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Elucidation of Halotolerant Mechanism and Improvement of Halotolerancy of Haloarchaeal Chitinase

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Summary

Chitin is a polysaccharide that made up of *N*-acetylglucosamine by β-1,4-linkages. Chitinase (EC 3.2.1.14) is an enzyme that can degrade chitin into small molecules. Chitinases from halophilic archaea have not been reported to date. Recently, the complete genome sequence of extremely halophilic archaeon *Halobacterium* sp. NRC-1 was reported, and a chitinase-homolog (named ChiN1) was found in the genome. The deduced amino acid sequence revealed that the mature ChiN1 was composed of three domains: a chitin-binding domain of carbohydrate-binding module (CBM) family 5, a functionally-unknown polycystic kidney disease domain and a catalytic domain of glycoside hydrolase (GH) family 18. *Haloarcula japonica* TR-1 is also an extremely halophilic archaeon and has a glycoprotein (CSG) on its cell surface. Because a large amount of CSG is produced by *Ha. japonica*, the promoter of *csg* gene is expected to be powerful. We have cloned the gene encoding ChiN1 from strain NRC-1, and *chiN1* gene has been successfully expressed in *Ha. japonica* by using the *csg* promoter. In this study, we have performed production and characterization of recombinant ChiN1 (wild-type) and some mutant enzymes.

Ha. japonica cells carrying *chiN1* gene were cultured and the extracellular fraction containing recombinant ChiN1 was used as a crude enzyme preparation. Optimal pH and temperature of ChiN1 are pH 4.5 and 55˚C, respectively. ChiN1 was most active at 1.0 M NaCl and stable over a wide range of NaCl concentration from 1.0 to 4.5 M. ChiN1 also showed transglycosylation activity, and the activity proved to improve in the presence of 40% DMSO.

ChiN1 contains a higher number of acidic amino acids than non-halophilic chitinases. Based on a 3D-structure model of ChiN1, these amino acids seemed to locate outside of the protein surface. Hence, ChiN1 could catch many water molecules to form a water shell and might be protected from salting-out. Since acidic amino acids are very important for the halotolerancy of ChiN1, we tried to introduce additional acidic amino acids on the surface of ChiN1 to improve halotolerancy. Mutants N239D (Asn239 was replaced by Asp) and Q242E (Gln242 was replaced by Glu) were expressed in *Ha. japonica*. Characterization of the mutants revealed that halotolerancy could be improved by introducing an acidic amino acid on the surface of ChiN1.