# Electronic Supplementary Information

# High-throughput Single-cell Cultivation Reveals Underexplored Rare Biosphere in Deep-sea Sediments Along the Southwest Indian Ridge†

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**Design and operation of the Droplet Picker.**

The first version of the MSP platform uses toothpicks to transfer droplet colonies into the well plates, which is not friendly for the operator because the droplet size was only hundreds micrometers and led to low isolation rate of microbes because many droplets were empty. In this work, we improved the platform with a semi-automatic Droplet Picker to accelerate the process. The picker used pump driven capillary Teflon tubing to aspirate droplet and then transfer cell suspensions to the microwell plates, which greatly reduce the contamination and also improve the recover ratios with more cells. Commercial available pickers were mostly designed for mammal cells pick which is expensive and based on air pressure and capillary glass which is not suitable for general application of MSP. So in this study, the Droplet Picker was made by ourselves.

The assembly of the picker is very simple, a syringe pump, a low cost manual X-Z translation stage, a Teflon tubing (capillary tip), a 3D printed support arm, tubing holder and dish clip are all what we need (Figure 2A). The process of picking droplets needs to touch the surface of MSP Petri dish which may cause deformation of tubing, so a 150-μm Teflon tubing was chosen rather than glass capillary. The Teflon tubing was prefilled with mineral oil and connected to the syringe, which can precisely control the aspirate. A 3D printed dish clip was used to fix the MSP Petri dish which can allow to track the spiral droplets array of MSP continually. After assembly and preparation, we can start droplet picking. First, we revolved the droplets Petri dish according to spiral tracks of droplets and the droplets were observed one by one with inverted microscope. Second, when droplets with bacteria growth was confirmed, we located the Droplet Picker to the target droplet by adjust the translation stage at x axes or by revolve the Petri at y axes. Third, after locating the target droplet, adjust the translation stage at z axes to touch the droplet and 200 nL liquid was aspirated immediately with a flow speed of 200 nL/s by the programmed Harvard pump. At last, 300 nL liquid in Teflon tube was discharged into a 96-well plate at a flow speed of 300 nL/s for scale up cultivation. Each microwell contained 100 μL M13 medium and 100 μL mineral oil. It took 20 seconds for one droplet, demonstrating that our droplet picker can save us time and liberate human hands at the same time compare to the previous MSP platform (Figure 2B, Supplementary Movie 1).

Since we used the same Teflon tubing repeatedly to pick up several droplets, the residual cells adhered on the inner-tubing wall might be a threaten to the next droplet, which often lead to cross-contamination. We confirmed the contamination with GFP-contained *E.coli* RP1616 cells, and showed a 94.4% cross-contamination. In order to overcome the contamination, we washed the tubing with 75%

ethanol three times and once with sterile water after one picking consecutively. The results showed that after the addition of washing step, the cross-contamination ratio decreased to zero. This indicated that the washing step can control the cross-contamination effectively. The whole process of washing was controlled by the programmed Harvard pump. In the later experiment, the washing step was incorporated (Supplementary Figure S1B).

**The microbial diversity of SWIR**

The microbial taxa detected in the original sediments showed highly diverse community compositions, including 56 phyla, 58 classes, 107 orders, 177 families, and 312 genera within the 2929 OTUs, and both archaea and bacteria were found to be abundant at the site. At the phylum level, *Thaumarchaeota* was the most abundant (40.3±18.6%) archaeal group, followed by *Proteobacteria* (33.7±16.0%) in bacterial group. Other taxa, including *Chloroflexi* (4.9±1.2%), *Actinobacteria* (3.8±2.0%), *Acidobacteria* (3.7±1.1%), *Firmicutes* (2.6±1.7%), *Gemmatimonade*tes (2.1±1.0%), *Nitrospirae* (1.4±0.5%), and *Bacteroidetes* (1.4±0.2%), were also detected in the site, but at much lower relative abundance (**Fig. S3C**). At the class level, the ammonia oxidation archaea, *Nitrososphaeria*, was the most abundant taxa. The *Alphaproteobacteria* (14.6±7.1%) and *Gammaproteobacteria* (14.6±6.7%) were the most abundant classes in the *Proteobacteria* phylum. At the order level, two unclassified orders were detected in *Proteobacteria*, accounting for 6.2% and 8.4% of the total reads, respectively (**Fig. S3C**). In the eight samples with culture-based sequencing methods (M1-M4, A1-A4), 19 and 14 phyla were detected in MSP pool and agar pool respectively. And 3 phyla including *Melainabacteria*, *Synergistetes* and *Deferribacteres* that only detected in MSP pool or agar pool were not fund in original samples. The relative abundance of major bacterial phyla presents in the MSP pool cultivation and agar pool cultivation varied considerably to the original sediments communities. The most abundant phylum in all samples were changed to *Proteobacteria* except for sample A1 which is *Bacteroidetes* (**Fig. S3B**). The community composition detected in this study is similar to that of previous studies. An investigative study of inactive hydrothermal vents in the SWIR found that *Thaumarchaeota* was the most abundant microbial group[1]. In the Central Indian Ridge, Arctic Mid-Ocean Ridge, and Western Pacific Ocean, *Thaumarchaeota* was also widely detected[2-4]. The abundant distribution of *Alphaproteobacteria* and *Gammaproteobacteria* were also reported in Iheya North, Iheya Ridge, and metal-rich vent sediments from Pacific Ocean hydrothermal fields[5, 6].

