Diversity of Sporosarcina-Like Bacterial Strains Obtained from Meter-Scale Augmented and Stimulated Bio-cementation Experiments

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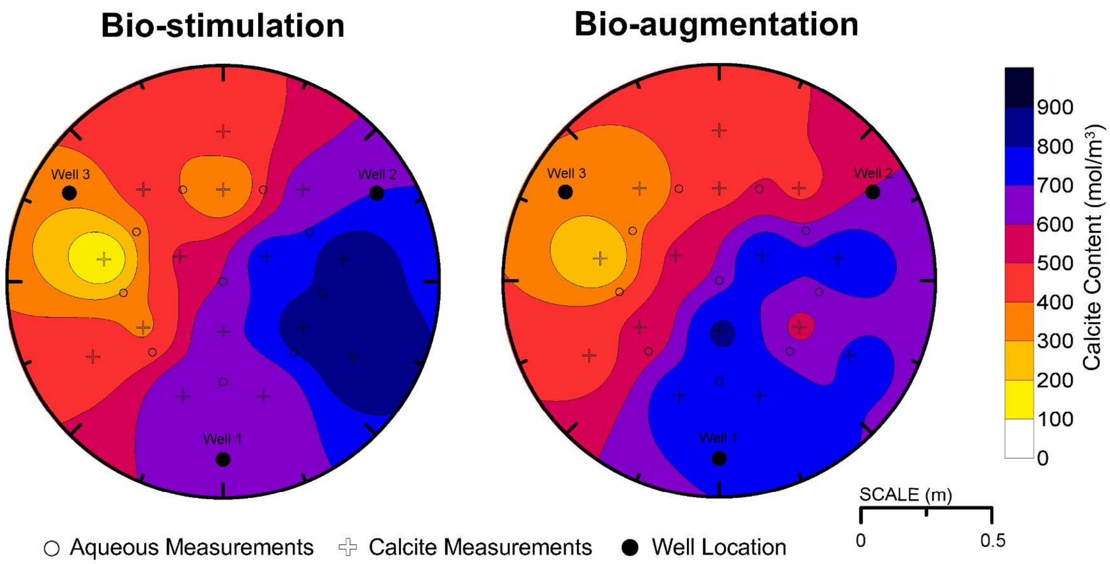
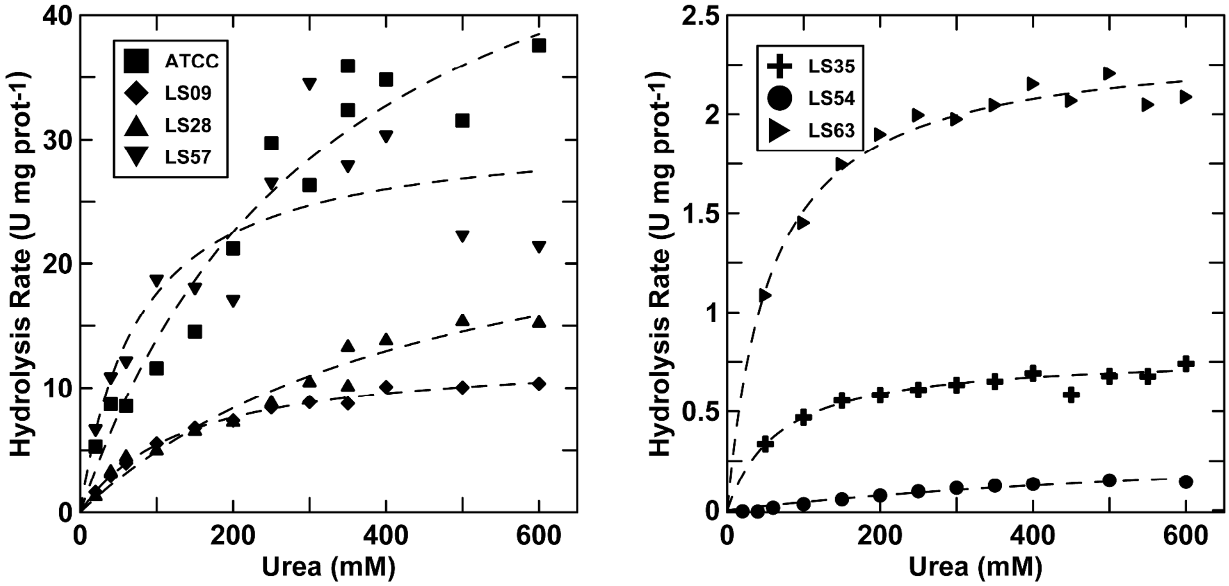
Supplemental Figure 1.





