CHARACTERISTICS OF BACTERIA ISOLATED FROM PENAEID SHRIMP

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CHARACTERISTICS OF BACTERIA ISOLATED FROM PENAEID SHRIMP[1])

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RÉSUMÉ

Des bactéries du tractus digestif de *Penaeus aztecus* et de son environnement ont été isolées, identifiées et caractérisées en fonction de leur capacité à utiliser des substrats distincts. Les bactéries de la crevette et des nourritures disponibles ont montré des capacités différentes à utiliser l'amidon, la gélatine, les acides gras, la cellulose et la chitine. Elles n'étaient pas inhibées par les composés phénoliques trouvés dans *Thalassia*.

Bacteria symbiotic in an animal's digestive tract often produce a complement of enzymes for digestion of plant foods as well as synthesize compounds that are assimilated by the host (McBee, 1971; Hungate, 1975). The role of intestinal flora has long been documented in ungulate herbivores (Hungate, 1975), and such terrestrial invertebrates as termites (Eutick et al., 1978; Schultz & Brezank, 1978). The possibility of a symbiotic gut flora is now being studied in several marine invertebrates. Such flora have been documented in herbivorous sea urchins (Lasker & Giese, 1954; Fong & Mann, 1980), copepods (Sochard et al., 1979), and penaeid shrimp (Hood et al., 1971; Dempsey, in review).

Prim & Lawrence, 1975, and Lasker & Geise, 1954, reported that large numbers of bacteria are capable of digesting many algal components. The ability of a gut flora to degrade algal polysaccharides or other complex plant polymers in an animal's diet can increase its host's digestive efficiency. Among polysaccharides, the major polymers of the ingested epiphytic algae include starch, found in the green algae, and cellulose in the green, brown and red algae (Chapman, 1968). Each of these algal phyla are available to the shrimp in seagrass meadows of our study region (Morgan & Kitting, 1984; Kitting et al., 1984). We hypothesized that the gut flora of penaeid shrimp is also capable of digesting many of these components.

Bacteria were also tested for their ability to digest chitin. Chitin is the main component of the shrimp's exoskeleton, including the lining of its intestine.

While foraging among epiphytic material on seagrass blades, shrimp often ingest pieces of seagrass and its detritus (Morgan & Kitting, 1984). Seagrasses contain various phenolic compounds (Burkholder et al., 1959; McMillan et al., 1980) and the leaching of these compounds in the shrimp digestive tract might be found to interfere with bacterial metabolism, or otherwise decrease the digestive efficiency of the shrimp.

Penaeid shrimp were collected from seagrass meadows in Redfish Bay near Port Aransas, Texas, from July to October, 1983. Thirty representative colonies were selected from Sea Water Complete plates. They reflected those colonies from plates of shrimp gut extract, which sampled the natural habitat, digestive tract, and surface of *Penaeus aztecus* Ives, 1891 (Dempsey et al., in review). These bacteria were identified (Oliver, 1982), and then tested for their ability to digest starch, gelatin, cellulose, and chitin. Selected bacteria from shrimp digestive tracts and from algal epiphytes were also tested for sensitivity to concentrated phenolic compounds extracted from common turtlegrass (*Thalassia testudinum*).

Nine genera of bacteria were identified. *Moraxella* was found only on plates from the sediment, epiphytic algae on turtlegrass, and water. *Flavobacterium*, *Cytophaga*, *Alcaligenes*, *Chromobacterium*, and the pseudomonas-like bacteria (*Pseudomonas*, *Xanthomonas*, *Altermonas*) were identified both in the shrimp as well as its environment. *Aeromonas*, *Vibrio*, and *Photobacterium* were all identified from colonies selected from plates of shrimp stomachs and hindguts (table I).

In a test for gelatines (protease), nutrient agar containing 0.4% gelatin was inoculated with each of thirty isolated bacteria, incubated for two days, then flooded with a gelatin precipitating agent, 15% $HgCl_2$ in 20% (vol/vol) concentrated HCl. A clear halo around the colonies indicated gelatin hydrolysis. In the test for starch hydrolysis (amylase activity), nutrient agar containing 0.2% starch was inoculated with each of the isolated bacteria, incubated for two days, then flooded with Gram-iodine (1 g I, 2 g KI, in 300 ml H_2O). A positive reaction was indicated by clear halos around the bacterial colonies. For the lipase test, nutrient agar containing 1% Tween 80® was inoculated with each of the isolated bacteria, and incubated for two days. The presence of lipase activity was indicated by insoluble oleic acid forming white halos around the bacterial colonies. Cellulase and chitinase were tested by overlaying non-nutritive seawater mineral base media with agar containing either 40% cellulose or chitin. Both the chitin (extracted from crab shells) (Reichenbach & Dworkin, 1981) and the cellulose (from filter paper) were hydrolyzed and finely suspended in seawater mineral base media. These media were inoculated and poured over the agar base. The opaque plates showed clear halos around areas of cellulase or chininase activity within 3 weeks.

