

Chitinase from an Antarctic Bacterium, *Sanguibacter* sp. KCTC10714

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Abstract

A chitinase-producing bacterium strain KCTC10714 was isolated from sea sand around the King Sejong Station, King George Island in Antarctica. It was identified as *Sanguibacter* sp., based on the biochemical properties and 16S rRNA gene sequence. KCTC10714 chitinase showed enzyme activity in broad range of temperature from 0 to 70°C. At 0°C, it showed 70.9% of relative activity in comparison with 100%. The chitinase gene of KCTC10714 was cloned using inverse PCR cloning method. KCTC10714 chitinase gene was designated as *chi21702*. The ORF of *chi21702* consisted of 1,449 bp (482 amino acid), and contained ChtBD3 (a chitin/cellulose binding domain) and an active site for chitinase family 18.

Introduction

Chitin is a polymer of β -(1-4)-linked *N*-acetylglucosamine (GlcNAc) and a very abundant polymer next to cellulose in nature and represents an important potential source of renewable biomass. Chitin is degraded by chitinase (EC 3.2.1.14) and β -*N*-acetyl-glucosaminidase (EC 3.2.1.30). Chitinase hydrolyze the β -1,4-linkages in chitin, yielding predominantly *N*-*N'*-diacetyl chitobiose, which in further degraded by chitobioses to the GlcNAc monomer. Chitinases are a diverse family of enzymes found in a wide variety of higher plants, in vertebrates, in fungi, and in bacteria. Marine bacteria are excellent sources of chitinase. In this study, a chitinase-producing bacterium strain KCTC10714 and the chitinase gene of KCTC10714 were reported.

Results

Identification of the isolate

From the sequence similarity analysis of 16S rDNA sequences, it was revealed that the closest relative of the strain KOPRI 21702 was *Sanguibacter inulinus* with 96.8% sequence similarity. KOPRI 21702 was included in the monophyletic lineage comprising all of the species of *Sanguibacter*, but it formed

a distinct lineage (Fig. 1). Considering sequence similarity lower than the guide line of distinct species (97%) and distinct phylogenetic lineage, it was concluded that the strain KOPRI 21702 was identified as *Sanguibacter* sp.

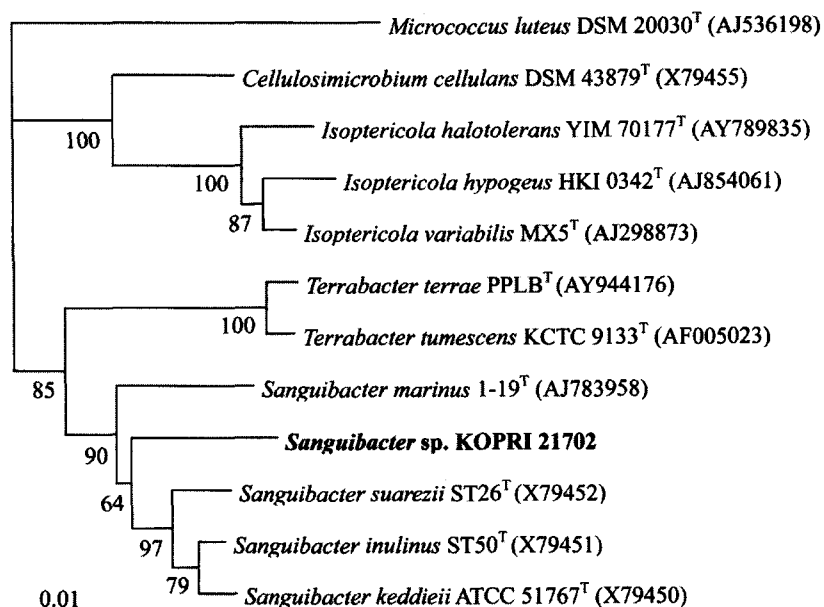


Fig. 1. Phylogenetic relationship of KOPRI 21702 and related species reconstructed by Neighbor-joining method. The numbers given under branches are the frequencies (>50%) with which a given branch appeared in 1,000 bootstrap replications.

