Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Materials

A novel bioemulsifier from *Geobacillus stearothermophilus* A-2 and potential application in microbial enhanced oil recovery

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Electronic Supplementary Material 1

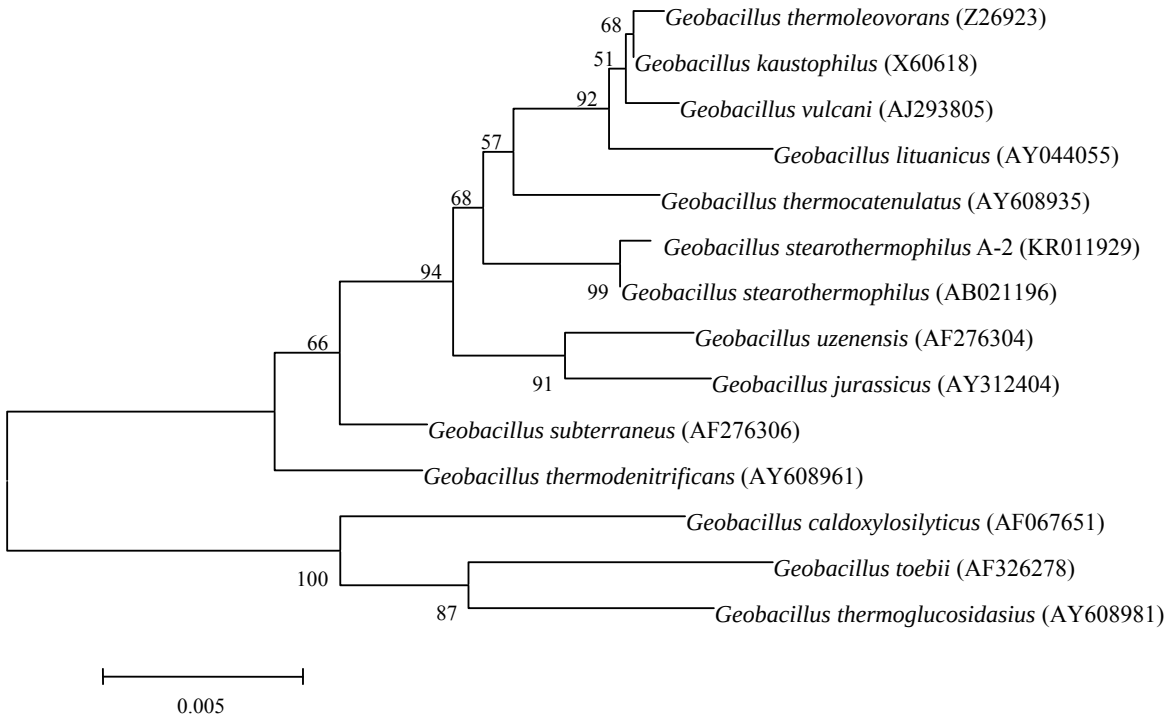

Fig. S1. The phylogenetic tree of strain A-2 constructed by neighbor-joining method with 16S rDNA gene

Electronic Supplementary Material 2

Methods:

The strain A-2 was incubated on a semi-solid Luria-Bertani medium (the concentration of agar was 0.6%) with puncture of inoculation method and cultivated for 24 hours at 60 °C. The phenomenon of strain growth along the puncture line was observed.

Results:

G. stearothermophilus A-2 could grow along the puncture line of semi-solid Luria-Bertani medium. Moreover, there were signs of growth at the bottom of puncture line which was an anaerobic zone. Thus the strain A-2 was facultative anaerobic.

