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Original article

Molecular cloning of novel chitinase genes from two species of crustaceans, red king crab *Paralithodes camtschaticus* and snow crab *Chionoecetes opilio*

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Introduction

Chitin, a β-1,4 linked straight-chain polymer of *N*-acetyl-D-glucosamine, the main component of exoskeleton of crustaceans, epidermis of insects, cuttlebone of molluscans and cell walls of fungi [1]. It is the second most abundant organic compound after cellulose. More than 80,000 metric tons of chitin is obtained per year from the marine waste [2]. Chitin oligosaccharide, the degradation products of chitin, is found to have various physiological function, immunopotentiative action and antitumor activity [3].

Chitinases (EC 3.2.1.14) are enzymes that randomly hydrolyze the β-1,4 glycosidic bonds of chitin and widely distributed in animals, plants, fungi, bacteria and viruses [4-6]. In crustaceans, chitinases play important physiological roles such as digestion of diets, ecdysis and protection against viral pathogens in vivo [7,8]. Although several studies have been investigated for the chitinase genes from some crabs and shrimps [6-10], there have been no reports on chitinase genes from hermit crab. In this study, therefore we tried the cloning of chitinase cDNA from the midgut gland of a hermit crab *Paralithodes camtschaticus* and a crab *Chionoecetes opilio*.

Materials and methods

Materials
Paralithodes camtschaticus and *C. opilio* were purchased from Shinpu Co., Ltd. (Japan). Samples were carried with living and kept at -80°C.

cDNA Cloning
Total RNA was extracted from midgut gland of the crabs using ISOGEN II (Nippon Gene, Tokyo). First strand cDNA was synthesized using 1.0 μg total RNA and oligo dT primers with PrimeScript™ Reverse Transcriptase (Takara Bio, Shiga, Japan). Internal sequences of chitinase genes from the crabs were amplified using the synthesized cDNA, Go Taq® Green Master Mix (Promega, Madison, WI), and degenerate primers which were designed based on the conserved region of amino sequences of GH family 18 chitinases from several species.

Phylogenetic Analysis
To classify the chitinases from the midgut gland of *P. camtschaticus* and *C. opilio* among crustaceans chitinases, we constructed a phylogenetic tree based on the sequences of enzyme precursors by the neighbor-joining method using the ClustalW program (http:// www.genome.jp/tools-bin/clustalw). A *Serratia marcescens* chitinase (GenBank: ADX33318.1) was used as an outgroup.

Results

cDNA Cloning
The chitinase gene fragments of approximately 480 bp were obtained after amplification from midgut gland of *P. camtschaticus* and *C. opilio*. The deduced amino acid sequences of the chitinase cDNA from the midgut gland of *P. camtschaticus* showed the similarity of 73% with the chitinase of *Eriocheir sinensis* (AKP18002.1). Similarly, those of *C. opilio* showed the similarity of 85% with the chitinase of *Portunus trituberculatus* (BAP28983.1). The deduced amino acid sequences of midgut gland of *P. camtschaticus* and *C. opilio* showed 69% of agreement.

Phylogenetic Analysis
The phylogenetic analysis revealed that chitinase of midgut gland of *C. opilio* belong to the group of crab chitinase in a group of crustacea chitinase and chitinase of midgut gland of *P. camtschaticus* didn't belong with the group of crab chitinase and shrimp chitinase (Fig. 1).

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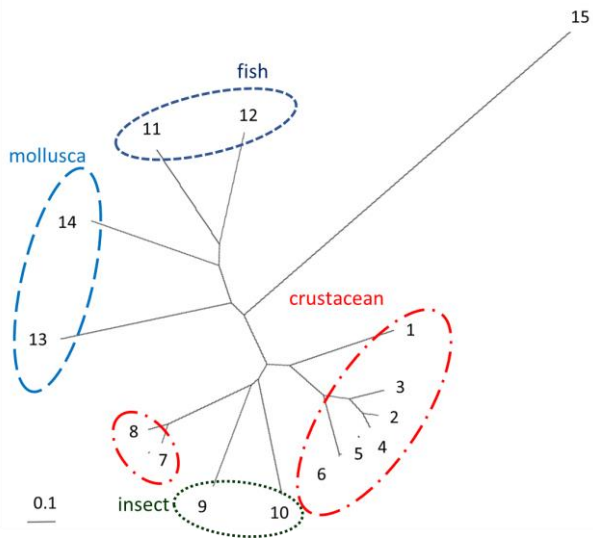

Fig. 1. Phylogenetic tree analysis of chitinase amino acid sequences by the neighbor-joining method using ClustalW. A bacterial chitinase, *Serratia marcescens* chitinase, was used as an outgroup. The names of species were as follows: 1. *Paralithodes camtschaticus*, 2. *Chionoecetes opilio*, 3. *Eriocheir sinensis* (AKP18002.1), 4. *Portunus trituberculatus* (BAP28983.1), 5. *Penaeus monodon* (ADG22163.1), 6. *Fenneropenaeus chinensis* (AAY44300.1), 7. *Daphnia pulex* (AFI40022.1), 8. *Daphnia magna* (KZS15151.1), 9. *Araneus ventricosus* (AAN39100.1), 10. *Tetranychus urticae* (XP_015789865.1), 11. *Sebastiscus marmoratus* (BAS29953.1), 12. *Scomber japonicas* (BAL40979.1), 13. *Octopus vulgaris* (BAV31378.1), 14. *Aplysia californica* (XP_012936350.1), 15. *Serratia marcescens* (ADX33318.1)

Discussion

The deduced amino acid sequences of the chitinase cDNA from midgut gland of *P. camtschaticus* showed the similarity of 73% with amino acid from 136 to 306 of chitinase 3 of *E. sinensis*. Chitinase 3 of *E. sinensis* was expressed in midgut gland, and it is reported that the enzyme participate in the digestion of diets [10]. Therefore, it is thought that the chitinase from midgut gland of *P. camtschaticus* participates in digestion. On the other hand, those of *C. opilio* showed the similarity of 85% with amino acid from 142 to 368 of chitinase of *P. trituberculatus*. Chitinase of *P. trituberculatus* was distributed in a wide variety of organs and expected to participate in the digestion and ecdysis [11]. Therefore, it is thought that the chitinase from midgut gland of *C. opilio* has a similar role. The phylogenetic analysis revealed that *P. camtschaticus* chitinase didn't belong with the group of crab chitinase and shrimp chitinase. It might be possible that hermit crab chitinase is constituted a new group in that of crustaceans chitinase.

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