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## Data Article

# Microbial biodiversity of Tang and Pirgal mud volcanoes and evaluation of bio-emulsifier and bio-demulsifier activities of Capnophile bacteria

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## ABSTRACT

The data presented in this article is related to the Master thesis; entitled “Survey Aerobic Microbial Diversity Mud Volcanoes in Chabahr and Khash Ports in Southern Iran” by the first author of this article, year 2011, Islamic Azad University, Iran (reference number (Parsia, 2011) [1] of this article). This article shows microbial biodiversity and evaluates bio-emulsifier and bio-demulsifier abilities of capnophile isolates, in order to introduce a superior isolate for the Microbial Enhanced Oil Recovery (MEOR) process in the petrochemical industry.

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## Specifications Table

Subject area	Microbiology, Biotechnology
More specific subject area	Use of superior isolates in Microbial Enhanced Oil Recovery (MEOR)
Type of data	Table, Text file

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How data was acquired	Screening of microbial groups based on their specific conditions (i.e., media culture, temperature, etc.). Biochemical identification of isolates. Evaluation of the bio-emulsifier and bio-demulsifier activities of capnophile isolates. Molecular identification and measurement of the surface tension of superior capnophile isolates in both activities.
Data format	Raw.
Experimental factors	Biochemical and microscopic tests were performed for all isolates for primary identification (biodiversity), to show some of their abilities, and then, evaluate the bio-emulsifier and bio-demulsifier activities of capnophile isolates.
Data source location	Pirgal and Tang mud volcanoes, Khash and Chabahr Ports, Southern Iran.
Data accessibility	The data is available in this article.

### Value of data

- This data would be valuable for the further studies of microbial diversity that exists in Tang and Pirgal mud volcanoes.
- This data would be valuable for further studies to find varieties of microbes with unique biotechnological applications from Tang and Pirgal mud volcanoes.
- This data would be valuable for further studies to optimize the bio-emulsifier and bio-demulsifier activities of recognized isolates.
- Used direct molecular identification methods to recognize species and compare with currently culture and biochemical methods.

## 1. Data

The dataset used in this article provides information on the microbial biodiversity of both mud volcanoes as well as the bio-emulsifier and bio-demulsifier activities of capnophile isolates, in order to use them in the Microbial Enhanced Oil Recovery (MEOR) process of the petrochemical industry. Presentation of data in this article is described in [Table 1](#).

**Table 1**

Presentation of data.

<i>Presented data</i>	<i>Tables</i>
<i>Name of group and number of microbial isolates from Tang and Pirgal mud volcanoes</i>	<a href="#">Table 2</a>
<i>Biochemical identification of gram-negative bacteria</i>	<a href="#">Table 3</a>
<i>Biochemical identification of spore forming gram-positive rods</i>	<a href="#">Table 4</a>
<i>Biochemical identification of irregular colony, non-sporing, gram-positive rod strains with different catalase tests (+ or -)</i>	<a href="#">Tables 5 and 6</a>
<i>Biochemical identification of regular colony, non-sporing, gram-positive rod strains with different catalase tests (+ or -)</i>	<a href="#">Tables 7 and 8</a>
<i>Biochemical identification of non-sporing gram-positive coccus strains with different catalase tests (+ or -)</i>	<a href="#">Tables 9 and 10</a>
<i>Identification of superior bio-demulsifier capnophile isolates based on degree of demulsification, followed by surface tension measurement and biochemical and molecular identification</i>	<a href="#">Tables 11, 12, and 13</a>
<i>Identification of superior bio-emulsifier capnophile isolates based on degree of emulsification, followed by surface tension measurement and biochemical and molecular identification</i>	<a href="#">Tables 14, 15 and 16</a>

**Table 2**  
Number of microbial isolates from Tang and Piral mud volcanoes.

Type of microbial group	Number of isolates
Mesophilic aerobic bacteria	21
Mesophilic facultative anaerobic bacteria	10
Mesophilic obligative anaerobic bacteria	0
Mesophilic capnophilic bacteria	25
Thermophile bacteria <sup>a</sup>	2
Sycrophile bacteria	3
Sulphate reducing bacteria	11
Yeast and mold	0
Nematode	0
Methylotroph bacteria	0
Methanotroph bacteria	0
<b>Total</b>	<b>72</b>

<sup>a</sup> Maximum growth temperature was 70 °C.

