Differences in Gut Microbial Communities Between Mussels and Oysters Reflect Differences in Food Resource Utilization

N-060

Dawn E. Loggans* and Shiao Y. Wang, The University of Southern Mississippi

deloggans@gmail.com
shiao.wang@usm.edu

Abstract

The ribbed mussel *Geukensia demissa* and the Eastern oyster *Crassostrea virginica* are commonly found in salt marshes along the east coast of the United States and the Gulf of Mexico. Although both bivalves are estuarine, *G. demissa* is intertidal whereas *C. virginica* is subtidal. Studies have shown that detrital lignocellulose derived from marsh grass is utilized by mussels to meet 26-80% of its carbon requirement but accounts for less than 3% of the oyster's carbon requirement. To determine whether this difference in food resource utilization may be reflected in a difference in their gut microbial community, we: 1) characterized the gut microflora of the two bivalves; 2) determined whether the gut microbial communities vary across season; and 3) determined whether cellulolytic microbes are present in the gut of the bivalves. Gut microbial communities were analyzed quarterly using universal bacterial primers to PCR amplify the 16S ribosomal RNA gene. Clone libraries were then analyzed using RFLP and the dominant members sequenced. Sequence results indicate significant differences in gut bacterial community between the two bivalve species that vary seasonally. Cyanobacteria are the only shared dominant community members in the bivalves during March and June, but are absent from both in September. *Mycoplasma* spp. are dominant community members in the gut of *G. demissa* during all seasons but not in that of *C. virginica*. Other gut bacteria in *G. demissa* included *Spiroplasma* spp., *Entoplasma somnilux*, and *Pseudomonas* sp. In contrast, dominant bacteria in oysters are *Wolbachia* sp., *Ralstonia* spp., *Haemophilus* sp., and *Escherichia coli*. A total of 172 cellulolytic CFUs were isolated from the bivalves and 96% were isolated from *Geukensia*. Our results show a substantial difference in the gut bacterial community between two bivalve species that often live only centimeters apart. Cellulolytic bacteria were present in bivalve guts but they were found almost exclusively in *G. demissa*.

Objectives

Ribbed mussels and Eastern oysters are both estuarine bivalves but ribbed mussels ingest cellulose-rich detritus whereas Eastern oysters do not[1-3]. We hypothesize that their gut microbial communities differ. The objectives of the study were to:

- Identify major members of their gut bacterial community
- Isolate cellulolytic bacteria from the intestine of the two bivalves

Methods

Extract genomic DNA from gut content

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PCR amplify 16S rDNA using universal bacterial Primers 63F and 1389R

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Construct 16S rDNA libraries

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Fingerprint 96 isolates and sequence those that are unique

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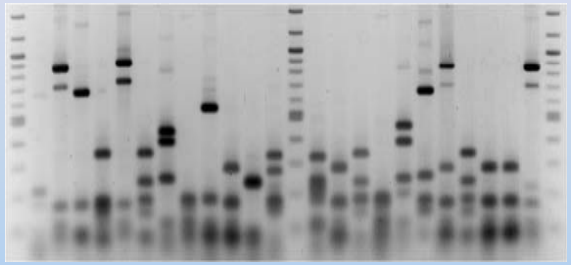
Assign identity using BLAST

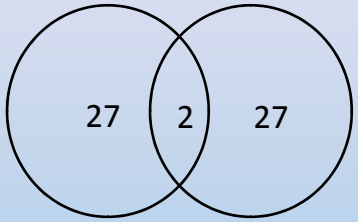
RFLP was used to Identify Unique Isolates



Intestinal Bacteria Differed In Mussels & Oysters

Mussel			Oyster		
	% of Community	BLAST result		% of Community	BLAST result
March	13.3	Uncultured cyanobacterium A	March	19.8	Cyanobacterium clone SIMO-832
	12.2	*Mycoplasma cottewii*		18.7	Uncultured bacterium A
	7.8	Uncultured bacterium B		8.8	Uncultured cyanobacterium
	5.6	Uncultured cyanobacterium B		4.4	*Wolbachia* endosymbiont
	5.6	Uncultured bacterium A		4.4	Uncultured soil bacterium
	3.3	*Spiroplasma sabaudiense*		3.3	*Pseudomonas* sp. RM2-2001
	3.3	*Mycoplasma cavipharyngis*		2.2	*Ehrlichia ruminantium* strain Umbanein
	3.3	*Spiroplasma* sp. CRW-1			
	2.2	*Synechococcus* sp. CC9605		2.2	*Spongiobacter* sp.
	2.2	*Pseudoxanthomonas* sp. 11	June	7.8	*Salmonella enteric*
June	22.2	Uncultured cyanobacterium clone balE6		5.6	*Ralstonia* sp. EF28
	22.2	*Mycoplasma pneumoniae* strain ATCC 29342		5.6	*Ralstonia* sp. HI3
				4.4	*Escherichia* sp. clone BR06BH06
	12.2	Uncultured cyanobacterium clone SIMO-838		3.3	cyanobacterium clone SIMO-832
				3.3	*Delftia* sp. WXZ-11
	7.8	*Entomoplasma somnilux*		2.2	*Escherichia coli* E24377A
	5.6	*Mycoplasma gallisepticum* strain R		2.2	*Derxia gummosa*
	2.2	Uncultured cyanobacterium clone LC3-54	Sept.	7.7	*Haemophilus* sp. clone IS022B59
				5.5	*Pseudomonas* sp. RM2-2001
Sept.	12.4	*Sphingomonas* sp. TSBY-38		5.5	*Ralstonia* sp. HI3
	11.2	*Pseudomonas* sp. AI1		4.4	*Pseudomonas* sp. MPU L18
	7.9	*Sphingomonas insulae* strain DS-28		3.3	*Propionibacterium acnes* isolate Asn14
	7.9	*Spiroplasma sabaudiense*			
	5.6	*Mycoplasma cottewii* strain VIS		3.3	*Ralstonia pickettii* strain 2000030635
	2.2	*Hymenobacter* sp. BSw20462			
	2.2	*Mycoplasma yeatsii* strain GIH		3.3	*Granulicatella* sp. clone BL027B79
	2.2	*Mycoplasma mycoides capri* PG3		2.2	*Sphingomonas* sp. 14_4K
Dec.	54.4	*Escherichia* sp. MSCB-10		2.2	*Escherichia coli* E24377A
	1.1	*Mycoplasma cottewii*	Dec.	67.4	*Escherichia coli* strain MM.1.9
	1.1	*Ralstonia* sp. P-4CB2		9	*Escherichia coli* strain O157:H7
	1.1	*Escherichia coli* W3110		3.3	*Escherichia coli* W3110
	1.1	*Escherichia* sp. clone 8817-D4-C-10B		1.1	*Escherichia coli* HS

The Bivalves had Few Bacteria in Common



96% of Cellulolytic Bacteria were Isolated from Mussels

Mussel Gut Isolates

Oyster Gut Isolates

Summary

- Gut microbial communities differed significantly between mussels and oysters reflecting likely differences in food resource utilization.
- Seasonal differences in bivalve gut bacteria indicate transient food resources.
- Most (96%) of the cellulolytic bacteria isolated originated from mussels suggesting that detritus is much more important in the diet of mussels than in that of oysters.

References

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2. Charles, F. & R.I.E. Newell. 1997. J. Exp. Mar. Biol. Ecol. 209: 201-213.
3. Peterson, B.J., R.W. Howarth & R.H. Garritt. 1986. Ecology 67: 865-874.