

## Salt Removal from Saline Soil by Halophilic Microbes

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### Summary

Currently, several million hectares of "salt-accumulated soils" (i.e., soils with salt accumulation on the soil surface) occur every year worldwide due to climate change and inappropriate irrigation. The large amount of salt (especially sodium ions) present in these saline soils causes plant growth problems, leading to desertification. This is a major problem on a global scale. Removal of this salt is based on washing away the salt with sufficient fresh water, which requires repetitive flooding and draining. In recent years, phytoremediation has also been practiced, in which salt-tolerant plants are used to absorb and remove salt from the soil. However, both fresh water washing and phytoremediation are said to require a huge amount of money and time to restore the soil. Therefore, the objective of this study was to remove salt from salt-accumulated soils using the extremely halophilic archaea.

In this study, we used a protein produced by the extremely halophilic archaeon as a sodium ion-binding protein for salt removal from salt-accumulated soils. Halophilic chitinase produced by halophilic archaea and mutant enzymes produced by introducing various mutations were prepared as sodium ion-binding proteins and used for salt removal. The chitinase found in the genome of the extremely halophilic archaeon *Halobacterium salinarum* has a large amount of acidic amino acids on its molecular surface, which are thought to bind sodium ions. Therefore, we hypothesized that more sodium ions would bind to the chitinase by increasing the amount of acidic amino acids on the surface of the molecule. First, recombinant chitinase was obtained and its properties were investigated. As a result, it was found that the chitinase is the halophilic enzyme that is active even at high salt concentrations. Furthermore, a molecular model of halophilic chitinase was constructed, and it was found that a large amount of acidic amino acids existed on the molecular surface of the halophilic chitinase. Therefore, it was thought that by further increasing the amount of acidic amino acids, a mutant halophilic chitinase with higher activity under high salt concentration and improved sodium ion-binding capacity could be obtained. We prepared a mutant chitinase with acidic amino acids on the surface of the molecule and investigated its properties. As a result, it was found that the mutant enzyme with improved salt tolerance was obtained. In the future, we will evaluate the sodium ion-binding ability of the mutant chitinase under high salt concentrations and analyze the effect of salt removal.