perature range of 25 to 55 C than at lower temperatures, ensibly due to the counteraction of the thermal denaturation -amylase by high pressure. This enzyme is more baroduric n most bacterial enzymes, but deep-sea pressures promoted its tivation at deep-sea temperatures, < 5 C.

108. Collagenolytic Activity of Some Marine Bacteria. STEPH R. MERKEL. Lehigh University, Bethlehem, Pa.

It has been reported that collagenolytic activity is quely associated with certain clostridia. Most claims of llagenolytic activity in other bacterial species were later ted on the grounds that native collagen was not used as the strate. The multiplicity of enzyme production by marine teria has led to an investigation of their collagenolytic ivities. The major difficulty in the search for collagenases is restrictive medium required by the rules laid down for a true llagenase. In the preliminary studies reported here, an acid-tracted calf skin collagen was used as the screening medium. hough this form of collagen cannot be considered truly native, medium was not digested by trypsin, crude pancreatin, nor active crude proteolytic enzymes from certain marine teria, but it was digested by *C. histolyticum* collagenase and enzyme preparations from certain marine bacteria. Many of marine bacteria exhibited collagenolytic activity only when wn in the presence of collagen. Those cultures that showed ivity against the acid-extracted collagen were then tested with ive bovine Achille's tendon, and the most active organisms re grown in 5-liter fermentors for enzyme characterizations.

109. Biological Nitrogen Fixation in an Estuarine Environment. RALPH H. BROOKS, JR., H. D. PUTNAM and P. L. RIZONIK. University of Florida, Gainesville.

Rates of biological nitrogen fixation in water and diments have been estimated in a small west coast Florida uary by the acetylene reduction technique of Stewart, zgerald, and Burris. Both laboratory and in situ incubation der a nitrogen-free aerobic and anaerobic atmosphere were ried out for periods of 1 to 6 hr. Ethylene production at levels to 200 ng/liter day^{-1} was detectable within the water lumn but only during the warm summer months. Highest rates ethylene production (12 to 60 ng/g dry weight day^{-1}) curred within the upper 5 cm of consolidated sediment. No tylene reduction was demonstrated within the flocculent diment-water interface. Using an average ratio of 1.5 for tylene/nitrogen reduction, it is estimated that 45 to 133 liter day^{-1} and 8 to 40 ng/g day^{-1} ammonia are fixed in the ter and sediment of this coastal estuary. The absence of anophycean algae suggests that the mechanism is bacterial in ture.

110. Psychrophilic Clostridia from Marine Sediments. J. NSTON, N. HOLMAN, and J. MATCHES. University of Washton Seattle.

There is little published information on the ability of stridia isolated from marine environments to grow at the low peratures normal in the sea. Clostridia isolated from sedints in Puget Sound have been found to grow at 10 C but most psychrotrophic rather than psychrophilic. Psychrophilic ains grew in enrichment cultures in a TPG meat broth ubated at 0 or 8 C of sediment samples from water depths of to 236 m and were isolated by streaking on seawater-blood-ar and incubating anaerobically at 0 or 8 C. Of the 29 strains lated, all grew at 0 C, 16 had a maximum growth temperature low 15 C, 8 below 22 C, 2 below 30 C, and 3 grew at 30 C. e organisms were tested biochemically at 0 C and 8, 15, or 30 depending on their maximum growth temperature. Most ains digested milk, produced H_2S, fermented dextrose, mal-se, and glycerol, but failed to reduce nitrate, hydrolyze starch, oduce lipase, or ferment other sugars. Gelatin was hydrolyzed indole produced by half the strains. More positive tests were tained at the higher temperature. Nevertheless the results icate that psychrophilic clostridia which can degrade organic aterial at ambient temperature occur in marine sediments.

111. Formation of Magnesium Ammonium Phosphate ystals in Cultures by a Marine Bacterium. R. N. BRAMLETT, WILLIAMS-WALLS, and E. GASPER. Georgia Institute of nology, Atlanta.

