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## Bioremediation of coast environment using halotolerant microorganism and clarification of mechanism for halotolerant

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### Summary

The pollution of marine environment and marine resources caused by toxic substances poses a grave problem, and there is a pressing need to remediate the heavily polluted environment as soon as possible. One of the methods to this end is bioremediation, which utilizes the activity of microorganisms as a remediation technique. Although this method has been applied to the treatment of oil spills and the remediation of dioxin-contaminated soil, technical issues remain regarding its use in treating combined pollution, that is, although its effect on specific toxic substances has been observed, no such effect has been observed on most systems containing multiple toxic substances. Because our environment is exposed to combined pollution, in this study, rather than focusing on individual pollutants as targets, we assume the case of combined pollution caused by various toxic substances and focus on lignin-degrading enzymes that have wide substrate specificity and high redox potential, and can degrade various toxic substances. The development of bioremediation technology that utilizes halotolerant microorganisms is desired because of the high concentration of salt in industrial wastewater and the marine environment. With these as the background, our objectives are to use halotolerant lignin-degrading microorganisms to treat the marine environment, specifically coastal waters, and to clarify the mechanism underlying the halotolerance of such microorganisms.

We report herein that SN-3 (Patent application 2005-068945), a halotolerant lignin-degrading fungus isolated from the sea coast, can significantly degrade toxic substances causing combined pollution in the marine environment, including tributyltin (TBT) and triphenyltin (TPT), which are endocrine-disrupting chemicals, and various dyes that inhibit algal photosynthesis. Moreover, SN-3 was identified as a filamentous fungus of genus *Pestalotiopsis* that grew well under such conditions as 0-12% NaCl (W/V), pH 2-11, and 4-37°C. Of the known lignin-degrading enzymes, SN-3 produces only laccase (EC 1.10.3.2), which has activity of 335 U/L under optimal conditions. Compared with the activity of the enzyme produced by the representative lignin-degrading fungus *Phanerochaete chrysosporium*, SN-3 laccase showed high activity within a short period; thus, SN-3 has high potential for use in coastal bioremediation. In addition, when laccase produced by SN-3 was compared with two commercially available preparations (originating from *Rhus vernificera* and *Trametes versicolor*, respectively), it was found that laccase showed much higher halotolerance and enzymatic activity than the two preparations in a high salinity environment. As far as we know, this is the first report of the halotolerance characteristic of laccase. Compared with hitherto reported treatments with manganese peroxidase and peroxidase, laccase treatment does not require hydrogen peroxide or manganese sulfate; thus, it is a favorable method for use in the environment.