Chitinase activity and chitinase gene

KCTC10714 chitinase showed enzyme activity in broad range of temperature from 0 to 70°C. At 0°C, it showed 70.9% of relative activity in comparison with 100% (Fig. 2). We cloned the chitinase gene from KCTC10714 using inverse PCR cloning method. KCTC10714 chitinase gene was 1,888 bp in length including 388 bp of 5'-UTR and 51 bp of 3'-UTR, and it was designated as *chi21702*. The ORF

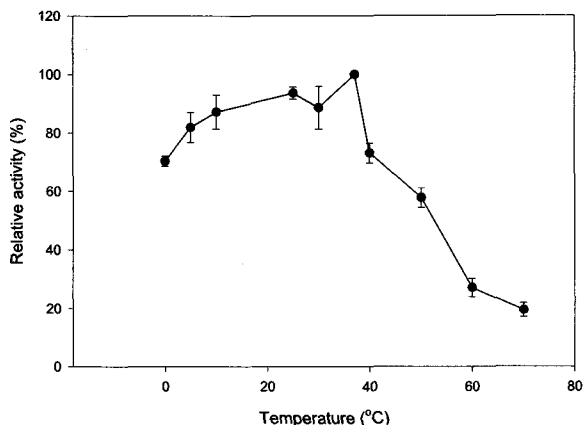


Fig. 2. Effect of temperature on the chitinase activity from KOPRI 21702.

of *chi21702* consisted of 1,449 bp (482 amino acid), and contained ChtBD3 (a chitin/cellulose binding domain) and an active site for chitinase family 18 (Fig. 3).

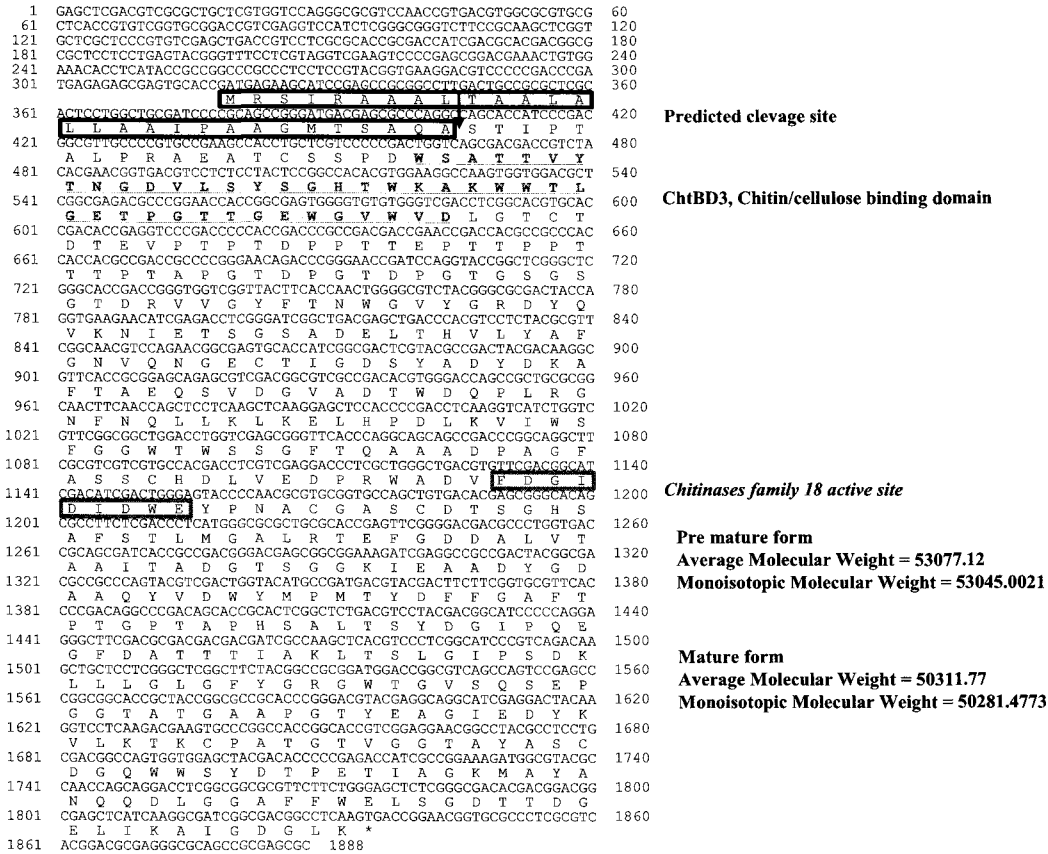


Fig. 3. Complete nucleotide sequence and deduced amino acid residues of *Sanguibacter* sp. chitinase gene.