During the isolation of marine bacteria from Antarctic Ocean sediments on a sulfate-reducing medium at 12 C, crystal formation was noted in certain cultures. Studies were performed to determine the chemical nature of the crystals and the conditions under which they were produced by one organism. This bacterium is a motile, gram-negative rod that will not grow in the absence of sodium chloride. On a salts-containing medium, it produces luxuriant growth within 48 hr at 12 C; crystals appear in the medium within 7 days. The crystals were harvested and, using infrared spectroscopy, identified as being either magnesium phosphate or magnesium ammonium phosphate. The nutrient content and salt concentrations of the growth medium were varied to investigate the role played by the chemical environment in the formation of the two types of crystals. It was concluded that the type of crystal formed was a function of the magnesium ion concentration and the *p*H of the medium.

G112. Microbial Ecology of the Salton Sea. JUHEE KIM and MARTIN K. NAKAJI. California State College, Long Beach

The Salton Sea, a saline lake in Southern California, was investigated to study the microbial ecology of certain selected groups. Characteristics of the bacteria such as optimum salt concentration for growth, bioluminescence, and abundance of gram-negative, motile rods resemble in nature marine bacteria. Media used for the study were prepared with the Salton Sea water. The highest aerobic bacterial count was obtained from 2.7% NaCl of the concentrations tested. Optimum temperature for growth was 25 to 37 C. Four isolates of luminous bacteria from the water were closely related to some of the species of *Photobacterium*. Two of the isolates had very dim light emission. *Desulfovibrio* spp. were found to be the most predominant type among the numerous sulfate-reducing bacteria. Species of sulfur-oxidizing thiobacilli that formed a pellicle in thiosulfate broth, non-sulfur purple bacteria, and green sulfur bacteria were observed by enrichment techniques. Distribution and survival of coliforms in the Salton Sea were also investigated.

G113. Effect of Oxygen on the Growth and Energy Metabolism of Heterotrophic Marine Bacteria. R. OHYE and K. GUNDERSEN. University of Hawaii, Honolulu.

A total of 150 strains of marine bacteria isolated under aerobic conditions from estuarine water and from surface and deep oceanic water were tested for ability to grow in media in equilibrium with atmospheres of different pO_2, viz., 0 (hydrogen gas), 0.02, 0.21 (air), and 1.0 atm. In general, a low pO_2 was tolerated better than a high pO_2 (1.0), which resulted in a substantial reduction in the growth rate in 50% of the organisms. Pure oxygen was toxic to 6% of the bacteria but stimulated the growth of another 6%; 59% of the organisms were facultative anaerobes and capable of growing in the complete absence of molecular oxygen. When the bacteria were classified according to the MOF test, 59% were again found to be facultative organisms producing acid from carbohydrate. A small fraction (5%) could be classified as microaerophiles, whereas the rest were aerobes. Acid production was predominant among the aerobes. Pigmented bacteria (yellow, orange, red, purple, green, black) did not develop pigment in the absence of oxygen. It is concluded that within the limits set by the oxygen minimum zone and oxygen supersaturated water in the marine environment, oxygen is an ecological factor of little significance for the majority of the heterotrophic bacteria.

G114. Sand Bacteria Characteristics Affecting their Selection by a Marine Worm. ROY M. JOHNSON and JOHN S. GRAY, Arizona State University, Tempe, and Wellcome Marine Laboratory, Robin Hood's Bay, England.

Bacteria isolated from beach sand at Robin Hood's Bay, Yorkshire, England, were tested for their ability to restore the attractiveness of autoclaved sand to that of the natural sand for the marine gastrotrich *Turbanella hyalina*. The strains completely restoring the attractiveness were gram-negative species of *Micrococcus*. Uniform gram-negative bacilli and pigmented cells had no effect. Of seven bacterial strains isolated from autoclaved sand exposed to natural seawater for 2 weeks, none was a *Micrococcus* sp. and only one, a *Vibrio* sp., individually restored attractiveness. Both the vibrio and the micrococcus grew in an 18% NaCl-peptone medium and were not sensitive to basic fuchsin.