24 **Supplemental Figure 2.**

Supplemental Table 1. Isolate List and Sample Source

Isolate	Tank	Day Isolated	Sample Location	Log Dilution	Urease	Closest Homologous Strain	Bases Sequenced
LS01	Stim	1	D	-2	+	Sporosarcina soli (T) I80	836
LS02	Stim	1	D	-2	+	Sporosarcina soli (T) I80	1424
LS03	Stim	1	D	-2	+	Sporosarcina soli (T) I80	1411
LS07	Stim	1	G	-2	+	Oceanobacillus polygoni (T) SA9	902
LS09	Stim	1	G	-2	+	Sporosarcina pasteurii WJ-5	1387
LS10	Stim	1	J	-2	+	Bacillus lentus UR41	1405
LS14	Stim	3	A	-4	+	Sporosarcina soli KNUC401	1421
LS15	Stim	3	D	-4	+	Sporosarcina soli KNUC401	1421
LS17	Stim	3	G	-5	+	Sporosarcina soli KNUC401	1416
LS18	Stim	3	G	-5	+	Sporosarcina soli KNUC401	1415
LS19	Stim	5	A	-3	+	Sporosarcina soli KNUC401	843
LS20	Stim	5	B	-4	+	Sporosarcina soli KNUC401	1418
LS21	Stim	5	B	-3	+	Sporosarcina soli KNUC401	1426
LS22	Stim	5	D	-4	+	Sporosarcina soli KNUC401	897
LS23	Stim	5	D	-3	+	Sporosarcina soli KNUC401	904
LS24	Stim	5	E	-3	+	Sporosarcina soli KNUC401	1423
LS25	Stim	5	E	-3	+	Sporosarcina soli KNUC401	1425
LS26	Stim	5	G	-3	+	Sporosarcina soli KNUC401	1425
LS27	Stim	5	J	-4	+	Sporosarcina soli KNUC401	912
LS28	Stim	5	J	-3	+	Sporosarcina soli KNUC401	1324
LS33	Stim	12	A	-3	+	Sporosarcina ginsengisoli Gsoil1433	839
LS34	Stim	12	B	-3	+	Sporosarcina ginsengisoli Gsoil1433	995
LS35	Stim	12	E	-1	+	Bacillus lentus (T) NCIMB8773	845
LS36	Stim	12	E	-1	+	Sporosarcina soli KNUC401	1423
LS37	Stim	12	G	-3	+	Bacillus lentus (T) NCIMB8773	948
LS38	Stim	12	G	-2	neg.	Oceanobacillus luteolus WM-4	1437
LS39	Stim	12	J	-1	+	Sporosarcina soli KNUC401	1446
LS40	Stim	12	J	-3	+	Bacillus hackensackii AY14842	1289
LS41	Stim	12	Well 2	-1	+	Sporosarcina soli KNUC401	1410
LS45	Stim	12	Well 2	-1	+	Sporosarcina saromensis KUDC1822	915
LS46	Stim	12	Well 2	-3	+	Sporosarcina luteola (T) Y1	955
LS47	Stim	12	Well 2	-3	neg.	Sporosarcina aquimarina KUDC1821	921
LS48	Stim	12	A	-2	+	Sporosarcina pasteurii IARI-J-21	1430
LS49	Stim	13	B	-3	+	Oceanobacillus luteolus (T) WM-1	1396
LS29	Aug	5	A	-3	+	Sporosarcina pasteurii (T) HQ676600	1095
LS31	Aug	5	G	-3	+	Sporosarcina pasteurii (T) HQ676600	1099
LS32	Aug	5	J	-3	+	Sporosarcina pasteurii (T) HQ676600	1096
LS70	Aug	5	E	-3	+	Sporosarcina pasteurii (T) HQ676600	1095
LS71	Aug	5	D	-4	+	Sporosarcina pasteurii (T) HQ676600	1089
LS72	Aug	5	D	-4	+	Sporosarcina pasteurii (T) HQ676600	1093
LS73	Aug	5	G	-4	+	Sporosarcina pasteurii (T) HQ676600	1093
LS52	Aug	12	A	-3	+	Sporosarcina soli (T) I80	1426
LS53	Aug	12	B	-3	neg.	Ornithinibacillus contaminans (T) CCUG 53201	1443
LS54	Aug	12	B	-2	+	Sporosarcina aquimarina FT1	1425
LS55	Aug	12	G	-1	+	Sporosarcina pasteurii WJ-5	1424
LS56	Aug	12	D	-2	+	Sporosarcina soli KNUC401	1426
LS57	Aug	12	E	-3	+	Sporosarcina soli KNUC401	1424
LS58	Aug	12	E	-2	+	Sporosarcina soli KNUC401	1422
LS61	Aug	12	G	-2	+	Sporosarcina soli KNUC401	1410
LS62	Aug	12	J	-2	+	Sporosarcina soli (T) I80	1422
LS63	Aug	13	A	-3	+	Sporosarcina soli (T) I80	1423
LS64	Aug	13	A	-3	+	Sporosarcina soli (T) I80	1425
LS65	Aug	13	B	-3	neg.	Oceanobacillus luteolus (T) WM-1	1435
LS66	Aug	13	D	-3	+	Sporosarcina soli (T) I80	1426
LS67	Aug	13	E	-3	+	Oceanobacillus luteolus WM-4	1438
LS68	Aug	13	G	-3	neg.	Oceanobacillus luteolus WM-4	1434
LS69	Aug	13	J	-3	+	Sporosarcina soli (T) I80	1424