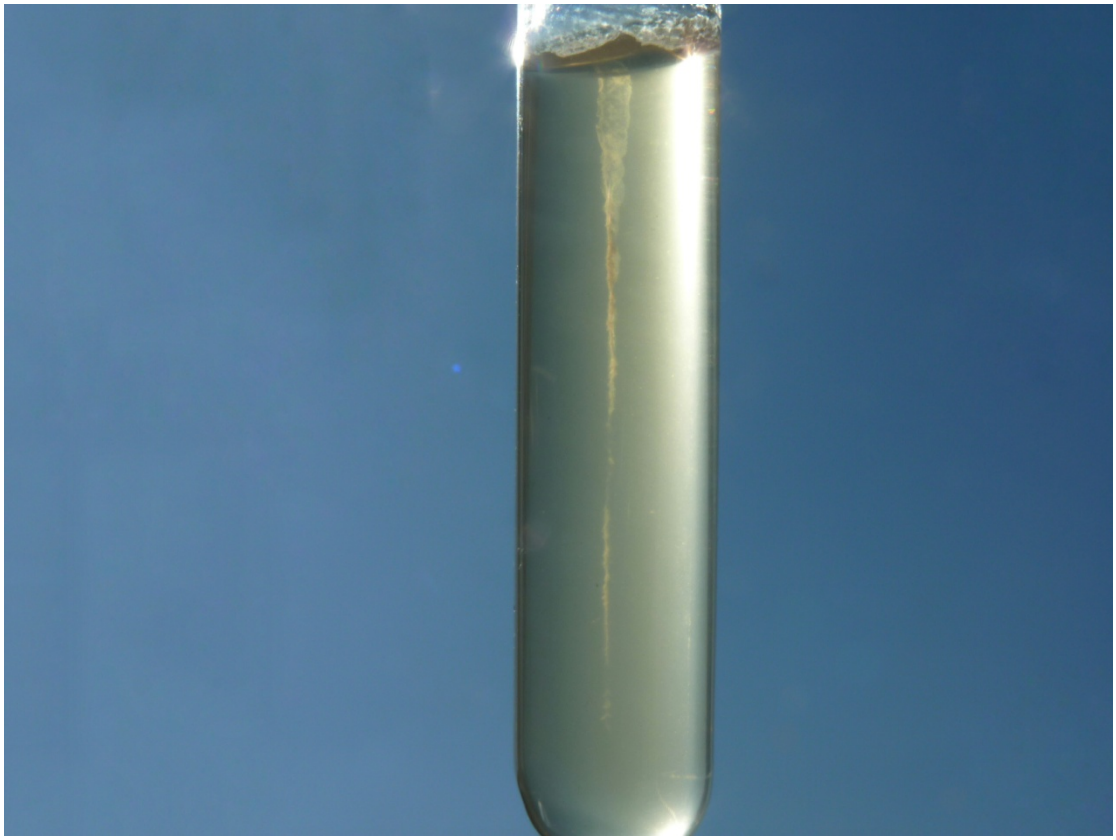

Fig. S2. The stab cultivation of strain A-2 in semi-solid Luria-Bertani medium

Electronic Supplementary Material 3

Table S1. Different characteristics between the strain A-2 and related species of genus *Geobacillus*

Characteristics	1	2	3	4	5	6	7	8	9	10
Temperature rang(℃)	40-75	37-65	35-78	45-70	37-68	45-65	45-70	45-70	45-70	55-70
pH range	4.0-9.0	6.0-8.0	6.0-8.0	6.5-8.5	6.0-8.0	6.2-7.8	6.0-7.6	5.5-9.0	6.0-8.0	6.5
NaCl range (%)	0-2	0-≤5	0-4	ND	0-≤5	0-4	0-≤5	0-3	0-3	0-0.5
Motility	+	+	+	-	ND	+	+	+	ND	+
Catalase	+	V	+	+	ND	+	ND	-	ND	+
Oxidase	-	V	+	+	ND	+	ND	-	ND	+
Hydrolysis of:										
Casein	-	V	W	+	+	-	-	-	V	+
Aesculin	+	V	+	ND	-	+	+	+	ND	ND
Gelatin	-	+	-	-	+	+	-	+	ND	+
Starch	+	+	-+	+	+	+	+	+	V	+
Gas from glucose	+	-	-	ND	-	-	-	-	V	-
$NO_3^- \rightarrow NO_2^-$	+	V	+	ND	+	+	+	-	+	+
Gas from nitrate	+	v	+	-	-	-	+	-	+	-
Production of acid from:										
L-Arabinose	+	-	-	-	-	+	-	-	+	+
Cellobiose	+	-	-	+	+	+	+	+	+	+
Galactose	-	-	-	+	V	+	+	+	+	+
Mannitol	+	V	+	+	+	+	ND	-	ND	+

Xylose	-	-	-	V	+	-	-	+	+	+
Glycerol	+	+	+	V	-	+	+	+	+	ND
Inositol	-	-	-	-	+	-	-	-	ND	ND
Lactose	-	-	-	-	-	-	-	+	+	ND
Rhamnose	-	-	-	-	-	-	-	-	V	ND
Sorbitol	-	-	+	-	-	-	-	-	ND	ND
Simmons	-	V	+	+	+	-	-	+	V	ND
Methyl red test	+	V	-	ND	+	-	+	-	ND	ND
Voges-Proskauer reaction	-	-	-	-	-	-	-	-	-	ND
Urease	-	-	-	ND	-	-	-	-	-	ND
DNA G+C content (mol %)	52.49	46-52	55.2	52-58	51-55	50.4-51.5	49.7-52.3	53.	48.2-52.3	52.5

Taxa: 1, Strain A-2; 2, *G.stearothermophilus*; 3, *G. thermocatenulatus*; 4, *G. thermoleovorans*; 5, *G.kaustophilus*; 6, *G. uzenensis*; 7, *G. subterraneus*; 8, *G. vulcani*; 9, *G. thermodenitrifican*; 10, *G.lituanicus*

+, All strains are positive; V, characteristic is variable; -, all strains are negative; ND, not determined.