**Table 3**  
Biochemical tests for the identification of gram negative strains.

Isolate	Test								
	Citrate utilization	TSI	Urease	Motility	H2S	Indol production	Growth on S.S Medium	Arginine hydrolysis	Result
C9	+	Acid/acid +gas	+	+	–	–	+	<sup>a</sup>	<i>Enterobacter cloacae</i>
S3	+	Alk/acid	+	+	–	–	+	y/y	<i>Enterobacteriaceae.sp</i>
S2	+	Alk/alk	+	–	–	–	+	y/y	<i>Pseudomonas.sp</i>
X9	+	Alk/alk	–	–	–	–	+	y/y	<i>Enterobacteriaceae.sp</i>
Y5	–	Acid/acid	–	+	–	–	+	y/y	<i>Enterobacteriaceae.sp</i>

<sup>a</sup> (no need to do); C: Capnophile; S: Sycrophile; X & Y: Mesophilic aerobic; y/y: Yellow/yellow.

**Table 4**  
Biochemical tests for the identification of spore forming gram positive rods.

Isolate	Test					
	LV reaction	Citrate utilization	V-P reaction	Growth in 7%NaCl	Starch Utilization	Result
X6	–	+	–	+	+	<i>Bacillus megaterium</i>
X10	–	+	–	+	+	<i>Bacillus megaterium</i>
X11	–	–	N.D	N.D	N.D	<i>Bacillus firmus</i>
Y1	–	+	–	–	+	<i>Bacillus brevis</i>
Y3	+	–	–	+	–	<i>Bacillus laterosporus</i>
Y4	+	–	+	+	–	<i>Bacillus laterosporus</i>
B2	+	+	N.D	N.D	N.D	<i>Bacillus cereus var.mycoides</i>
B10	–	+	–	+	+	<i>Bacillus megaterium</i>

B: Mesophilic facultative anaerobic; X & Y: Mesophilic aerobic; N.D: Not determined.

**Table 5**

Biochemical tests for the identification of irregular colony, non-sporing, gram positive rod strains, catalase positive.

Isolate	Test									
	Oxygen	Motility	Acid fast staining	LV reaction	VP reaction	Growth in 7% NaCl	Starch hydrolysis	OF	Oxidase	Result
X3	A	–	–	–	+	+	–	+	+	Arthrobacter.sp
X4	A	–	–	+	–	+	–	+	+	Arthrobacter.sp
X5	A	–	–	+	–	+	–	+	+	Arthrobacter.sp
X2	A	–	–	–	+	+	–	+	+	Arthrobacter.sp
Y2	A	–	–	+	–	–	–	+	+	N.D
Y7	A	–	–	–	–	+	–	+	+	Arthrobacter.sp
Y8	F	–	–	+	–	–	–	+	+	N.D
Y9	A	–	–	+	–	+	–	+	+	Arthrobacter.sp
B1	F	–	–	–	+	–	–	+	+	N.D
B3	F	–	–	+	–	–	–	+	+	N.D
B6	F	+	–	+	–	+	–	+	–	Jonesia denitrificans
B7	F	+	–	+	+	+	–	+	–	Jonesia denitrificans
C21	F	+	–	–	–	+	–	+	–	Jonesia denitrificans

B: Mesophilic facultative anaerobic; X &amp; Y: Mesophilic aerobic; C: Capnophile; F: facultative; A: aerobic; N.D: Not determined.

**Table 6**

Biochemical tests for the identification of irregular colony, non-sporing, gram positive rod strains, catalase negative.

Isolate	Test									
	Oxygen	Motility	Acid fast staining	LV reaction	Citrate utilization	VP reaction	Growth in 7% NaCl	Starch hydrolysis	Oxidase	
X1	F	–	–	+	+	N.D	–	–	+	
B4	F	–	–	+	+	–	–	–	+	
C8	F	–	–	–	+	–	–	+	+	
C12	F	–	–	–	+	–	–	+	+	
C13	F	–	–	+	+	–	–	+	+	
C24	F	–	–	–	+	–	–	+	+	

B: Mesophilic facultative anaerobic; X: Mesophilic aerobic; C: Capnophile; F: facultative; N.D: Not determined. All strain except x1 and B4 showed 90% &lt; similarity to Aeromicrobium.sp.