**References**

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**Supplementary Figures**

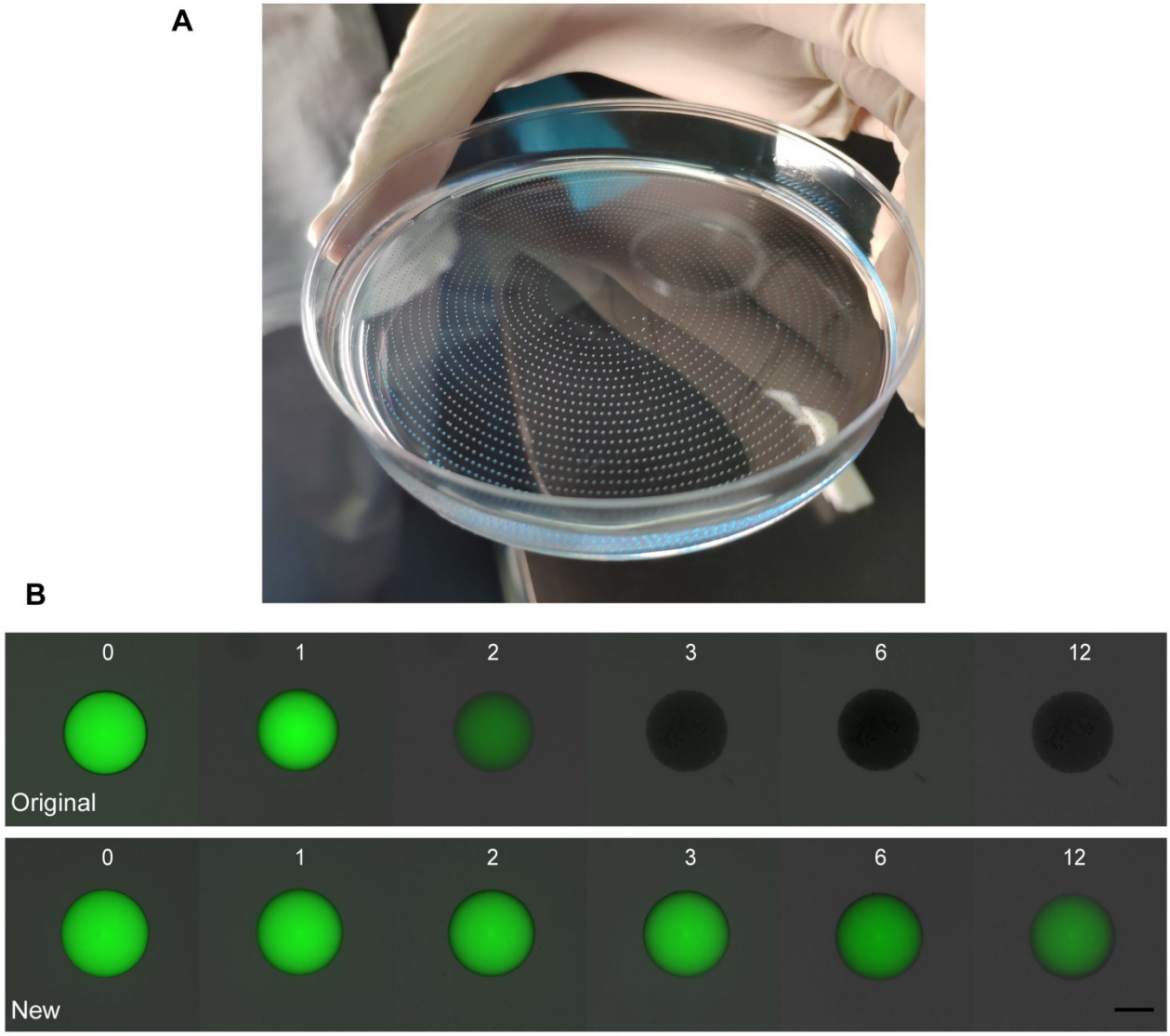


**Figure S1.** Droplet evaporation comparison of improved method and the original MSP method. (A) a picture of the MSP Petri dish; (B) Original means the method to prevent droplet evaporation in previous study (2016) and New means the method used in this study. The MSP droplets were put in 30 °C isothermal incubator with for up to 12 days. The time-lapse fluorescence photomicrographs were taken at T=0, 1, 2, 3, 6, 12 days. Scale bar is 150 μm.

