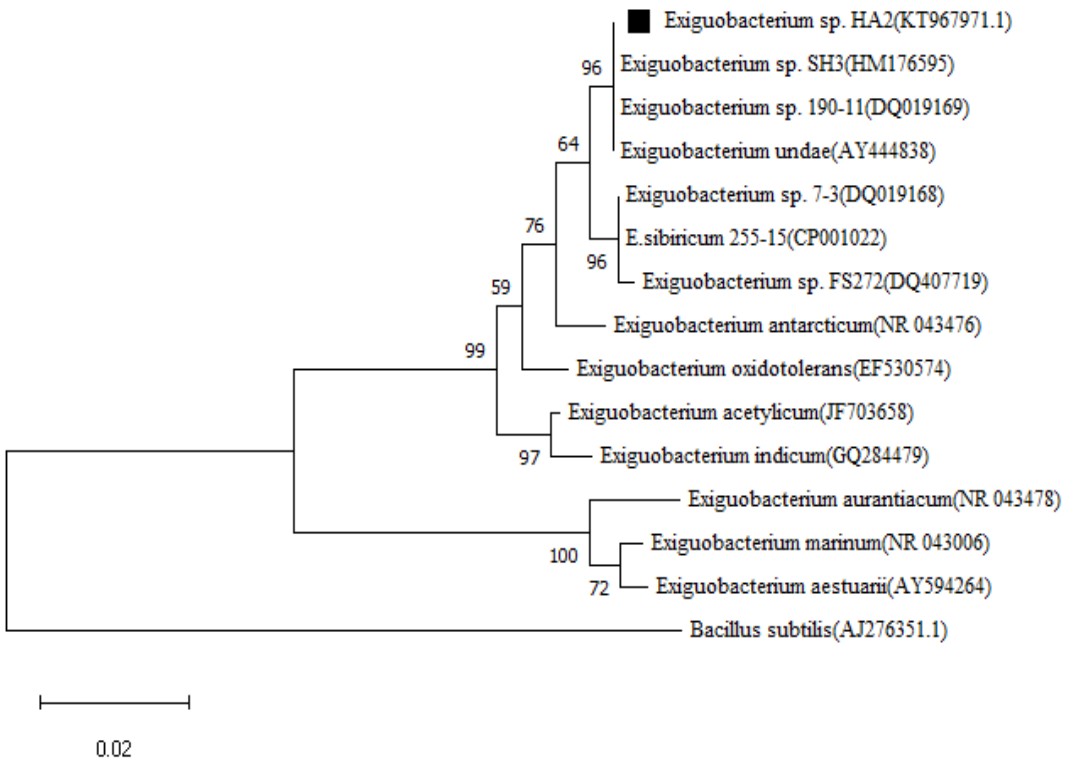

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence. Numbers at the nodes indicate the bootstrap values on neighbour joining analysis

