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Acquisition of Novel Glycoside Hydrolyase from Marine Using eDNA-PCR

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Summary

The aim of this study was to identify novel glycoside hydrolyase genes from mixed genome DNA of seawater samples without bacterial cultivation. I attempted to obtain directly novel chitinase genes from mixed genome DNA of seawater, including bacteria. A short region (about 280-bp) of family-18 chitinase genes was amplified by PCR from the seawater sample with two degenerated primers ChiF1 and ChiR1, cloned into the plasmid vector, and introduced into *E. coli*. A total of 85 colonies in two samples from seawater (T30 and OKI) were subjected to sequence analysis with subsequent on line homology searches using databases. Fourteen different family 18 chitinase genes were recovered from these clones. These chitinases exhibited low sequence similarity (less than 88%) with the other known chitinase, were thought to be novel chitinase. Out of 85 clones, 61 had same chitinase genes. These chitinase (acids deduced from about 280-bp) exhibited amino acid sequence similarity to *Bacillus circulans* chitinase A1 with sequence identity of about 52% and were not detected in other seawater samples. The same samples (T30 and OKI) were characterized by the 16S rRNA gene libraries and terminal restriction fragment length polymorphism (T-RFLP) analysis. A large number of species that are not yet known exist in the T30 and OKI samples. The 16S rRNA gene libraries and T-RFLP included the "*Gammaproteobacteria*", "*Alphaproteobacteria*", *Bacteroidetes*, *Flavobacteriaceae*, *Verrucomicrobia*, *Actinobacteria*, *Roseobacter* sp., *Synechococcus* sp.. Bacteria, which related to a lot of detected chitinase (61 clones) were not detected from 16S rRNA gene libraries and T-RFLP.