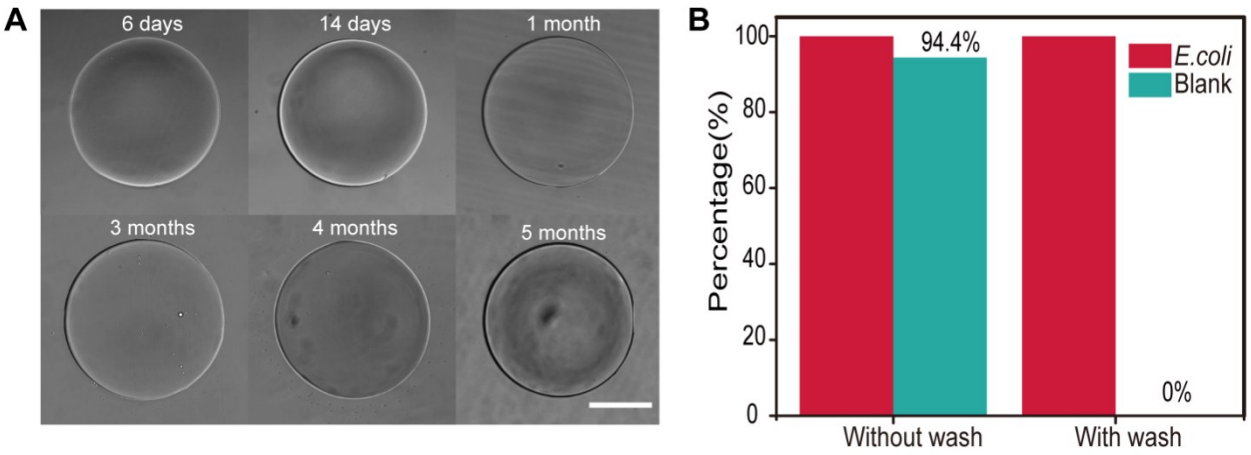


**Figure S2.** (A) The droplets evaporation control by recording the microscopic images of MSP droplets with long-term observation up to 5 months at room temperature (~20 °C); Scale bar is 150 μm; (B) Contamination control with Droplet Picker: droplets with GFP-*E.coli* encapsulated called *E.coli* and without bacteria called blank, the two types of droplets were picked continually one by one.

