南極淡水湖からの低温活性プロテアーゼ産生菌の分離と同定

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Proteolytic bacteria isolated from freshwater lakes in Antarctica

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Proteases are ubiquitous enzymes that hydrolyze peptide bonds of other proteins. These enzymes have been used for various industrial applications. Cold-active protease may have potentials for food processing or organic wastewater treatment at low temperature. In this paper, we report the isolation and identification of bacteria that secret cold-active protease from freshwater lakes in Antarctica.

The water samples including surface sediments were collected from three lakes: Yukidori-Ike, Hotoke-Ike, and Skallen-Oike during December 2012 through January 2013 in the 54th Japanese Antarctic Research Expedition. The water temperatures were 5.4-6.1°C and pH at around 7.5. The 0.1 mL of the water samples were spread onto LB- or MBS-agar plates supplemented with 30 g/L skim milk and incubated at 4°C. The colonies appeared with clear zones, indicating proteolytic activity, were selected and purified by repeated single colony isolation. The purified strains were identified by partial nucleotide sequences of 16S rDNA. To evaluate proteolytic activity of the isolates, the 1 μL of culture of each strain was inoculated onto MBS-agar-skim milk plates and incubated at 4 and 25°C. The proteolytic activity of each strain was evaluated based on the ratio of the diameter of colony to the radius of clear zone.

Total 63 strains were isolated as cold-active protease producing bacteria, and they were grouped into genera *Flavobacterium* (28 isolates), *Pseudomonas* (14 isolates), *Arthrobacter* (10 isolates), *Psychrobacter* (7 isolates), *Cryobacterium* (2 isolates), *Hymenobacter* (1 isolate) and *Polaromonas* (1 isolate) based on the partial 16S rDNA sequences. Among these isolates, strains ANS4-1, ANS4-2, ANH4-1 and ANH4-27 showed the highest proteolytic activity at 4°C, and they were close relatives of *Pseudomonas prosekii* AN/28/1^T, *Pseudomonas frederiksbergensis* JAJ28^T, *Psychrobacter cryohalolentis* K5^T and *Cryobacterium psychrophilum* DSM 4854^T, respectively. Strain ANS4-1, ANS4-2 and ANH4-1 were also able to grow and show proteolytic activity at 25°C. In contrast, strain ANH4-27 was not able to grow at 25°C indicating that ANH4-27 was proteolytic psychrophile.

These results expanded our knowledge about microbial diversity in Antarctic fresh water lakes and provide additional sources for cold-active proteases. Further study is needed in order to reveal the detailed characteristics of the isolates and their proteases.