**Table 7**

Biochemical tests for the identification of regular colony, non-sporing, gram positive rod strains, catalase positive.

Isolate	Test											
	Oxygen	Motility	Acid fast staining	H2S production	growth at 35 °C	VP reaction	Growth in 7%NaCl	Starch hydrolysis	OF	Oxidase	Gelatin hydrolysis	
X7	F	+	–	–	+	–	+	–	+	+	–	
Y10	A	–	–	–	+	N.D	+	–	+	+	+	
C2	F	–	–	–	+	+	+	–	O	–	N.D	

X &amp; Y: Mesophilic aerobic; C: Capnophile; F: facultative; A: aerobic; O: Oxidative; N.D: Not determined. Strain C2 showed 80% &lt; similarity to Listeria.sp.

**Table 8**

Biochemical tests for the identification of regular colony, non-sporing, gram positive rod strains, catalase negative.

Isolate	Test					
	Oxygen	Motility	Growth at 35 °C	LV reaction	Citrate utilization	TSI
B5	F	–	+	–	+	A/A+gas+H2S
C5	F	–	+	+	+	A/A+gas+H2S
C20	F	–	+	+	+	A/A+gas+H2S
C25	F	–	+	+	+	A/A+gas+H2S

B: Mesophilic facultative anaerobic; C: Capnophile; A/A: Acid/acid.

All isolates showed 98% < similarity to *Erysipelothrix.sp.***Table 9**

Biochemical tests for the identification of non-sporing, gram positive coccus strains, catalase positive.

Isolate	Test								
	Oxygen	Motility	Acid fast staining	CAMP	OF	LV reaction	VP reaction	Citrate utilization	Oxidase
B8	F	+	–	–	+	+	–	–	+
B9	F	+	–	+	+	+	–	–	–
X8	F	+	–	–	+	+	–	+	–
S1	F	–	–	–	+	–	–	+	+
C3	F	+	–	–	+	–	+	+	+
C4	F	–	–	–	+	–	–	+	+
C6	F	–	–	–	+	+	–	+	–
C7	F	–	–	–	+	+	–	+	–
C14	F	–	–	+	O	–	–	+	+
C16	F	–	–	+	O	+	–	+	+
C17	F	–	–	–	+	+	–	+	–
C19	F	–	–	+	+	+	–	+	–
C10	F	+	–	–	+	–	+	+	+
C22	F	–	–	+	+	–	+	+	–
C23	F	–	–	–	O	+	–	+	+

B: Mesophilic facultative anaerobic; X: Mesophilic aerobic; C: Capnophile; S: Sycrophile; F: facultative; O: Oxidative.

C3 and C10 strains showed 80% < similarity to *Planococcus.sp.***Table 10**

Biochemical tests for the identification of non-sporing, gram positive coccus strains, catalase negative.

Isolate	Test							
	Oxygen	Motility	Acid fast staining	LV reaction	Citrate utilization	Oxidase	Vancomycin sensitive	Growth at 10 °C
C1	F	–	–	+	+	–	S	–
C15	F	–	–	+	+	–	R	–
Y6	F	+	–	+	–	+	S	–

Y: Mesophilic aerobic; C: Capnophile; F: facultative; S: Sensitive; R: Resistance.

C 1, C15 and Y6 showed 90% < similarity to *Gemella.sp.*, *Pediococcus.sp.* and *Trichococcus.sp.*, respectively.**Table 11**

Degree of demulsification of capnophile isolates.

Strain	Degree of demulsification	Strain	Degree of demulsification	Strain	Degree of demulsification	Strain	Degree of demulsification
C1	0	C8	1	C15	2	C22	1
C2	0	C9	0	C16	0	C23	1
C3	1	C10	2	C17	0	C24	3
C4	0	<b>C11</b>	<b>5</b>	C18	0	C25	2
C5	1	C12	2	C19	0		
C6	3	C13	1	C20	3		
C7	1	C14	1	C21	2		

**Table 12**  
Surface tension and identification tests of C11 (superior bio-demulsifier isolate).

Isolate	Anaerobic growth	Motility	Acid fast	LV reaction	VP utilization	Citrate utilization	Growth in 7% NaCl	Starch hydrolysis	Indol	Gelatin hydrolysis	Gram-stain	Morphology	Molecular identification	Surface tension (mN/m)	
														S	C
C11	+	-	-	+	+	+	+	+	-	+	+	Bacilli with spore	<i>Bacillus thuringiensis</i> strain B4(1)	27.7	40.1

S:Sample; C:Control.