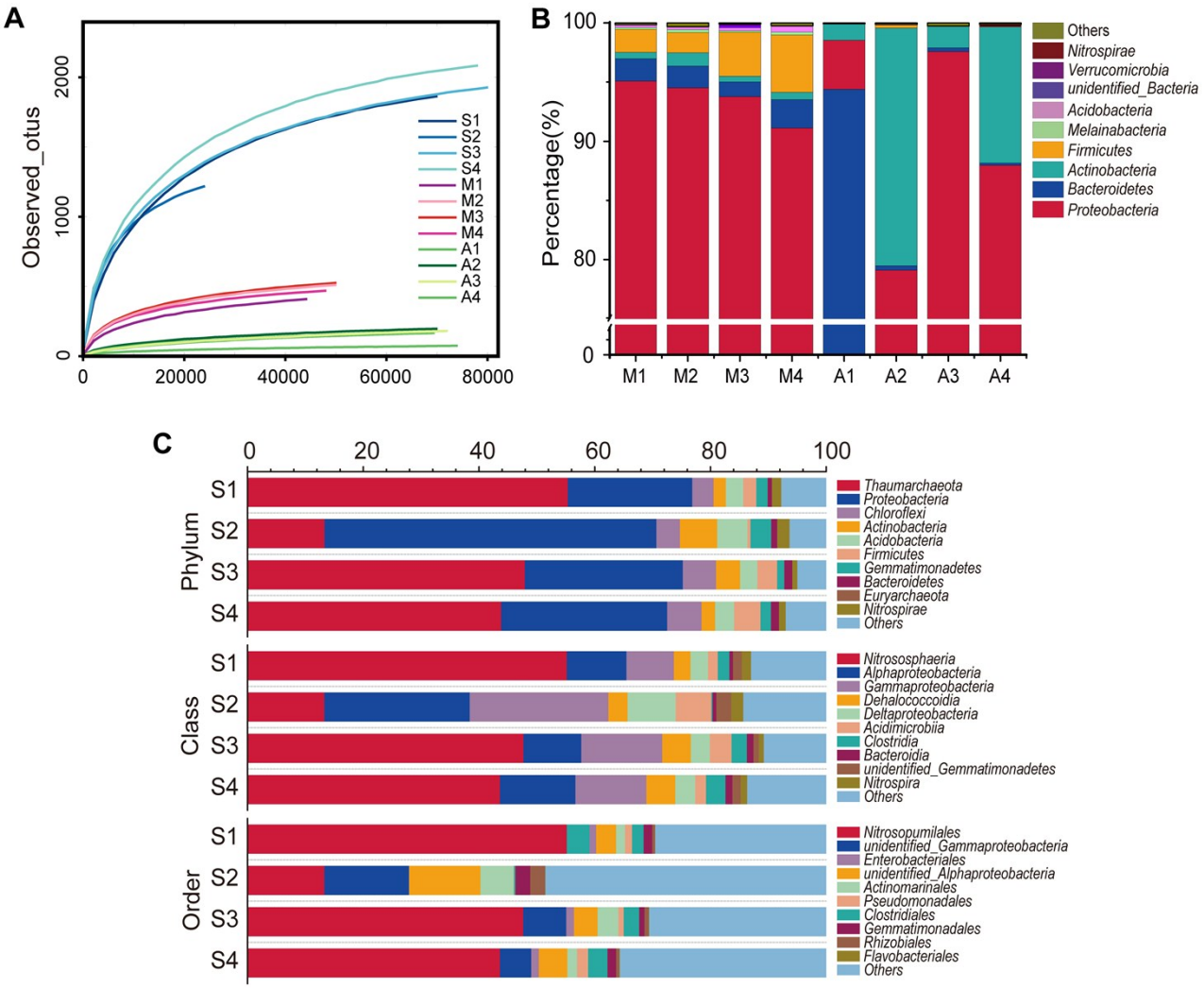


**Figure S3**. (A)Rarefaction curves based on 16S rRNA gene high throughput sequencing of bacterial communities of Samples, MSP Pool and Agar Pool; (B) Microbial diversity of MSP pool and Agar pool: top ten abundant phylum were showed in the bar charts. (C) The microbiome composition of the original sediment samples at the phylum, class and order levels.

