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Bioremediation of cadmium-contaminated paddy soil using an autotrophic and heterotrophic mixture

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SM: Materials and methods

Isolation and characterizations of heterotrophic isolates

High throughput sequencing technology was used to further identification of the 7 isolates. Total genomic DNA was extracted with HP Fungal DNA Kit (OMEGA) by reported methods.[1] Internal transcribed spacer (ITS) sequences were amplified by using primer pairs ITS4R(5'-TCCTCCGCTTATTGATATGC-3') and forward primers ITS7F (5'-GTGARTCATCGARTCTTTG-3'). The GTGARTCATCGARTCTTTG-3'). The polymerase chain reaction (PCR) amplification procedures were performed as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 62 °C for 45 s and 72 °C for 30 s, with final extension at 72 °C for 10 min[2, 3]. PCR products were quantified and purified by electrophoresis on agarose gel (1.0%) and purified using E.Z.N.A. DNA Gel Extraction KIT (OMEGA). The sequencing process was performed at Sangon Biotech (Shanghai) Co., Ltd.

Analysis of community succession in agitating bioleaching

The soil slurry was then centrifuged at 10000×*g* for 5 minutes. Subsequently, the total genomic DNA of soils was extracted using Fast DNA® SPIN Kit for Soil (MP Biomedicals Inc., USA) according to the instruction. The quality of DNA was examined according to the reported methods [4]. The V4 region of 16S ribosomal RNA (rRNA) was amplified by PCR using primers 515F(5'-GTGCCAGCMGCCGCGGTAA-3') and 806R(5'-GTGCCAGCMGCCGCGGTAA-3') combined with unique Illumina adapter sequences [5]**.** PCR products were purified by aforementioned methods and the 16S DNA sequencing process was performed on Illumina MiSeq sequencing platform of *Key Laboratory of Biometallurgy of Ministry of Education*.

SM: Tables

Table S1 Growth conditions of 7 acid leaching strains

Strains	Energy source	Temperature /℃	pH
A.caldus DX	S	45	2.0
A.thiooxidans AO1	S	30	2.0
Athiooxidans ZJ	S	30	2.0
L.ferriphilum DX	$FeSO_4$	40	1.6
A. thiooxidans DX	S	30	2.0
A. caldus S1	S	45	2.0
F. acidiphilum DX	$FeSO_4$	45	1.0

Table S2 Alpha-diversity indexes of soils using different treatments on day 4.

Item	Shannon index	Simpson index	Evenness	OTU Number[a]
Origin Soil	3.7±0.05 a	0.75±0 a	0.5±0 a	1597±38 a
CK	3.1±0.18 ab	0.74±0 a	0.44±0.02 ab	1225±118 bc
9K Control	3.2±0.19 ab	0.73±0.01 a	0.44±0.02 ab	1378±54 ab
Att-sys	2.9±0.3 b	0.73±0.01 a	0.41±0.04 bc	1023±136 c
Htt-sys	1.8±0.07 c	0.69±0.01 b	0.36±0.01 c	176±22 d
Co-sys	3.1±0.21 b	0.74±0 a	0.44±0.02 ab	1074±97 c

[a] Mean ± standard deviation (n = 3). Means within a column followed by the same letter are not significantly different according to the LSD test ($P>0.05$). The indexes were calculated from OTU relative abundance of each replicate

