SUPPORTING INFORMATION

Potential Application of *Paenibacillus* sp. C1 to the Amelioration of Soda Saline-alkaline Soil

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1.1 Isolation of strains

Firstly, 10 g of fresh soil was weighed and added into a triangular flask containing 90 mL of sterile water and glass beads. After shaking for 10 min at 28℃ and 160 rpm, 1 mL of soil suspension was absorbed and added to a 20-mL modified LB medium. After culture for 24 h, 1 mL of fermentation broth was absorbed and added to 9 mL of 0.2% normal saline. After shaking and mixing evenly, the dilution under three gradients of 10^{-2}, 10^{-4} and 10^{-6} was prepared in turn, and 200 μL of each dilution was absorbed and coated on the soil broth culture medium. Each gradient was repeated for three times, and cultured in a constant temperature incubator at 28℃ for 2 days. Single colonies with obvious differences in appearance were selected and purified on the soil broth culture medium. The purified strains were inoculated into the fresh LB culture medium to obtain fermentation broth, and then made into a bacterial suspension, which was inoculated into the modified NBRIP culture medium at the ratio of 2%, and cultured at 28℃ and 160 rpm for 5 days. The pH value of the fermentation broth was measured every 24 h, and the strains with the pH value less than 5.5 of the fermentation broths were preserved. Acid-producing strains were inoculated into an inorganic salt culture medium with a small rectangular filter paper (1.5 cm × 1 cm), and the filter paper collapse experiment was carried out at 28℃ for 6 days. Strains with strong cellulose degradation ability were screened according to the degree of filter paper collapse. The supernatant was centrifuged at 8000 rpm for 15 min and mixed with absolute ethanol at a ratio of 1:3 and placed at 4℃ overnight. Then the supernatant was poured out, and the precipitate was dried at 60℃ and weighed. Finally, the strains that could produce acid, degrade filter paper and produce exopolysaccharide were taken as target strains.

1.2 Sequence identification of 16S rRNA

PCR amplification was carried out by using 2 × TSINGKE Master Mix (code No.: TSE003) system with 16S rRNA as primer and ddH_2O as negative control. The reaction system includes 1 μL of DNA, 25 μL of 2 × TSINGKE Master Mix, 1 μL of Forward Primer (10 μM), 1 μL of Reverse Primer (10 μM), 22 μL of ddH_2O. PCR thermal conditions were as follows: 94℃ for 10 min; 30 cycles of 94℃ for 30 s, 55℃ for 30s, 72℃ for 1.5 min; and a final extension cycle at 72℃ for 10 min. After the PCR reaction, 2 μL was taken for 1% agarose gel electrophoresis, and the PCR product was purified and stored at 4℃ after 40–60 min electrophoresis at a constant voltage of 160V. Based on the BLAST function in NCBI, the strain gene sequence similar to the determined gene sequence was selected after homology comparison and analysis. The phylogenetic

tree of the compared sequence was constructed by MEGA7 software, and the positions of the species and genus (or genera) of target strains in the classification system were identified through the confidence test (Tamura et al., 2013).

1.3 Main culture media

The soil broth medium consisted of 5 g of glucose, 5 g of $Ca_3(PO_4)_2$, 0.0584 g of KH_2PO_4, 0.1547 g of K_2HPO_4, 15–18 g of agar and 1000 mL of soil extract, and the pH value was 7.

The modified NBRIP medium consisted of 10 g of glucose, 5 g of $Ca_3(PO_4)_2$, 0.5 g of $(NH_4)_2SO_4$, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.03 g of $MnSO_4 \cdot 7H_2O$, 0.03 g of $FeSO_4 \cdot 7H_2O$, 0.3 g of KCl, 20 g of NaCl, 18 g of agar and 1000 mL of distilled water, and the pH value was 7.

The filter paper collapse inorganic salt culture medium consisted of 1 g of filter paper, 1 g of KNO_3, 0.5 g of $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of NaCl, 15–18 g of agar and 1000 mL of distilled water, and the pH value was 7.

The tryptone medium (LB medium) consisted of 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl and 1000 mL of distilled water, and the pH value was 7.