Electronic Supplementary Material 4

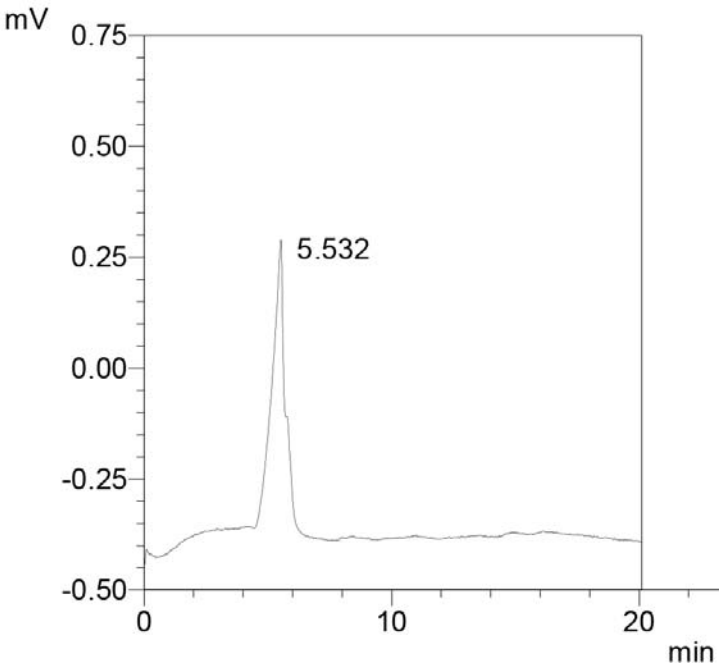

Fig. S3. The Gel-permeation chromatography (GPC) analysis of bioemulsifier produced by *G. stearothermophilus* A-2

Electronic Supplementary Material 5

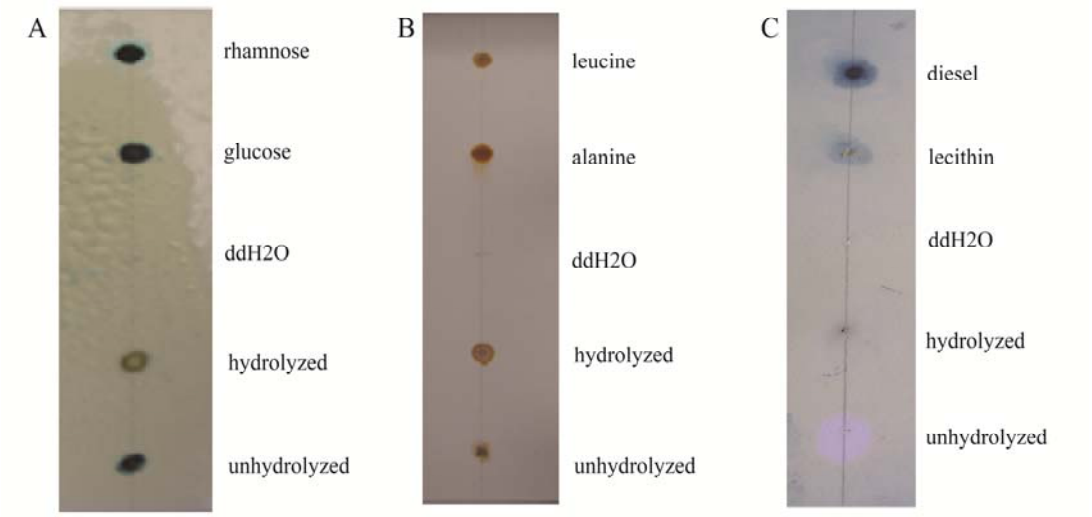

Fig. S4. Qualitative analysis of bioemulsifier produced by *G. stearothermophilus* A-2

(A) Sulfuric acid-anthrone coloration; (B) Ninhydrin coloration; (C) Ammonium molybdate-perchloric acid coloration.