Supplemental Table 2: Alkalinity and Carbon Dioxide Generating Microbial Processes*

Process	Substrate	Product	Representatives	Specific activity (substrate) (U mg prot^{-1})	OH- production** (μmol min^{-1} mg prot^{-1})	CO_2 production** (μmol min^{-1} mg prot^{-1})
Denitrification[41]	NO_3^-	0.5 N_2	*Paracoccus denitrificans*	0.0764	**0.0764 - 0.171**	**0.096 - 0.214**
			Pseudomonas stutzeri	0.1712		
Sulfate Reduction[42]	SO_4^{2-}	H_2S	*Desulfovibrio desulfuricans* CSN	0.0285	**0.057 - 0.122**	**0.057 - 0.122**
			D. vulgaris Marburg	0.041		
			Desulfobulbus pripionicus	0.061		
			Desulfobacter hydrogenophilus	0.036		
Iron Reduction[43]	Fe(III)	Fe(II)	*Geobacter metallireduciens*	0.210 - 0.870	**0.0196 - 1.74**	**0.0024 - 0.218**
			Desulfuromonas acetoxidans	0.414 - 0.570		
			Pseudomonas sp.	0.175		
			Shewanella putrefaciens MR-1	0.0098 - 0.833		
			Shewanella putrefaciens MR-4	0.478 - 0.510		
			Shewanella putrefaciens sp200	0.250 - 0.310		
			strain BrY	0.46		
Ureolysis	urea	$2NH_4^+$ CO_3^{2-}	*Sporosarcina soli* LS28**	12	**0.230 - 24**	**0.115 - 12**
			S. pasteurii LS09**	9.26		
			Bacillus hackensacki i LS35**	0.662		
			S. aquimarina LS54**	0.115		
			S. pasteuri i ATCC 11859**	30.7	**61.4**	**30.7**

*For denitrifying, sulfate-reducing and iron-reducing bacteria, whole cell rates (μmol min-1 mg prot-1) are based on pure culture literature maxima and ranges. Literature rates for denitrification derived assuming protein = 50% of total dry weight. Whole cell ureolysis rates are based on kinetic parameters for Table 5 data at 350 mM urea, pH 8.5. Ranges shown are for 4 tank isolates; S. pasteurii ATCC 11859 data shown separately.

**Rates of OH- production (or H+ consumption) and CO2 production for tabulated processes are derived from balanced equations shown below, with acetate as electron donor for respirations, i.e. first 3 reactions. The derived ratios are:

OH-/substrate = 1:1 for denitrification; 2:1 for iron reduction, sulfate reduction, and ureolysis.

CO2/substrate = 1.25:1 for denitrification; 2:1 for sulfate reduction; 0.25:1 for iron reduction; 1:1 for ureolysis.

Reaction Equations:[44]

$5/8C_2H_4O_2 + NO_3^- + H^+ \rightarrow 5/4CO_2 + 1/2N_2 + 7/4H_2O$ $1/8C_2H_4O_2 + FeOOH + 1/4H_2O \rightarrow 1/4CO_2 + Fe^{2+} + 2OH^-$

27 $C_2H_4O_2 + SO_4^= + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$ $H_2NCONH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_2 + 2OH^-$

Figure S1. Mid-tank height calcite contours (mol/m^3) at mid-height in each tank at the end of cementation phase. Both tanks were dissected and core tubes were driven at 15 locations (shown here as + symbols) to collect soil samples for calcite quantification by a pressure chamber method that employed an acid dissolution reaction.[56] Resulting calcite values were inverse-distance interpolated to generate these spatial contours of final mid-depth calcite content.

Figure S2. Urea hydrolysis rates over a range of urea concentrations at pH 8.5 with Michaelis-Menten kinetic fits. Panel (a) shows higher rate strains: ATCC 11859 (type strain of *S. pasteurii*), LS09 (*S. pasteurii*), LS28 (*S. soli*) and LS57 (*S. soli*). Panel (b) shows data for LS35, LS54 and LS63 (*B. lentus, S. aquimarina* and *S. soli*, respectively).

Table S1. Isolate List and Sample Source for strains discussed within.

Table S2. Alkalinity and Carbon Dioxide Generating Microbial Processes