The modified LB medium consisted of 10 g of tryptone, 5 g of yeast extract, 10 g NaCl and 1000 mL of distilled water, and the pH value was adjusted to 8 with 1 mol/L NaOH.

The straw degradation medium consisted of 1 g of straw, 1 g of KNO_3, 0.5 g of $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of NaCl, 15–18 g of agar and 1000 mL of distilled water, and the pH value was 7.

1.4 Determination of cellulase activity

The fermentation solution was centrifuged at 4000 rpm for 10 min, and 0.5 mL crude enzyme solution was taken. Then, 1.5 mL of CMC-Na buffer solution was added and placed in water bath at 50℃ for 30 min. Afterwards, 1.5 mL of DNS reagent was added and placed in water bath at 100℃ for 5 min, after which the solution was quickly cooled. Finally, the volume was adjusted to 25 mL, and the absorbance was measured at 540 nm.

1.5 Determination of polysaccharide content

The fermentation broth was centrifuged to remove thalli, and the supernatant was added with 3 times of absolute ethanol at 4℃ overnight. The solution was centrifuged at 4000 rpm for 10 min, and the collected precipitate was washed with ethanol twice, after which the precipitate was diluted to an appropriate concentration. Then, 1 mL of

distilled water, 1 mL of 6% phenol and 5 mL of 98% concentrated sulfuric acid were added into 1 mL of solution. The solution was shaken uniformly and cooled to room temperature. After 20 min, the absorbance was measured at 490 nm.

1.6 Determination of available phosphorus, organic phosphorus and alkaline phosphatase activity of soil

The soil samples were extracted with NaOH solution and the available phosphorus (AP) content in soil was determined by the molybdenum antimony colorimetric method. After sieving with a 60-mesh sieve, the organic phosphorus (OP) content was determined by the ignition method. The alkaline phosphatase (ALP) activity was determined by the disodium phenyl phosphate colorimetric method.

1.7 Determination of total organic carbon of soil

The soil samples were screened by a 100-mesh sieve, and the organic matter (OM) content was determined by the potassium dichromate method. The total organic carbon (TOC) content in soil was calculated as the OM content was divided by 1.724.

1.8 Determination of polysaccharide content of soil

To determine polysaccharide content, 1 g of soil sample was accurately weighted and put into a 250-mL triangular flask, and the polysaccharide content in soil was determined by the phenol-sulfuric acid method.

1.9 PCR amplification

All PCR reactions were conducted in a reaction system with a total volume of 25 μL containing 12.5 μL of 2xTaq Plus Master Mix, 3 μL of BSA (2 ng/μL), 1 μL of Forward Primer (5 μM), 1 μL of Reverse Primer (5 μM), 2 μL of template DNA (30 ng), and 5.5 μL of ddH_2O. Thermal cycling was consisted of initial denaturation at 95℃ for 5 min, followed by 28 cycles of denaturation at 95℃ for 45 s, annealing at 55℃ for 50 s, and elongation at 72℃ for 45 s. Eventually, the PCR system was held at 72℃ for 10 min.

1.10 Sequence processing

Raw 16S rRNA data of soil samples in CK1, C1, CK2 and C1+RS groups were demultiplexed, quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME 1.8.0) software package with the following three criteria. Firstly, low quality reads with scores < 20 or with a read length < 230 bp were filtered out. Secondly, barcodes were matched, while ambiguous bases and unmatched barcodes were

removed. Thirdly, reads that could not be assembled were discarded. Only overlaps sequences longer than 10 bp were assembled according to their overlap sequences. The operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU115) 16S rRNA database using a confidence threshold of 70% (Amato et al., 2013).

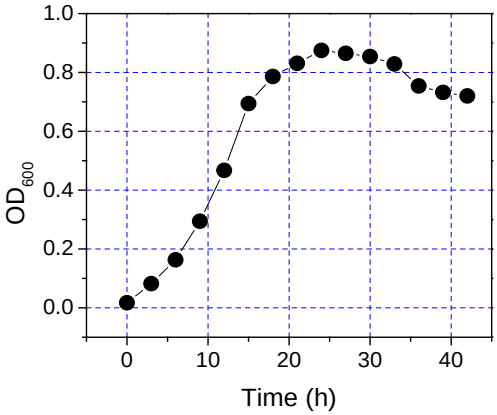

Figure S1. Growth curve of *Paenibacillus* sp. C1.

