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**TITLE:** Diversity of Benzyl- and Alkylsuccinate Synthase Genes in Hydrocarbon-Impacted Environments and Enrichment Cultures

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**Table SI-1.** Oligonucleotide primer set combinations and sequences targeting *assA* and *bssA* genes.

**Table SI-2.** Description of isolates, hydrocarbon-impacted sites, and enrichment cultures.

“NT” designates that samples were not analyzed for metabolites; “ND” designates that metabolites consistent with octacosane addition to fumarate were not detected; and “N/A” means “not applicable”.

**Table SI-3.** Amplification results for primer sets used to target *assA* and *bssA* genes in environmental samples and enrichment cultures. (+) designates positive amplification; (-) designates that *assA* and *bssA* genes were not detected; and “NT” designates that the samples were not tested with those primers. The number of OTUs per number of screened clones (OTUs/clones screened) is reported for environmental samples from which clone libraries were generated.

Table SI-1

Primer Set	Forward (F) and Reverse (R) Oligonucleotide Primer Sequences*	Expected amplicon size (bp)
<b>1 (targets <i>assA</i> and <i>bssA</i>)</b>	<i>ass/bss</i> F: 5'-TTTGAGTGCATCCGCCAYGGICT-3' <i>ass/bss</i> R: 5'-TCGTCRTTGCCCCATTTIGGIGC-3'  (Note: The <i>ass/bss</i> F positions relative to <i>assA1</i> and <i>bssA</i> are 1294 and 1321, respectively; the <i>ass/bss</i> R positions in <i>assA1</i> and <i>bssA</i> are 1933 and 1981, respectively.)	<i>assA</i> : 661 <i>bssA</i> : 682
<b>2 (targets <i>bssA</i>)</b>	1213F: 5'-GACATGACCGAYGCCATYCT-3' 1987R: 5'-TCRTCCTTGCCCCAYTT-3'	793
<b>3 (targets <i>assA</i>)</b>	1294F: 5'-TTSGARTGCATCCGNCACGGN-3' 1936R: 5'-TCRTCATTNCCCCAYTTNGG-3'	661
<b>4 (targets <i>assA</i>)</b>	1294F; 5'-TTSGARTGCATCCGNCACGGN-3' 2457R: 5'-TTGTCCTGNGTYTTGCCG-3'	1180
<b>5 (targets <i>assA</i>)</b>	1294F: 5'-TTYGAGTGYATNCGCCASGGC-3' 1936R: 5'-TCRTCATTNCCCCAYTTNGG-3'	661
<b>6 (targets <i>assA</i>)</b>	1294F: 5'-TTYGAGTGYATNCGCCASGGC-3' 2457R: 5'-TTGTCCTGNGTYTTGCCG-3'	1180
<b>7 (targets <i>assA</i>)</b>	1432F: 5'-CCNACCACNAAGCAYGG-3' 1936R: 5'-TCRTCATTNCCCCAYTTNGG-3'	523
<b>8 (targets <i>assA</i>)</b>	1432F: 5'-CCNACCACNAAGCAYGG-3' 2457R: 5'-TTGTCCTGNGTYTTGCCG-3'	1042
<b>9 (targets <i>assA</i>)</b>	1432F: 5'-CCNACCACNAAGCAYGG-3' <i>ass/bss</i> R: 5'-TCGTCRTTGCCCCATTTIGGIGC-3'  (Note: The <i>ass/bss</i> R positions relative to <i>assA1</i> and <i>bssA</i> are 1933 and 1981, respectively.)	523

\*Positions within the *assA* gene are relative to *assA1* (2,505 bp) in *Desulfatibacillum alkenivorans* AK-01 (Accession number DQ826035); Positions within the *bssA* gene are relative to *bssA* (2,586 bp) in *Thauera aromatica* K172 (Accession number AJ001848).

Table SI-2.

Sample	DNA Source	Metabolites Identified in Groundwater or Culture	Reference*
<i>Desulfatibacillum alkenivorans</i> AK-01	Pure culture	Hexadecylsuccinic acid	[17]
<i>Desulfoglaeba alkanedexens</i> ALDC	Pure culture	Alkylsuccinic acids (C <sub>6</sub> , C <sub>8</sub> , C <sub>10</sub> , C <sub>12</sub> )	[8, 14, 28]
Passaic River	Sediment	NT	N/A
Newtown Creek	Sediment	NT	N/A
Arthur Kill Waterway	Sediment	NT	N/A
Gowanus Canal	Sediment	NT	N/A
Casper, Wyoming (MW-439)	Sediment	Benzylsuccinic acid, methylbenzylsuccinic acids, dimethylbenzylsuccinic acids, alkylsuccinate (C <sub>5</sub> ), alkylsuccinic acids with unsaturation (C <sub>5</sub> , C <sub>6</sub> , C <sub>7</sub> )	[9]
Fort Lupton, Colorado	Sediment	Alkylsuccinic acids (C <sub>6</sub> , C <sub>7</sub> , C <sub>8</sub> , C <sub>9</sub> ), alkylsuccinic acids with unsaturation (C <sub>6</sub> , C <sub>7</sub> , C <sub>8</sub> ), 3-methylbenzylsuccinic acid, 4-methylbenzylsuccinic acid	[6-8]
SDB paraffin-degrading enrichment culture	Enrichment culture	ND	N/A
Propane-utilizing mixed culture	Enrichment culture	<i>n</i> -Propylsuccinate, <i>iso</i> -propylsuccinate	[20]

\*References cited in research article

