

南極淡水湖由来のタンパク質分解菌とそれらが産生する低温活性プロテアーゼの解析

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Proteolytic microbes in Antarctic freshwater lakes and their cold active proteases

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Antarctica is one of the extreme environments for creatures. However, in the Antarctic coastal freshwater lakes, the water temperature increases to 5-10°C in summer, and the biological activity is accelerated. In this environment, cold active protease-producing microbes as decomposer may play a large role to hydrolyze protein produced by a kinds of protists. In this paper, we report the results of cultivation of proteolytic microbes derived from three Antarctic coastal freshwater lakes.

The water samples including surface sediments were collected from Lake 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 MBSY-agar plates supplemented with 30 g/L skim milk and incubated at 4°C. The colonies appeared with clear zones, indicating protease activity, were purified by single colony isolation. The purified strains were identified by the partial SSU-rDNA sequences. The crude enzymes were recovered from pure cultures of 19 representative strains, and their specific activities and sensitivity to the several protease inhibitors were examined.

Total 71 strains were isolated as cold-active protease-producing microbes, and they were grouped into bacteria (63 isolates) and eukaryote (8 isolates). Bacterial isolates were classified in genera *Flavobacterium* (28 isolates), *Pseudomonas* (14 isolates), *Arthrobacter* (10 isolates), *Psychrobacter* (7 isolates), *Cryobacterium* (2 isolates), *Hymenobacter* (1 isolate) and *Polaromonas* (1 isolate). Some of the bacterial isolates assumed to be novel species. All eukaryotic isolates were identified as yeast *Leucosporidium antarcticum*. A half of the representative strains were psychrophilic and did not grow above 25°C. Protease secreted by *Pseudomonas prosekii* strain ANS4-1 showed the highest activity among all proteases of the representative isolates. From the results of inhibitor tests, nearly all of the isolates secreted metalloproteases. These results expanded our knowledge about microbial protein degradation in Antarctic freshwater lakes.