

Appendix A. Supplementary data

Effect of phosphate-solubilizing bacteria on the mobility of insoluble cadmium and metabolic analysis

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The supplementary material includes Figure S1, Figure S2, FigureS3, Table S1, Table S2, Table S3, Text S1, and Text S2.

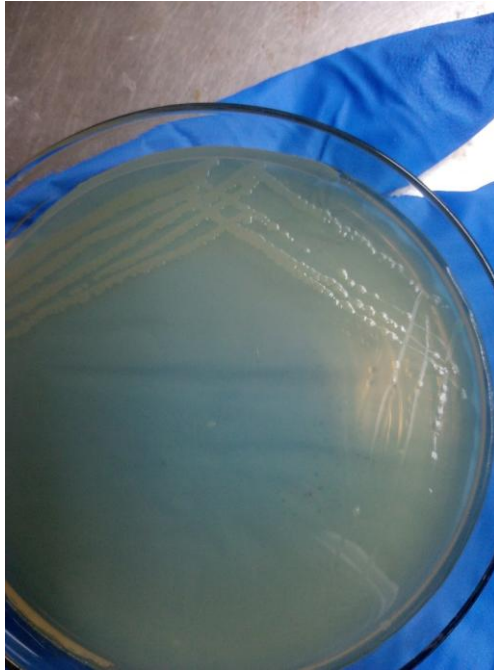


Figure S1. Colony morphology of *Bacillus cereus* qh-35.

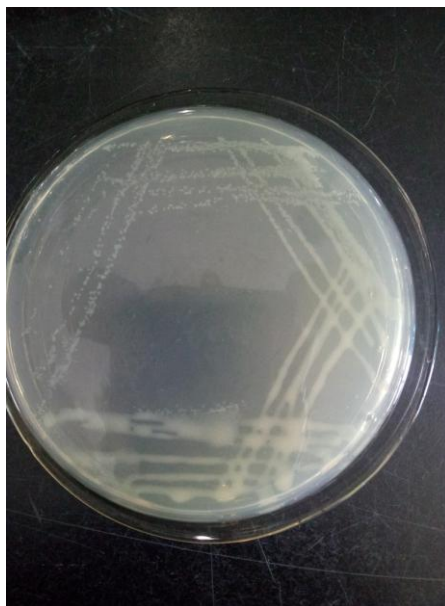


Figure S2. Colony morphology of *Pseudomonas fluorescens* gim-3.