**Table 13**Sequences producing significant alignments *Bacillus thuringiensis* strain B4(1).

Select for downloading or viewing reports	Description	Max score	Total score	Query cover	E value	Ident	Accession
Select seq gb   FJ236808.1	<i>Bacillus thuringiensis</i> strain B4(1) 16S ribosomal RNA gene, partial sequence	1168	1168	83%	0.0	88%	FJ236808.1

## 2. Experimental design, materials and methods

In the summer of 2011, sampling was performed at Tang and Pirgal mud volcano craters, in aseptic conditions, using sterile plastic pipes (in sizes of 5, 10, 15 and 30 cm) [1].

Each sample was diluted in 9cc strilled Ringer's solution. Next, 1 cc of the solution was added to 9 cc of strilled nutrient broth medium and incubated at 30 °C for 48 h. Each microbial group used specific conditions, such as medium culture (MC), temperature (tem) and time (T) of incubation [1]. For biochemical identification, isolates were classified based on their colony shape, morphology and gram-stain. They were then identified using tests for gram negative bacteria, gram positive non-sporing and spore-forming bacilli (A colour Atlas of *Bacillus* species) and cocci bacteria based on table and diagram references [1–4].

The bio-emulsifier test used the Francy method (year 1991) and assessed their stabilizing emulsification capacity (degree 0–4) [5,6]. In the bio-demulsifier test, 1 ml from Erlenmeyer flasks was added to tubes containing stable emulsions of water/diesel and diesel/water. They were then properly vortexed and incubated at 30 °C for the assessment of demulsification degree (0 to 5). The surface tensions of superior isolates were measured by Tensiometer (TD1C LAUDA) [7,8]. Superior isolates were identified with molecular tests. Their genomes were extracted by kit. The universal primers used to amplify 16S rDNA, were 27 F(5' AGA GTT TGA TCC TGG CTC AG 3') and 1492 R(5' CGG TTA CCT TGT TAC GAC TT 3'). These amplified a 1500-base pair region of the 16S rDNA gene. The amplified DNA was visualized by gel electrophoresis and sequenced. A 16S rDNA sequence was analysed using Chromas LITE. The most similar bacterial species was found in the GenBank using BLAST search. Neighbours joining phylogenetic trees were constructed based on 16S rDNA sequences using ClustalW [1].

**Table 14**  
Degree of emulsification of capnophile isolates.

Strain	Degree of emulsification	$\beta$ -hemolysis	Strain	Degree of emulsification	$\beta$ -hemolysis	Strain	Degree of emulsification	$\beta$ -hemolysis	Strain	Degree of emulsification	$\beta$ -hemolysis
C1	0	+	C8	2	+	C15	0	-	C22	0	-
C2	1	+	C9	1	+	C16	0	+	C23	0	-
C3	0	-	C10	0	-	C17	0	+	C24	2	+
C4	0	-	C11	0	+	<b>C18</b>	<b>4</b>	+	C25	2	+
C5	3	+	C12	0	-	C19	0	-			
C6	0	-	C13	2	+	C20	1	+			
C7	0	-	C14	0	-	C21	0	-			

**Table 15**  
Identification and surface tension tests of C18 (superior bio-emulsifier isolate).

Isolate	Oxygen	Motility	Oxidase	LV reaction	VP utilization	Catalase	OF	Starch hydrolysis	Indol	Gelatin hydrolysis	Gram-stain	Morphology	Molecular identification	Surface tension (mN/m)	
														S	C
C18	F	–	–	+	+	+	+	+	–	+	+	Bacilli with endospore	Bacillus anthracis strain EFF-G51	22.6	40.1

F: Facultative; S:Sample; C:Control.

**Table 16**Sequences producing significant alignments *Bacillus anthracis* strain EFF-G51.

Select for downloading or viewing reports	Description	Max score	Total score	Query cover	E value	Ident	Accession
Select seq gb   KP813652.1	<i>Bacillus anthracis</i> strain EFF-G51 16S ribosomal RNA gene, partial sequence	1210	1210	89%	0.0	87%	KP813652.1

**Transparency document. Supporting information**

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2017.08.041>.

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