## Supplementary tables

**Table S1:** Bacterial strains isolated from the SWIR sediments with MSP

| Phylogenetic affiliation | Isolate name | GenBank acc.No | Isolates | Closest Cultivated Species | Similarity (%) |
|---|---|---|---|---|---|
| *Proteobacteria* | P13-D3 | MK318603 | 1 | *Aquabacter spiritensis* DSM 9035(T) | 96.67 |
| | P9-C10 | MK318595 | 1 | *Sphingomonas astaxanthinifaciens* DSM | 97.35 |
| | P13-B11 | MK318604 | 52 | *Novosphingobium aromaticivorans* DSM | 97.78 |
| | P8-D1 | MK318630 | 2 | *Sphingomonas aquatilis* JSS7(T) | 98.1 |
| | P4-E9 | MK318586 | 2 | *Sphingomonas sanxanigenens* DSM 19645(T) | 98.26 |
| | P3-F12-3 | MK318596 | 1 | *Novosphingobium lindaniclasticum* LE124(T) | 98.46 |
| | P7-D12 | MK318593 | 16 | *Sphingomonas melonis* DAPP-PG 224(T) | 98.61 |
| | P9-A2 | MK318627 | 1 | *Paracoccus sediminis* DSM 26170(T) | 98.62 |
| | P7-B6 | MK318617 | 1 | *Sphingomonas wittichii* RW1(T) | 98.66 |
| | P1-H3 | MK318607 | 5 | *Sphingomonas leidyi* ATCC 15260(T) | 98.75 |
| | P2-A8 | MK318600 | 1 | *Alcanivorax jadensis* T9(T) | 98.79 |
| | P1-D1 | MK318568 | 8 | *Thalassospira tepidiphila* 1-1B(T) | 99.13 |
| | P7-D5 | MK318629 | 1 | *Methylobacterium aquaticum* DSM 16371(T) | 99.15 |
| | P1-C3 | MK318571 | 113 | *Sulfitobacter pontiacus* DSM 10014(T) | 99.19 |
| | P2-D6 | MK318609 | 1 | *Pseudomonas xanthomarina* DSM 18231(T) | 99.21 |
| | P3-C10 | MK318594 | 5 | *Erythrobacter pelagi* UST081027-248(T) | 99.34 |
| | P1-C4 | MK318602 | 1 | *Pseudomonas zhaodongensis* NEAU-ST5- | 99.43 |
| | P14-E8 | MK318637 | 4 | *Stenotrophomonas maltophilia* MTCC 434(T) | 99.47 |
| | P3-B7-2 | MK318579 | 6 | *Pseudomonas hunanensis* LV(T) | 99.58 |
| | P2-F3-1 | MK318585 | 2 | *Celeribacter manganoxidans* DY2-5(T) | 99.63 |
| | P5-A11 | MK318624 | 3 | *Sphingomonas leidyi* ATCC 15260(T) | 99.63 |
| | P8-F3 | MK318636 | 29 | *Pseudomonas hibiscicola* ATCC 19867(T) | 99.68 |
| | P3-B7-1 | MK318601 | 3 | *Brevundimonas vesicularis* NBRC 12165(T) | 99.7 |
| | P5-A12 | MK318623 | 2 | *Sphingomonas ginsenosidimutans* KACC | 99.7 |
| | P2-C6 | MK318578 | 1 | *Thalassospira indica* PB8B(T) | 99.71 |
| | P2-A11 | MK318581 | 1 | *Stenotrophomonas rhizophila* DSM 14405(T) | 99.72 |
| | P9-A4 | MK318598 | 4 | *Methylobacterium oryzae* CBMB20(T) | 99.78 |
| | P5-D10 | MK318618 | 5 | *Sphingomonas zeae* JM-791(T) | 99.78 |
| | P2-C8 | MK318584 | 1 | *Alcanivorax venustensis* ISO4(T) | 99.79 |
| | P3-A10 | MK318599 | 3 | *Sphingomonas paucimobilis* NBRC 13935(T) | 99.85 |
| | P6-H3 | MK318611 | 2 | *Caulobacter vibrioides* CB51(T) | 99.85 |
| | P13-H3 | MK318622 | 6 | *Stenotrophomonas indicatrix* WS40(T) | 99.86 |
| | P2-D3 | MK318582 | 1 | *Citreicella marina* CK-I3-6(T) | 99.93 |
| | P2-A5 | MK318590 | 4 | *Erythrobacter citreus* RE35F/1(T) | 99.93 |
| | P3-A7-2 | MK318597 | 24 | *Brevundimonas aurantiaca* DSM 4731(T) | 99.93 |
| | P4-F11 | MK318628 | 1 | *Halomonas alkaliantarctica* CRSS(T) | 99.93 |
| | P8-A7 | MK318613 | 2 | *Methylobacterium radiotolerans* JCM 2831(T) | 100 |
| | P5-F9 | MK318619 | 1 | *Methylorubrum rhodesianum* DSM 5687(T) | 100 |
| | P8-E7 | MK318626 | 1 | *Sphingomonas melonis* DAPP-PG 224(T) | 100 |
| *Actinobacteria* | P14-E7 | MK318616 | 1 | *Nocardioides iriomotensis* IR27-S3(T) | 97.31 |
| | P8-F7 | MK318633 | 1 | *Geodermatophilus africanus* CF11/1(T) | 98.5 |
| | P5-F1 | MK318625 | 1 | *Microbacterium ginsengisoli* DSM 18659(T) | 98.53 |
| | P1-A4 | MK318592 | 1 | *Pseudarthrobacter siccitolerans* 4J27(T) | 99 |
| | P14-G11 | MK318631 | 1 | *Nocardioides furvisabuli* SBS-26(T) | 99.04 |
| | P3-D3 | MK318605 | 130 | *Williamsia serinedens* DSM 45037(T) | 99.05 |
| | P4-H12 | MK318621 | 34 | *Microbacterium proteolyticum* RZ36(T) | 99.12 |
| | P10-E4 | MK318567 | 1 | *Blastococcus saxobsidens* BC448(T) | 99.13 |
| | P1-B12-2 | MK318572 | 1 | *Arthrobacter bambusae* GM18(T) | 99.14 |
| | P12-C8 | MK318577 | 1 | *Pseudonocardia tropica* YIM 61452(T) | 99.21 |
| | P1-D12 | MK318583 | 2 | *Paenarthrobacter nitroguajacolicus* G2-1(T) | 99.21 |
| | P9-D8 | MK318569 | 12 | *Mycobacteroides saopaulense* EPM 10906(T) | 99.22 |