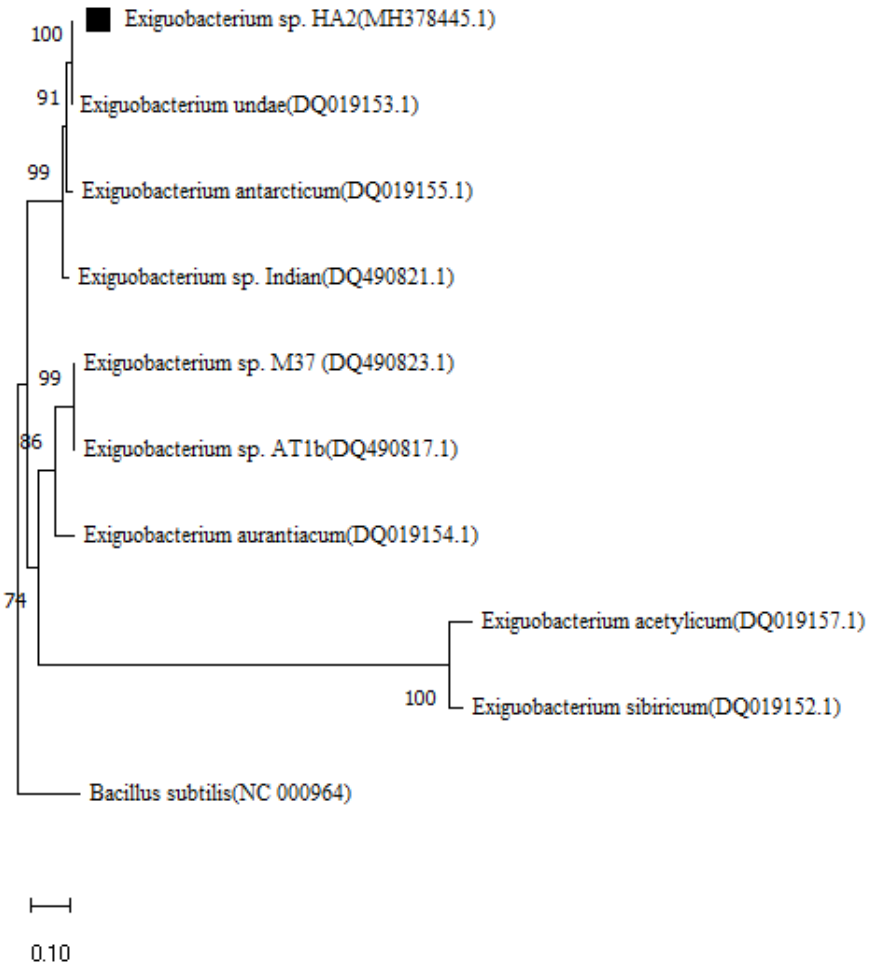

Fig. 2. Phylogenetic tree based on *gyr*B gene sequence. Numbers at the nodes indicate the bootstrap values on neighbour joining analysis

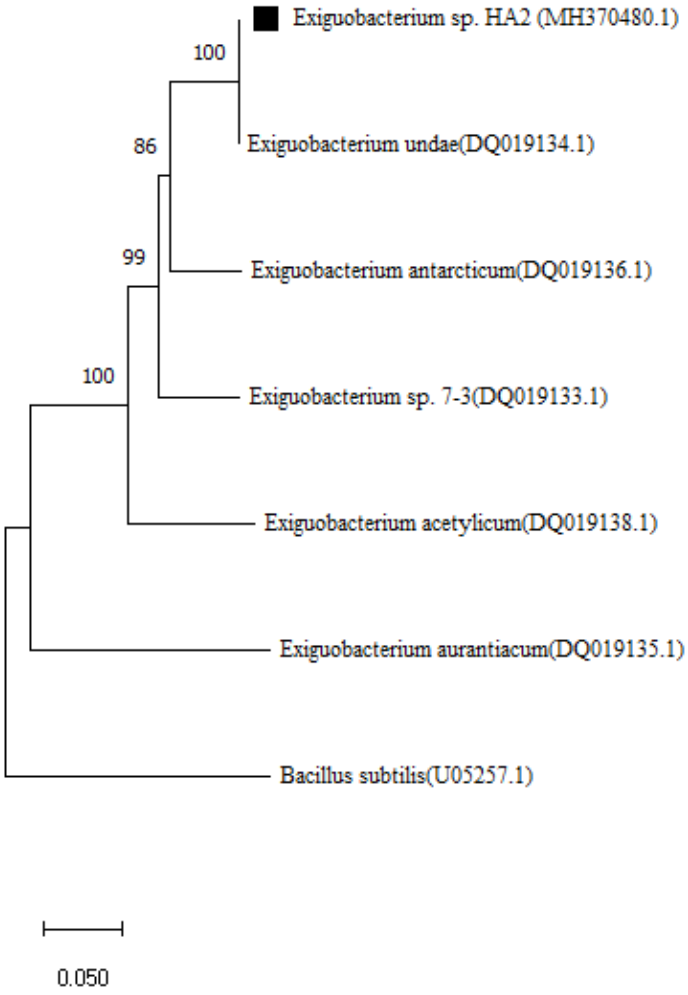

Fig. 3. Phylogenetic tree based on *cit*C gene sequence. Numbers at the nodes indicate the bootstrap values on neighbour joining analysis