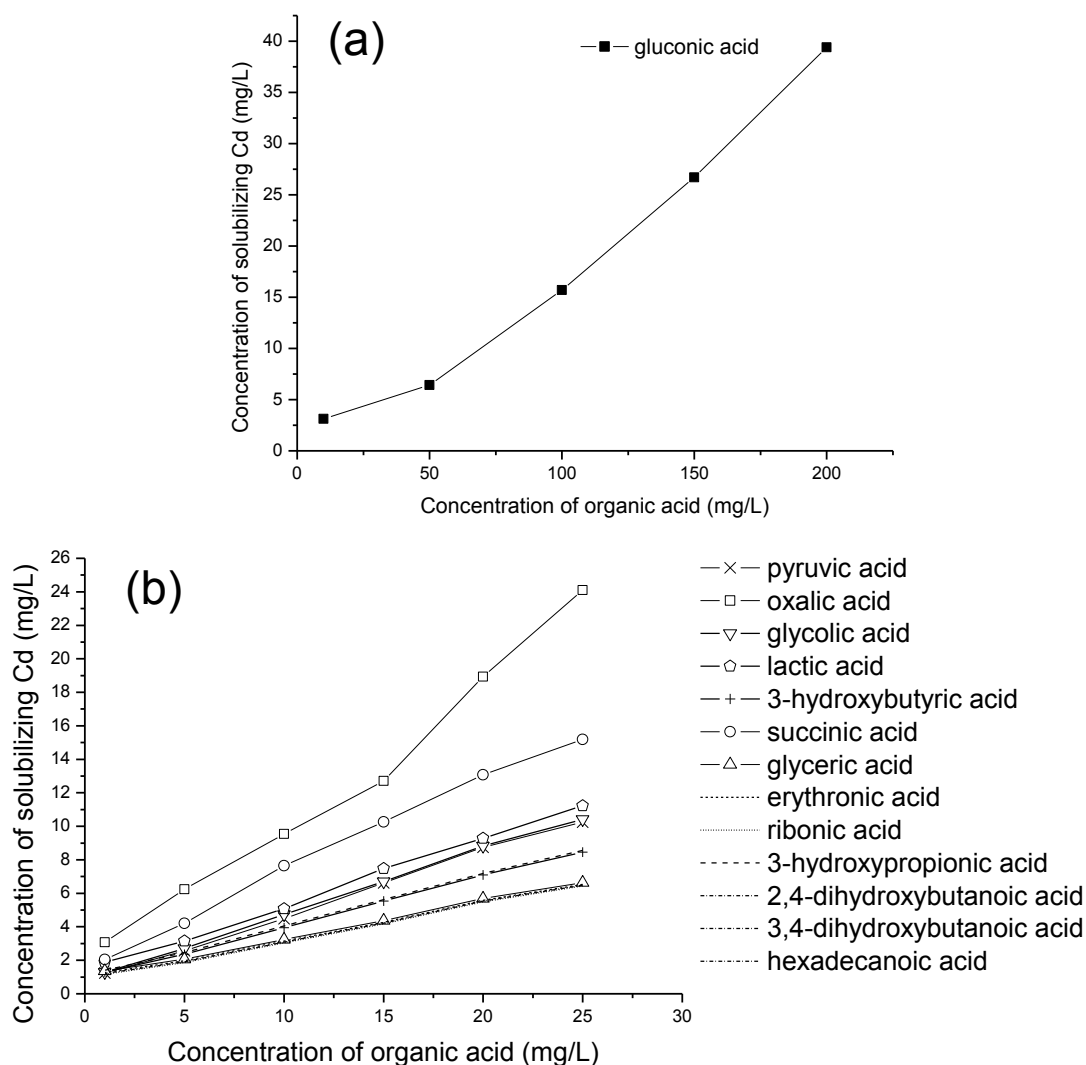


Figure S3. Curve of the cadmium (CdCO_3) solubilization ability of organic acids.

Dicarboxylic acid exhibits higher efficiency in dissolving Cd than monocarboxylic acids, although succinic acid has high pK_a ($pK_{a1} = 4.21$). The acid dissolution and coordination reactions of organic acids can stimulate the release of non-soluble compounds. Acid dissolution depends on pK_a value and concentrations of organic acids. The coordination reaction depends on the structure of organic acids. Obviously the first carboxyl-dissociated and -coordinated Cd greatly promote the dissociation and coordination of the second carboxyl. Although pyruvic acid has a small pK_a ($pK_a = 2.49$), the solubilizing Cd effect is similar to that of alpha-hydroxyl carboxylic acids, because the carbonyl group is much weaker than hydroxyl coordination. Gluconic acid ($pK_a = 3.86$), oxalic acid ($pK_{a1} = 1.22$), glycolic acid ($pK_a = 3.83$), lactic acid ($pK_a = 3.86$), 3-hydroxybutyric acid ($pK_a = 4.36$), glyceric acid ($pK_a = 3.42$), erythronic acid ($pK_a = 3.47$), ribonic acid ($pK_a = 3.38$), 3-hydroxypropionic acid ($pK_a = 4.39$), 2,4-dihydroxybutanoic acid ($pK_a = 3.71$), 3,4-dihydroxybutanoic acid ($pK_a = 4.27$), and hexadecanoic acid ($pK_a = 4.78$).

Table S1. The physiological and biochemical reactions of *Bacillus cereus* qh-35.