Most of the bacteria tested exhibited amylase, lipase and gelatinase activity;

TABLE I

Characteristics of isolated Bacteria from penaeid shrimp and their environment

Genera: isolate number & source	Cellulase	Lipase	Amylase	Gelatinase	Chitinase
Flavobacterium					
1 Exoskeleton	+	+	+	+	-
2 Hindgut	-	-	-	-	-
4 Water	-	+	+	+	-
7 Stomach	+	-	-	-	-
8 Stomach	-	-	-	-	-
20 Epiphytes	-	-	-	-	-
Moraxella					
3 Water	-	+	+	+	-
6 Water	-	-	+	+	-
13 Epiphytes	-	-	+	+	-
19 Epiphytes	-	+	-	+	-
Cytophaga					
5 Water	+	-	-	+	+
7a Stomach	+	-	+	+	-
12 Epiphytes	+	-	+	+	-
Photobacterium					
26 Hindgut	-	+	-	+	-
Alcaligenes					
9 Stomach	-	+	+	+	-
10 Stomach	-	+	+	+	-
15 Epiphytes	-	-	+	+	-
25 Hindgut	-	+	+	+	-
30 Hindgut	+	-	+	+	-
Pseudomonas, Caulebacter, Xanthamonas, Altermonas					
11 Epiphytes	-	+	+	+	-
21 Epiphytes	-	-	-	-	-
24 Hindgut	-	+	+	+	-
27 Hindgut	-	-	-	-	-
28 Hindgut	-	+	+	+	-
29 Hindgut	-	+	+	+	-
Chromobacterium					
14 Epiphytes	-	+	+	+	-
22 Stomach	-	+	+	+	-
Aeromonas					
16 Stomach	-	+	+	+	-
18 Stomach	-	+	+	-	-
Vibrio					
17 Stomach	-	+	+	+	-
23 Stomach	-	+	-	+	-

few showed the ability to digest cellulose or chitin (table I). The bacteria identified as *Cytophaga* all showed cellulase activity and were the only bacteria isolated here that had the ability for digestion of chitin. Hood et al., 1971, found numerous bacteria in the digestive tract of the shrimp capable of digesting chitin. The dissimilarity in the numbers found here is probably due to sampling techniques, and colony selection.

Isolated colonies were tested for inhibition by phenolic compounds extracted from *Thalassia* (in various stages of decomposition) to which the shrimp is naturally exposed. Young unepiphytized blades, old attached blades, sloughed off green blades and decomposed brown blades were collected and cleaned of epiphytes. Phenolic compounds were extracted in methanol by the method described by Harrison & Chan, 1980. The extracted compounds were concentrated at ten times their naturally occurring concentrations and filter discs were saturated with these extracts. These discs were placed on SWC media plates that had been inoculated with bacteria from either shrimp stomachs, hindguts or epiphytic algae from *Thalassia*. Blades of *Thalassia* were also macerated and placed in holes bored in inoculated SWC plates. The plates with the filter discs and the macerated blades were both incubated for two days at 20°C. At the end of two days no inhibition was seen in either type of test. No inhibition of growth was observed; bacteria grew up to the filter disc perimeter, with all blade extracts as well as with the macerated blades themselves.

The lack of inhibition by the phenolic compounds on the bacteria supports the findings of Harrison & Chan, 1980, who showed that bacteria intimately associated with *Zostera marina* were resistant to phenolic compounds extracted from it.

The ability of the intestinal flora to withstand natural levels of phenolic compounds that might appear in the shrimp diet, as well as degrade various components of the shrimp's ingested plant material, may prove beneficial to the shrimp. Certain of these quickly growing bacteria found in large numbers in the digestive tract of the shrimp thus may play a significant role in the shrimp's digestion, as in the gut flora found in many terrestrial herbivores.

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