N-60

Prevalence and Diversity of a Ring-Cleaving Dioxygenase Gene, *pcaH*, in a Salt Marsh Community

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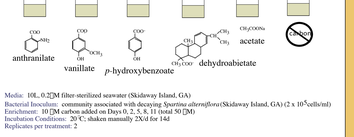
Abstract

The degradation of lignin-related aromatic compounds is an important ecological process in the highly productive salt marshes of the southeastern U. S., yet little is known of the mediating organisms and their catabolic pathways. Here we report the diversity of a gene encoding a key ring-cleaving enzyme of the β-ketoadipate pathway, *pcaH*, amplified from bacterial communities associated with decaying *Spartina alterniflora*, the salt marsh grass dominating these coastal systems, as well as from enrichment cultures with aromatic substrates (*p*-hydroxybenzoate, anthranilate, vanillate or dehydroabietate). Sequence analysis of 149 *pcaH* clones revealed 59 unique sequences. Fifty-eight percent of the clones matched sequences amplified from a collection of 36 bacterial isolates obtained from seawater or from senescent *Spartina*. Fifty-two percent of the *pcaH* clones could be assigned to the *Roseobacter* group, a lineage of marine α-Proteobacteria abundant in coastal ecosystems. Another 6% matched genes retrieved from non-*Roseobacter* isolates cultured from decaying *Spartina* in the genera *Acinetobacter*, *Bacillus* and *Stappia*, and 42% could not be assigned to a cultured bacterium based on sequence identity. These results suggest a very high diversity of genes encoding a single step in aromatic compound degradation in this coastal marsh, and that many of these genes may be harbored by members of the *Roseobacter* lineage.

Salt Marsh Community (SMC)

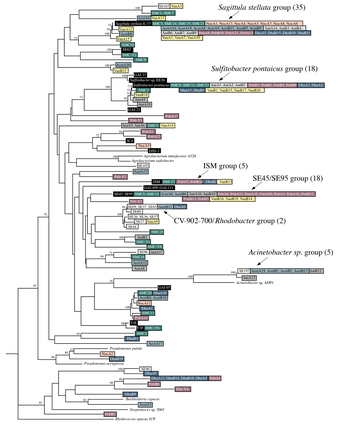
pcaH from natural communities



Media: 10L, 0.2μM filter-sterilized seawater (Skidaway Island, GA)
Bacterial Inoculum: community associated with decaying *Spartina alterniflora* (Skidaway Island, GA) (2 x 10^5 cells/ml)
Enrichment: 10 μM carbon added on Days 0, 2, 5, 8, 11 (total 50 μM)
Incubation Conditions: 20 °C; shaken manually 2X/d for 14d
Replicates per treatment: 2

Enrichments were established to monitor the response of *pcaH* genes in the natural bacterial community associated with decaying *Spartina* to aromatic substrates representative of compounds produced during lignin decay.

pcaH from enriched communities



Diversity of *pcaH* genes in *Spartina*-associated bacteria was quite high. Of the 25 clones sequenced from the natural community, 18 unique sequences were found. Of the 124 enrichment clones, 43 were unique, and only 6 of these matched sequences from the natural community. Homology searches with sequences from GenBank confirm these sequences have highest similarity to PcaH from various bacterial genera, with deduced amino acid similarities ranging between 82-100% and identities between 73-100%.

pcaH from marine isolates

Phylogenetic affiliations of marine isolates with amplifiable *pcaH* genes.

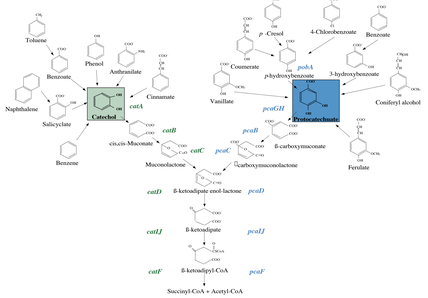
Isolate	Subdivision	Group	Closest relative (accession number)	Similarity (%)
DSS-3	α Proteobacteria	*Roseobacter*	*Roseovarius* sp. AS-36	97
EE-36	α Proteobacteria	*Roseobacter*	*Sulfitobacter pontiacus*	99
GAI-05	α Proteobacteria	*Roseobacter*	marine isolate JP88.1	98
GAI-37	α Proteobacteria	*Roseobacter*	*Sulfitobacter* sp. GAI-21	97
GAI-111/109	α Proteobacteria	*Roseobacter*	*Roseobacter* clone NAC11-6	94
IC4	α Proteobacteria	*Roseobacter*	hydrothermal vent strain TB66	98
ISM	α Proteobacteria	*Roseobacter*	*C. virginica* symbiont CV919-312	96
YJF/Y4I	α Proteobacteria	*Roseobacter*	marine bacterium PP-154	97
Sagittula stellata E-37	α Proteobacteria	*Roseobacter*	NA	NA
SE03[a]	α Proteobacteria	*Rhizobium/Agrobacterium*	*C. virginica* symbiont CV902-700	97
SE09	α Proteobacteria	*Rhizobium/Agrobacterium*	*C. virginica* symbiont CV902-700	98
SE11	α Proteobacteria	*Rhizobium/Agrobacterium*	*C. virginica* symbiont CV902-700	97
SE65	α Proteobacteria	*Rhizobium/Agrobacterium*	*C. virginica* symbiont CV902-700	99
SE37	α Proteobacteria	*Rhodobacter*	marine isolate Sippewissett 2-21	99
SE45/SE95	α Proteobacteria	*Roseobacter*	hydrothermal vent strain AG33	98
SE62	α Proteobacteria	*Roseobacter*	Isolate GAI-37	96
SE197	α Proteobacteria	Moraxellaceae	*Acinetobacter calcoaceticus*	99
SE96	γ Proteobacteria	Halomonadaceae	*Nesticlaea scintillans* endocyte	97
SE98	Firmicutes	*Bacillus/Clostridium*	*Bacillus cereus*	99
SE105	Firmicutes	*Bacillus/Clostridium*	*Bacillus* sp. OS-5	99
SE165	Firmicutes	*Bacillus/Clostridium*	*Bacillus subtilis*	91