API 50 CHB	Strain qh-35
<i>Assimilation of</i>	
Glycerol (GLY)	-
Erythritol (ERY)	-
D-Arabinose (DARA)	-
L-Arabinose (LARA)	-
Ribose (RIB)	+
D-Xylose (DXYL)	-
L-Xylose (LXYL)	-
Adonitol (ADO)	-
Methyl- β -D-xylopyranoside (MDX)	-
Galactose (GAL)	-
Glucose (GLU)	+
Fructose (FRU)	+
Mannose (MNE)	-
Sorbose (SBE)	-
Rhamnose (RHA)	-
Dulcitol (DUL)	-
Inositol (INO)	-
Mannitol (MAN)	-
Sorbitol (SOB)	-
Methyl- α -D-mannopyranoside (MDM)	-
Methyl- α -D-glucopyranoside (MDG)	-
N-acetyl-glucosamine (NAG)	+
Amygdalin (AMY)	-
Arbutin (ARB)	-
Esculin (ESC)	+
Salicin (SAL)	-
Cellobiose (CEL)	-
Maltose (MAL)	+
Lactose (LAC)	-
Melibiose (MEL)	-
Saccharose (SAC)	-
Trehalose (TRE)	+
Inulin (INU)	-
Melezitose (MLZ)	-
Raffinose (RAF)	-
Acid modified starch (AMD)	+
Glycogen (GLYG)	+
Xylitol (XLT)	-
Gentiobiose (GEN)	-
Turanose (TUR)	-
Lyxose (LYX)	-
Tagatose (TAG)	-
D-Fucose (DFUC)	-
L-Fucose (LFUC)	-
D-Arabinitol (DARL)	-
L-Arabinitol (LARL)	-
Potassium gluconate (GNT)	-
Potassium 2-ketogluconate (2KG)	-

Potassium 5-ketogluconate (5KG)	-
API 20 E	Strain qh-35
o-Nitrophenyl-β-D-galactosidase (ONPG)	-
Arginine dihydrolase (<u>ADH</u>)	-
Lysine decarboxylase (<u>LDC</u>)	-
Ornithine decarboxylase (<u>ODC</u>)	-
Assimilation of citrate (<u>ICIT</u>)	-
H ₂ S production (<u>H₂S</u>)	-
Urease (<u>URE</u>)	+
Tryptophan deaminase (TDA)	-
Indole production (IND)	-
Voges-Proskauer test (<u>VP</u>)	+
Gelatin hydrolysis (<u>GEL</u>) (protease)	+
Glucose fermentation or oxidation (GLU)	-
Identification	<i>Bacillus cereus</i>

Table S2. The physiological and biochemical reactions of *Pseudomonas fluorescens* gim-3.

API 20 NE	Strain gim-3
Nitrate reduction (NO ₃)	+
Tryptophan producing indole (TRP)	-
Glucose fermentation (<u>GLU</u>)	-
Arginine dihydrolase (<u>ADH</u>)	+
Urease (<u>URE</u>)	-
Esculine hydrolysis (ESC) (β -glucosidase)	-
Gelatin hydrolysis (GEL) (protease)	+
p-Nitrophenyl- β -D-galactosidase (PNPG)	-
<i>Assimilation of</i>	
Glucose (GLU)	+
Arabinose (ARA)	+
Mannose (MNE)	+
Mannitol (MAN)	+
N-acetyl-glucosamine (NAG)	+
Maltose (MAL)	-
Potassium gluconate (GNT)	+
Capric acid (CAP)	-
Adipic acid (ADI)	-
Malic acid (MLT)	+
Citrate (CIT)	+
Phenylacetic acid (PAC)	-
Oxidase (OX)	+
Identification	<i>Pseudomonas fluorescens</i>

Table S3. Secretions of *B. cereus* and *P. fluorescens*.