SM: Figures

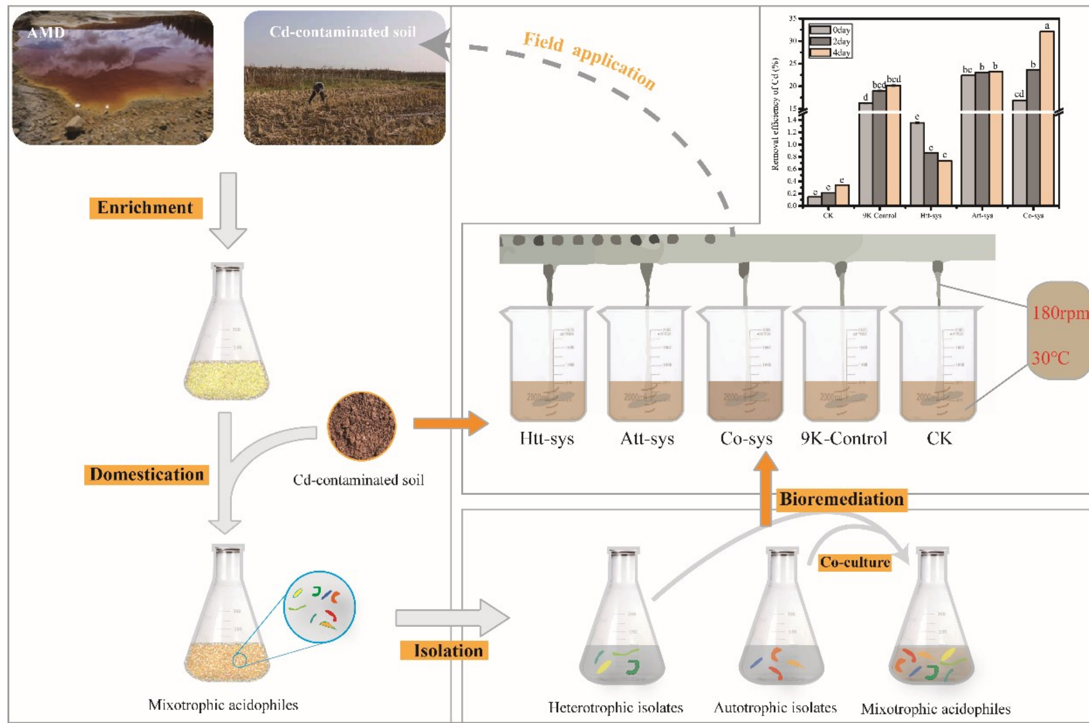

Fig. S1 Graphic Abstract of the experimental process in this paper

Fig. S2 The growth of 7 isolates on plates with bromocresol green indicator

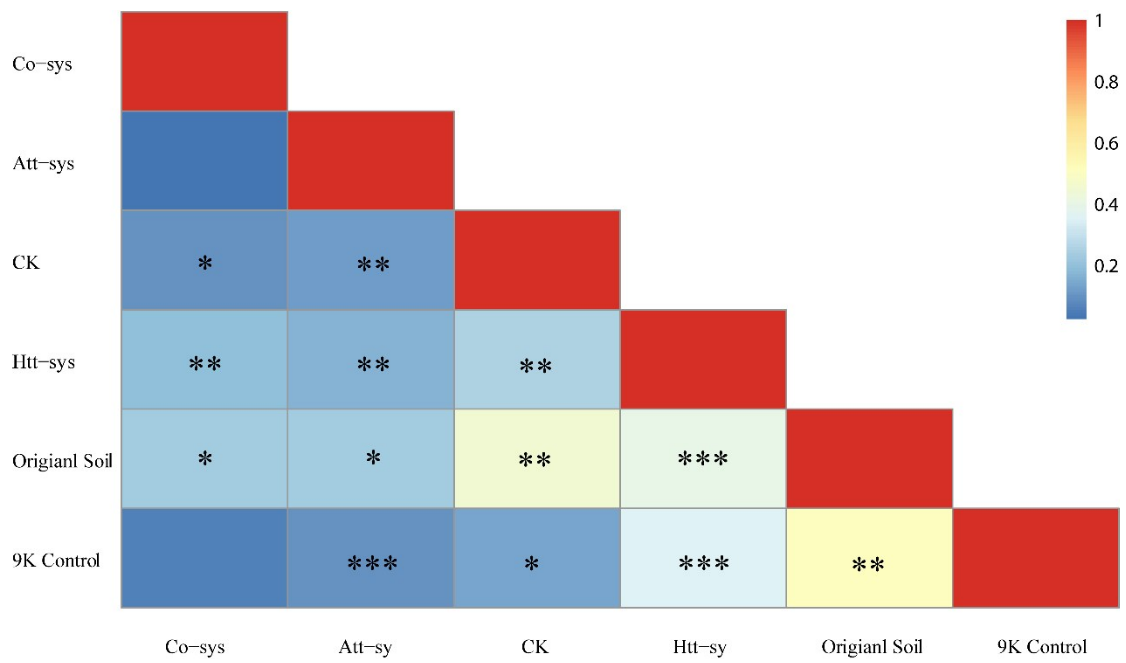

Fig. S3 PerMANOVA using distance matrices, “*” means $p<0.05$, “**” means $p<0.01$ and “***” mean $p<0.001$.

Reference

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