NA = not applicable
a = The following isolates had identical 16S rRNA gene sequences to SE03: SE22, SE26, SE27, SE32, SE35, SE36, SE39, SE44, SE49, SE55, SE57, SE60, SE83, SE97, SE114.

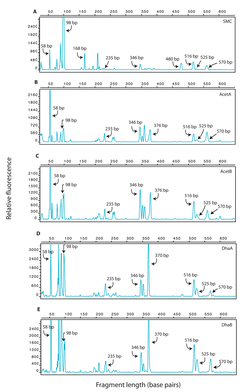
For comparative purposes, a culture collection of some of the *pcaH*-containing bacteria associated with decomposing *Spartina*, as well as *Roseobacter* isolates from a pre-existing collection, was assembled.

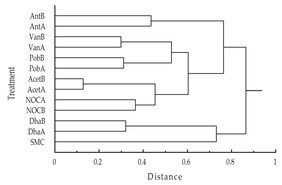
Conclusions

- The abundance and diversity of *pcaH* genes suggests the β-ketoadipate pathway is an important route for the degradation of aromatic compounds in southeastern marsh systems.
- Over half (58%) of the genes retrieved directly from natural and enriched communities can be matched to *pcaH* genes from cultured marine isolates.
- A predominance of *Roseobacter*-like *pcaH* sequences suggests members of this lineage may be pivotal in the processing of aromatic compounds in the salt marsh environments.
- Members of the *Roseobacter* lineage are ideal candidates for laboratory-based physiological and genetic studies to obtain critical information on the mechanisms of and ecological controls on aromatic compound degradation in marine ecosytems.

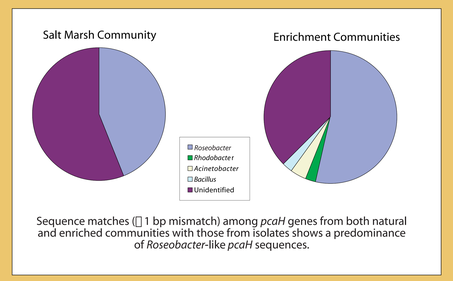


The β-ketoadipate pathway is a major catabolic route for lignin-derived aromatic compounds in terrestrial soils. The importance of this pathway in marine systems is currently unknown.

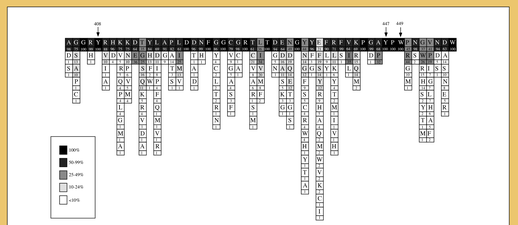




T-RFLP profiles of 16S rDNA amplicon pools indicated distinctive bacterial communities developed in response to each enrichment substrate.



Sequence matches (≤ 1 bp mismatch) among *pcaH* genes from both natural and enriched communities with those from isolates shows a predominance of *Roseobacter*-like *pcaH* sequences.



Analysis of all deduced amino acid sequences, including those in GenBank, shows complete conservation of 2 residues demonstrated to be involved in Fe2+ binding, Tyr408 and Tyr447, and a residue involved in substrate specificity, Trp449. The conservation of 10 additional residues suggests a required catalytic or structural function for these amino acids.

Dedicated to Dr. L. Nicholas Ornston

in gratitude for his outstanding scientific leadership and in appreciation for his constant support

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