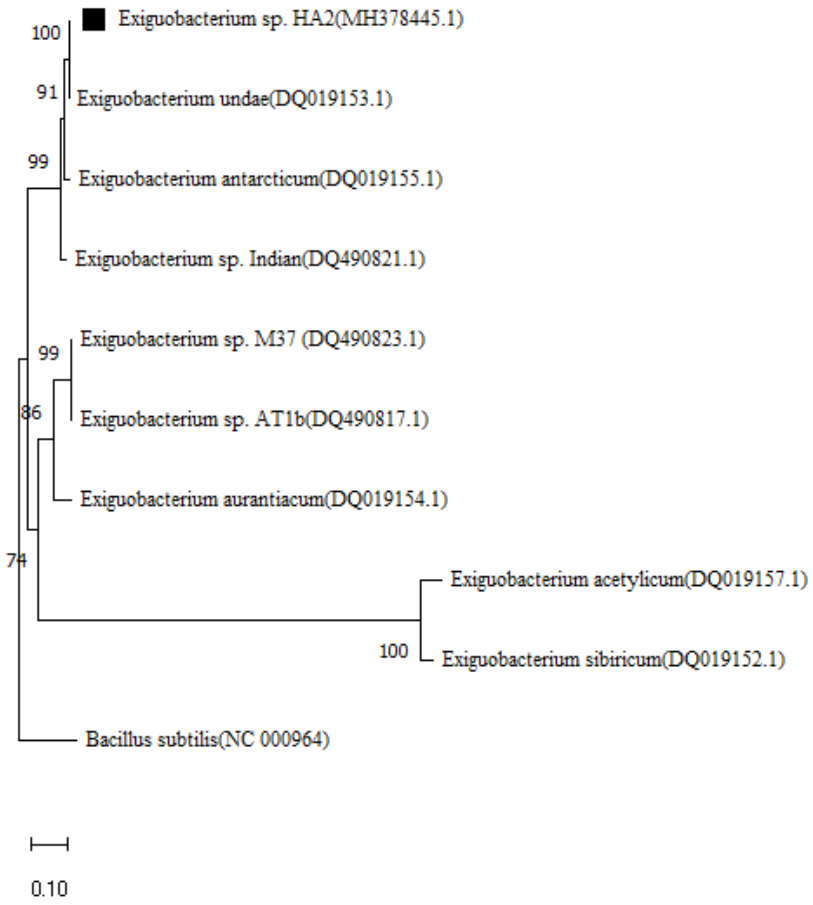

Fig. 4. Phylogenetic tree based on *rpo*B gene sequence. Numbers at the nodes indicate the bootstrap values on neighbour joining analysis

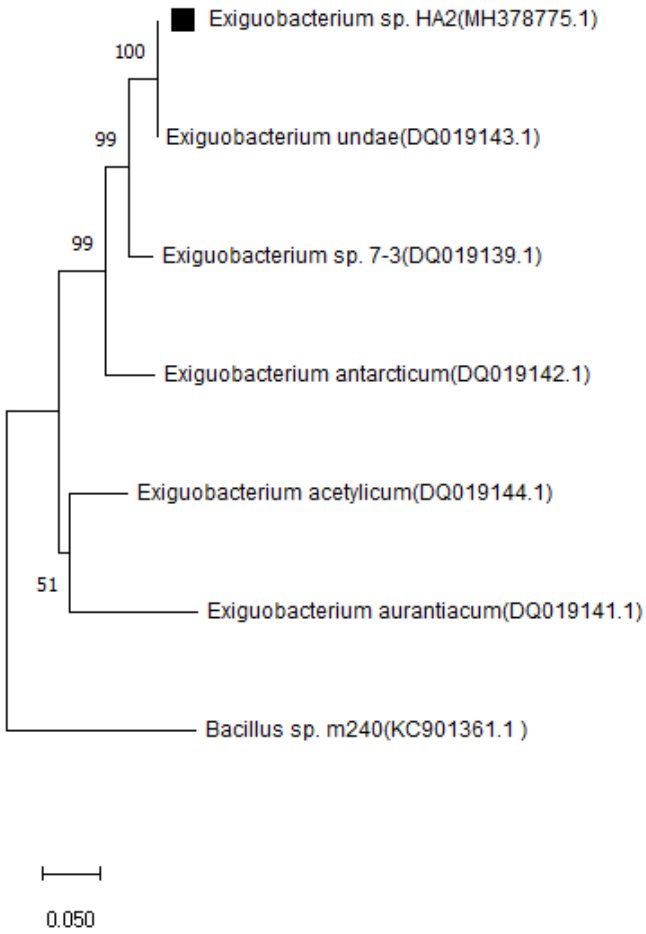

Fig. 5. Phylogenetic tree based on *hsp*70 gene sequence. Numbers at the nodes indicate the bootstrap values on neighbour joining analysis

