

Characterization of Halobacillus Strains from a Carbonate Rock at a Deep-sea Methane Seep

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Sixteen strains of gram-positive rod-shaped moderately halophiles were obtained from a carbonate rock collected at a methane seep at 642 m deep off Ishigakijima Island, south-western Japan, and affiliated with the genus *Halobacillus* based on their 16S rRNA gene sequences closely (97-99%) similar to those of *Halobacillus. trueperi* and *H. litoralis*. Cellular fatty acid profiles of the strains cultured at 10% NaCl, and 28 degree C were clearly distinguished from those of the known species, and showed high proportions of branched acids such as anteiso 15:0 and anteiso 17:0 as well as only little unsaturated acids. In contrast, the obtained strains showed increased proportions of straight branched and unsaturated acids when grown at optimal conditions. Cardinal salinity and temperature ranges for growth of the isolates in general were 0-25% NaCl (optimal 3-12%) and 4-40 degree C (optimal 20-35 degree C), respectively. The isolated strains were characteristically able to grow at 4 degree C or under an anaerobic condition, reduce nitrate to nitrite, and utilize Tween 80 as a single carbon source; these characteristics also distinguished the isolated from *H. trueperi* and *H. litoralis*. Utilization of 101 substrates as single carbon sources was tested using the Biolog microplates at 3% and 10% NaCl, and resulted generally in the utilization of larger numbers of substrates with higher intensity at the higher salinity. Tween 40, Tween 80 and i-erythritol were commonly utilized at both salinities. A view of similar genotypes but distinct phenotypes of the strains is discussed in relevance to adaptation to deep-sea methane seep conditions, particularly high ionic strength in the carbonate rock-forming condition.