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|---|---|---|---|---|---|
| | P1-E12 | MK318573 | 1 | *Microbacterium paraoxydans* NBRC | 99.23 |
| | P3-C5 | MK318580 | 1 | *Blastococcus aggregatus* DSM 4725(T) | 99.36 |
| | P1-B4 | MK318587 | 16 | *Arthrobacter subterraneus* CH7(T) | 99.36 |
| | P6-H2 | MK318612 | 1 | *Mycolicibacterium phocaicum* CIP 108542(T) | 99.42 |
| | P9-H5 | MK318634 | 3 | *Mycobacteroides abscessus subsp. bolletii* BD(T) | 99.47 |
| | P14-F5 | MK318635 | 4 | *Janibacter melonis* CM2104(T) | 99.47 |
| | P2-B10 | MK318606 | 2 | *Dietzia maris* DSM 43672(T) | 99.49 |
| | P1-A7 | MK318591 | 2 | *Micrococcus yunnanensis* YIM 65004(T) | 99.5 |
| | P13-A9 | MK318608 | 3 | *Microbacterium laevaniformans* DSM | 99.55 |
| | P2-E8 | MK318615 | 1 | *Micrococcus antarcticus* T2(T) | 99.56 |
| | P9-C11 | MK318570 | 18 | *Mycobacteroides saopaulense* EPM 10906(T) | 99.65 |
| | P1-A12 | MK318588 | 7 | *Microbacterium phyllosphaerae* DSM | 99.71 |
| | P10-E5 | MK318614 | 1 | *Agrococcus citreus* IAM 15145(T) | 99.78 |
| *Firmicutes* | P3-H5 | KY800370 | 6 | *Oceanobacillus profundus* CL-MP28(T) | 95.86 |
| | P3-B8 | KY800371 | 48 | *Ornithinibacillus contaminans* CCUG | 95.89 |
| | P2-C2 | KY800369 | 1 | *Virgibacillus halodenitrificans* DSM 10037(T) | 96.77 |
| | P3-E4 | MK318575 | 1 | *Staphylococcus capitis subsp. urealyticus* GTC 727(T) | 99.38 |
| | P13-G7 | MK318632 | 1 | *Staphylococcus hominis subsp. novobiosepticus* GTC 1228(T) | 99.69 |
| | P12-B3-1 | MK318620 | 1 | *Staphylococcus hominis subsp. hominis* DSM 20328(T) | 99.71 |
| | P3-B2 | MK318574 | 1 | *Terribacillus goriensis* CL-GR16(T) | 99.93 |
| | P10-H1-2 | MK318589 | 1 | *Bacillus altitudinis* 41KF2b(T) | 99.93 |
| | P3-F12-2 | MK318610 | 2 | *Staphylococcus warneri* ATCC 27836(T) | 100 |
| *Deinococcus-Thermus* | P10-H1 | MK318576 | 1 | *Deinococcus taklimakanensis* X-121(T) | 99.28 |