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Bacteriological Study of the Natural Flora of the Eastern Oyster, *Crassostrea virginica*

Recent progress in the culture of bivalve larvae (V. L. Loosanoff and H. C. Davis, *In* "Advances in Marine Biology," **1**, 1–136, Academic Press, New York and London, 1963) has made it possible to rear economically important bivalve mollusks under controlled conditions in hatcheries. Before a study of the bacterial flora of the larvae themselves was undertaken, a study was made of the bacterial flora of the Eastern oyster, *Crassostrea virginica*, in Long Island Sound. In the past, oyster bacteriology has dealt chiefly with coliforms since their presence gives an index of fecal pollution and, therefore, is of public health significance. We are now concerned with the bacterial flora of the oyster, not in terms of its containing organisms associated with human beings, but potential pathogens of the oyster and its progeny.

The natural bacterial flora of the Pacific oyster, *Crassostrea gigas*, was determined in a study by R. R. Colwell and J. Liston (*Appl. Microbiol.*, **8**, 104–109, 1960). Our study of Eastern oysters has shown the presence of the same bacterial genera, with the exception that no gram-positive bacteria were found. Aseptically prepared oyster homogenates were inoculated on an enriched seawater agar and incubated at 18°C for 7 days. A total of 96 isolates were obtained for taxonomic and physiological study. All isolates were gram-negative, short, or pleomorphic, rods; 34 (35.4%) were pigmented (yellow or orange) and 55 (57.3%) were motile (all but one was monotrichous, polar flagellated). With the scheme of J. M. Shewan, G. Hobbs, and W. Hodgkiss (*J. Appl. Bacteriol.*, **23**, 379-390, 1960) 31.2% of the isolates belonged to the genus *Pseudomonas*, 26.0% to the genera *Flavobacterium-Cytophaga*, 25.0% to the genus *Vibrio*, and 17.7% to the genera *Achromobacter–Alcaligenes*. The inability to distinguish conclusively between *Flavobacterium* and *Cytophaga* or between *Achromobacter* and *Alcaligenes* made it more realistic to pair these genera.

Physiologically the isolates were more proteolytic (87.4%) than amylolytic (49.0%) or lipolytic (70.8%). Reactions in the MOF (marine oxidation-fermentation) medium of E. Leifson (*J. Bacteriol.*, **85**, 1183–1184, 1963) showed that 35 (36.4%) of the isolates attacked glucose oxidatively and 25 (26.0%) fermentatively; 26 (27.1%) produced no reaction in the medium. Ten isolates (10.4%) did not grow in the medium, presumably because of inhibition by phenol red. Tests of the ability of the isolates to reduce nitrate to nitrite, produce ammonia from peptone, and produce indole from tryptophan showed 54 (58.1%), 40 (43.0%), and 19 (20.4%), respectively, to be active. Only 4.3% of the isolates grew in a broth medium prepared with distilled water as diluent; 41.9% grew in a basal medium consisting of seawater enriched with glucose and ammonium chloride. Disc and lawn sensitivity tests showed all isolates to be sensitive to chloramphenicol and tetracycline, where 90.4% were sensitive to penicillin, and 79.6% to streptomycin. The genera enzymically most active were *Vibrio* and *Pseudomonas* in that order. All the isolates are common inhabitants of marine waters (S. J. Altschuler and G. A. Riley, *Bull. Bingham Oceanogr. Coll.*, **19**, 81–88, 1967; J. M. Sieburth, *J. Exptl. Marine Biol. Ecol.*, **1**,

98–121, 1967; U. Simidu and K. Aiso, *Bull. Japan. Soc. Sci. Fish.*, 28, 1133–1141, 1962). Although gram-positive bacteria are usually not considered abundant in seawater, it is surprising that a sessile invertebrate, such as the oyster, does not concentrate gram-positive bacteria probably present in Long Island Sound bottom sediments. It will be of interest to learn if hatchery-reared larval and juvenile oysters harbor a bacterial flora similar to that of the adult animals from Long Island Sound.

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