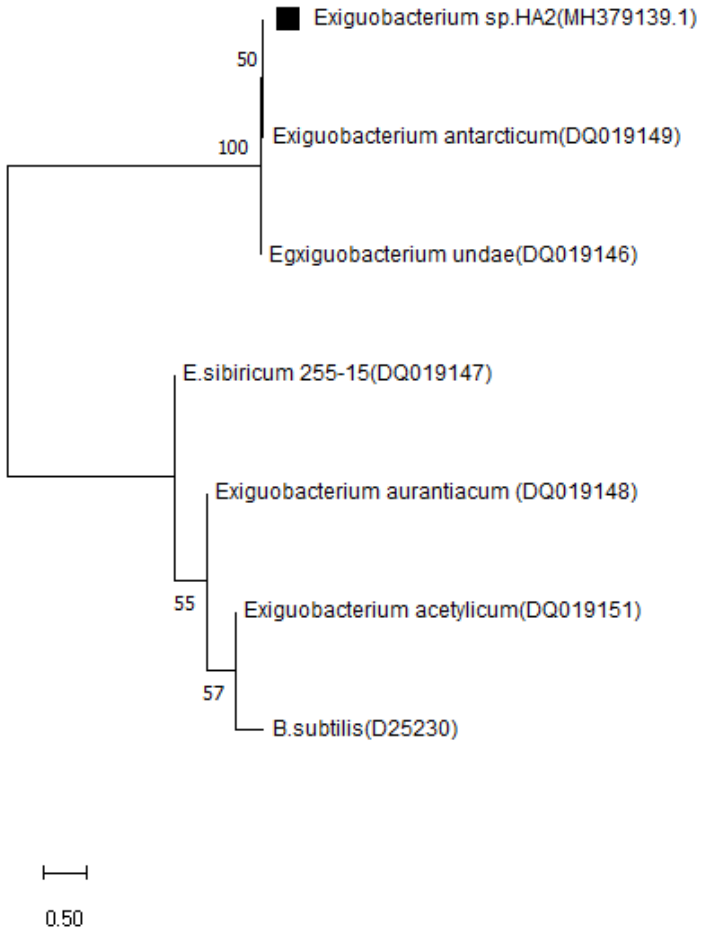

Fig. 6. Phylogenetic tree based on *csp* gene sequence. Numbers at the nodes indicate the bootstrap values on neighbour joining analysis

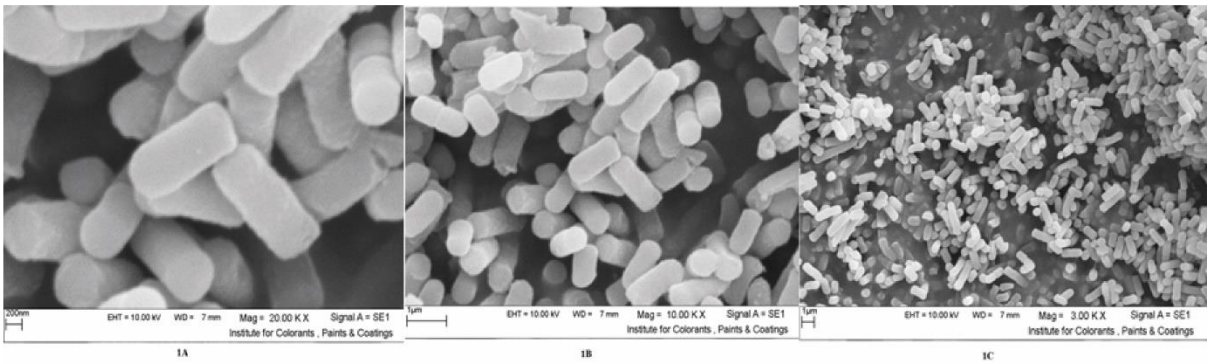

Fig. 7. Scanning electron microscopy images of *Exiguobacterium* sp. HA2 with different resolutions