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Table S2. Amino acid components of bioemulsifier produced by *G.stearothermophilus* A-2

Amino acid		Content (g /100 g)
Aspartate	Asp	2.69
Methionine	Met	1.8
Threonine	Thr	1.48
Isoleucine	Ile	1.22
Serine	Ser	0.98
Leucine	Leu	1.83
Glutamate	Glu	4.8
Tyrosine	Tyr	0.75
Glycine	Gly	1.52
Phenylalanine	Phe	1.12
Alanine	Ala	2.73
Lysine	Lys	1.64
Cysteine	Cys	0.32
Histidine	His	0.83
Valine	Val	1.98
Arginine	Arg	1.32
Proline	Pro	0.75
Total content		27.75

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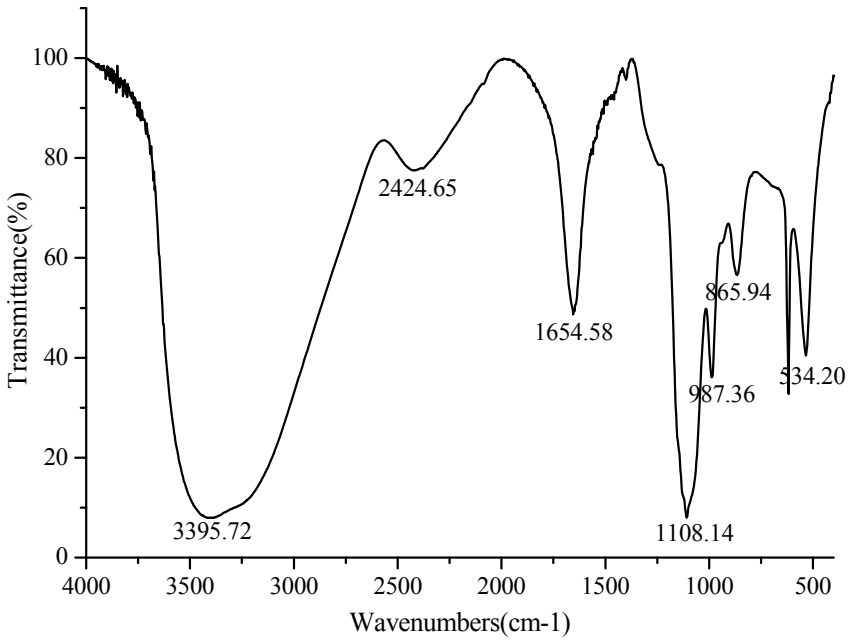

Fig. S5. FT-IR spectra of bioemulsifier produced by the *G. stearothermophilus* A-2

Electronic Supplementary Material 8

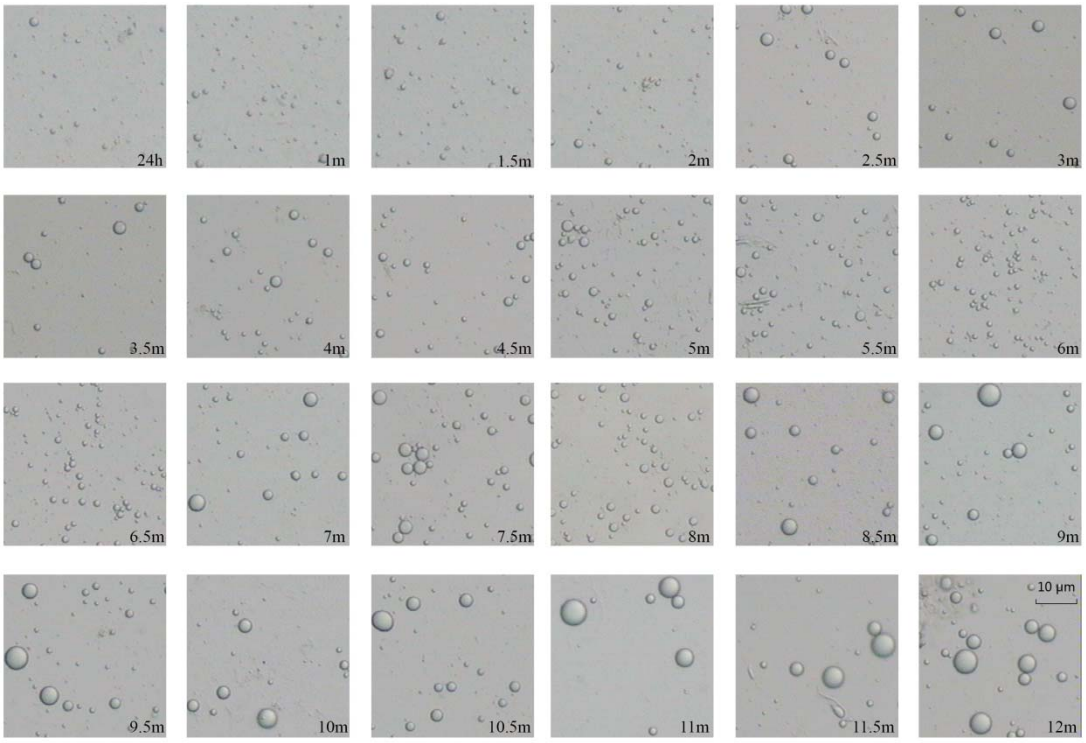

Fig. S6. Effects of placing time on the emulsifying activity of bioemulsifier (24 h-12 month) (10 × magnification, μm)