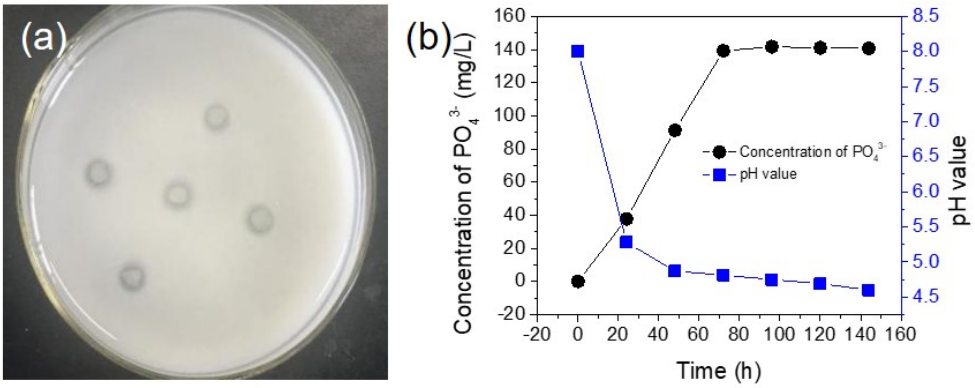

Figure S2. Phosphorus-dissolving circles (a) and characteristics (b) of *Paenibacillus* sp. C1.

Table S1. Main physicochemical parameters of soil samples.

Parameters	AP (mg/kg)	OP (mg/kg)	TOC (g/kg)	pH	EC (mS/cm)	Alkalinity (%)	Salinity (g/kg)
Values	13.22	15.68	4.85	8.42	0.522	43.17	6.31

AP: available phosphorus; OP: organic phosphorus; TOC: total organic carbon; EC: electrical conductivity.

Table S2. Groups in soil experiment.

Groups	CK1	C1	CK2	C1+RS
Composition	Saline-alkaline soil	Saline-alkaline soil and *Paenibacillus* sp. C1	Saline-alkaline soil, rice straws, $(NH_4)_2SO_4$ and $Ca_3(PO_4)_2$	Saline-alkaline soil, *Paenibacillus* sp. C1, rice straws, $(NH_4)_2SO_4$ and $Ca_3(PO_4)_2$

Table S3. Richness and diversity indices of bacterial communities for all soil samples. CK1: saline-alkaline soil; C1: saline-alkaline soil with *Paenibacillus* sp. C1 bacterial cells; CK2: saline-alkaline soil with rice straws, $(NH_4)_2SO_4$ and $Ca_3(PO_4)_2$; C1+RS: saline-alkaline soil with rice straws, $(NH_4)_2SO_4$, $Ca_3(PO_4)_2$ and *Paenibacillus* sp. C1 bacterial cells.

Groups	Chao1	Goods coverage	Observed species	PD whole tree	Shannon	Simpson
CK1	3409.41	0.99	3062.00	244.06	9.81	1.00
C1	3506.23	0.99	3081.00	243.73	9.69	1.00
CK2	3468.18	0.99	3024.90	243.00	9.49	0.99
C1+RS	3451.14	0.99	2927.70	241.12	9.56	1.00

Table S4. Relative abundance of *Paenibacillus* in different samples. CK1: saline-alkaline soil; C1: saline-alkaline soil with *Paenibacillus* sp. C1 bacterial cells; CK2: saline-alkaline soil with rice straws, $(NH_4)_2SO_4$ and $Ca_3(PO_4)_2$; C1+RS: saline-alkaline soil with rice straws, $(NH_4)_2SO_4$, $Ca_3(PO_4)_2$ and *Paenibacillus* sp. C1 bacterial cells.

Name	Relative abundance (%)			
	CK1	C1	CK2	C1+RS
Paenibacillus	0.0193	0.2239	0.1486	0.2046

References

Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A. 2013. Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. ISME J 7:1344−1353.

Tamura K, Stecher G, Peterson D. 2013. MEAG6: molecular evolutionary genetics analysis version 6.0. Molecul Biol Evol 30:2725–2729.