Time	Compound	Formula	RI calculation	RI reference	Derivatization	Mark peak	Peak area ($\times 10^5$) ^a	Peak area ($\times 10^5$) ^b
6.476	1-Hexanol	C ₆ H ₁₄ O	991.1	982	O-TMS	159	89.62626	84.26175
8.732	Pyruvic acid ^a	C ₃ H ₄ O ₃	1050.2	-	MO, O-TMS	73	3.12031	-
9.116	Lactic acid	C ₃ H ₆ O ₃	1060.2	1051	O,O-TMS	73	3.96691	1.76053
9.739	Glycolic acid	C ₂ H ₄ O ₃	1076.5	1078	O,O-TMS	73	11.16354	0.51969
10.708	Alanine	C ₃ H ₇ O ₂ N	1102.1	1093	N,O-TMS	116	1.20397	17.27514
11.008	Hydroxylamine ^b	H ₃ ON	1110.6	1123	N,N,O-TMS	73	-	0.32077
12.046	Oxalic acid ^b	C ₂ H ₂ O ₄	1140.3	1131	O,O-TMS	73	-	1.37809
12.238	3-Hydroxypropionic acid ^a	C ₃ H ₆ O ₃	1145.7	1144	O,O-TMS	177	0.07348	-
12.783	3-Hydroxybutyric acid ^a	C ₄ H ₈ O ₃	1161.3	1163	O,O-TMS	73	0.55035	-
14.569	Valine ^b	C ₅ H ₁₁ O ₂ N	1213.8	1221	N,O-TMS	144	-	0.30108
14.806	Glyceraldehyde	C ₃ H ₆ O ₃	1221.4	-	MO, O,O-TMS	73	1.88081	0.60568
16.143	Ethanolamine	C ₂ H ₇ ON	1264.3	1281	N,N,O-TMS	174	0.21725	0.55529
16.309	Phosphoric acid	H ₃ O ₄ P	1269.5	1283	O,O,O-TMS	299	9.72631	22.67024
16.494	Glycerol	C ₃ H ₈ O ₃	1275.5	1282	O,O,O-TMS	73	2.81253	2.62104
17.614	Succinic acid	C ₄ H ₆ O ₄	1312.6	1314	O,O-TMS	73	0.85247	0.49004
18.093	Glyceric acid	C ₃ H ₆ O ₄	1329.5	1336	O,O,O-TMS	73	1.73067	0.55222
20.369	2,4-Dihydroxybutanoic acid	C ₄ H ₈ O ₄	1410.9	1431	O,O,O-TMS	103	0.64563	0.78120
20.934	3,4-Dihydroxybutanoic acid	C ₄ H ₈ O ₄	1432.7	1448	O,O,O-TMS	73	0.60173	0.73906
21.101	Erythrose	C ₄ H ₈ O ₄	1439.1	-	MO, O,O,O-TMS	73	2.73090	1.76661
21.483	Erythrose	C ₄ H ₈ O ₄	1453.7	-	MO, O,O,O-TMS	73	13.75517	8.82034
22.773	Salicylic acid	C ₇ H ₆ O ₃	1503.4	1507	O,O-TMS	73	0.95474	0.7060

	(internal reference)				MS			8
23.705	Erythronic acid	C4H8O5	1541.8	-	O,O,O, O-TMS	73	2.88888	5.3010 7
25.782	Ribono-gamma-lactone ^b	C5H8O5	1629.0	-	O,O,O- TMS	73	-	2.2126 6
26.523	Ribose	C5H10O 5	1661.4	-	MO, O,O,O, O-TMS	73	18.5698 4	17.653 98
28.077	Putrescine ^b	C4H12N 2	1731.0	-	N,N,N, N-TMS	174	-	16.813 08
28.466	2-Desoxy-pentose-3-ulose	C5H8O4	1749.1	-	2MO, O,O-T MS	73	4.64678	2.5702 7
28.993	3-Deoxy-arabino-hexonic acid 1,4-lactone	C6H10O 5	1773.3	-	O,O,O- TMS	73	1.24872	1.5223 0
29.078	Ribonic acid	C5H10O 6	1777.2	1799	O,O,O, O,O-T MS	73	3.23952	2.6260 6
29.751	3-Desoxy-pentitol ^a	C5H12O 4	1808.7	-	O,O,O, O-TMS	73	2.71802	-
30.153	Galacto-hexodialdose ^a	C6H12O 6	1828.3	-	2MO, O,O,O, O-TMS	160	2.76357	-
30.454	Galactofuranose ^a	C6H12O 6	1842.9	1852	O,O,O, O,O-T MS	73	14.9376 2	-
30.993	Fructose ^a	C6H12O 6	1869.1	-	MO, O,O,O, O,O-T MS	103	55.7691 7	-
31.214	Gluconolactone ^b	C6H10O 6	1879.8	1916	O,O,O, O-TMS MO,	73	-	10.255 91
31.225	Fructose ^a	C6H12O 6	1880.3	-	O,O,O, O,O-T MS	103	49.8506 9	-
31.407	Glucose ^a	C6H12O 6	1889.1	-	O,O,O, O,O-T MS	204	129.386 53	-
31.422	Glucono-gamma-lactone ^b	C6H10O 6	1889.8	-	O,O,O, O-TMS	73	-	6.5060 6
31.688	Glucose ^a	C6H12O 6	1902.9	-	MO, O,O,O, O,O-T MS	319	169.757 58	-
31.966	Glucose ^a	C6H12O 6	1917.1	-	MO, O,O,O,	73	144.251 18	-

33.217	Galactopyranose ^a	C ₆ H ₁₂ O ₆	1980.8	-	O,O-TMS	204	119.51640	-
33.456	Gluconic acid ^b	C ₆ H ₁₂ O ₇	1993.0	1997	O,O,O,O,O,O-TMS	73	-	35.72410
34.431	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	2044.7	2047	O-TMS	117	0.62709	0.34125

Note: Superscript a means only *B. cereus* secretion, superscript b means only *P. fluorescens* secretion, and no superscript means both secretions. MO was methoximation, TMS was trimethylsilyl, O was oxygen, and N was nitrogen. RI reference was derived from NIST05. Peak area data include the mean of six replicates.

Text S1. The 16S rDNA gene sequences of *Bacillus cereus* qh-35.

GGGGAGTGGCGGCAGCTATACATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTAT
GAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCATAAGACTGGGAT
AACTCCGGGAAACCGGGGCTAATACCGGATAACATTTGAACTGCATGGTTCGAAATT
GAAAGGCGGCTTCGGCTGTCACCTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTG
AGGTAACGGCTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC
ACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCG
CAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCG
TAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACTGACGGTA
CCTAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGG
CAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGAT
GTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCA
GAAGAGGAAAGTGGAATTCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAAC
ACCAGTGGCGAAGGCGACTTCTGGTCTGTAACGACTGAGGCGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGT
GTTAGAGGGTTTCCGCCCTTATGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGG
AGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTTCGAAGCAACCGGAAGAACCTTACCAGGTCTTGACATCCTCT
GAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGT
TGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTG
ATCTAGTTGCCATCATTAAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGA
GGAAGGTGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGC
TACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAAC
CGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAA
TCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAAACACCCGAAGTCGGTGGGGTAACCTTTTTGGAGCCAGCCG
CCTAAGTGACAGC

Text S2. The 16S rDNA gene sequences of *Pseudomonas fluorescens* gim-3.

TGGGGGCGCTACCATGCAGTCGAGCGGTAGAGAGAAGCTTGCTTCTCTTGAGAGCGG
CGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAA
CGGACGCTAATACCGCATAACGTCCTACGGGAGAAAGCAGGGGACCTTAGGGCCTTGC
GCTATCAGATGAGCCTAGGTTCGGATTAGCTAGTTGGTGAGGTAATGGCTACCAAGGC
GACGATCCGTAAC TGGTCTGAGAGGATGATCAGTCACACTGGA ACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATC
CAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAG
GAAGGGTACTTACCTAATACGTGAGTATTTGACGTTACCGACAGAATAAGCACCCGGC
TAACTCTGTGCCAGCAGCCGCGTAATACAGAGGGGTGCAAGCGTTAATCGGAATTAC
TGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAA
CCTGGGAACTGCATCCAAA ACTGGCGAGCTAGAGTATGGTAGAGGGTGGTGGAAATT
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACC
ACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTT
AGTGGCGCAGCTAACGCATTAAGTTGACCCCTGGGGAGTACGGCCGCAAGGTTAAA
ACTCAAATGAATTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATTTCGAA
GCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGATGGATT
GGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGTA
GATGTTGGGTAAAGTCCCGTAACGAGCGCAACCCTTGTCTTAGTTACCAGCACGTTAT
GGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCCGAGGAAGGTGGGGATGACGT
CAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAGA
GGGTTGCCAAGCCGCGAGGTGGAGCTAATCCATAAAACCGATCGTAGTCCGGATCG
CAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTC
GCGGTGAATACGTTCCCGGGCCTTGTAACACACCCGCCGTCACACCATGGGAGTGGGTT
GCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGTACCAGCGGTGATTACCG