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Gene structure of goose-type lysozyme in the mandarin fish *Siniperca chuatsi* with analysis on the lytic activity of its recombinant in *Escherichia coli*

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Abstract

A goose-type lysozyme (*g*-lysozyme) gene has been cloned from the mandarin fish (*Siniperca chuatsi*), with its recombinant protein expressed in *Escherichia coli*. From the first transcription initiation site, the mandarin fish *g*-lysozyme gene extends 1307 nucleotides to the end of the 3′ untranslated region, and it contains 5 exons and 4 introns. The open reading frame of the *g*-lysozyme transcript has 582 nucleotides which encode a 194 amino acid peptide. The 5′ flanking region of mandarin fish *g*-lysozyme gene shows several common transcriptional factor binding sites when compared with that from Japanese flounder (*Paralichthys olivaceus*). The recombinant mandarin fish *g*-lysozyme was expressed in *E. coli* by using pET-32a vector, and the purified recombinant *g*-lysozyme shows lytic activity against *Micrococcus lysodeikticus*.

Keywords: *g*-type lysozyme; Gene organization; Promoter; Recombinant protein; Mandarin fish; Chinese perch; *Siniperca chuatsi*

1. Introduction

Lysozyme is an enzyme which can hydrolyze 1, 4-beta-linkages between *N*-acetyl-*D*-glucosamine and *N*-acetylmuramic acid in peptidoglycan heteropolymers of prokaryotic cell walls, leading to breakdown of bacteria (Jollés and Jollés, 1984). The research on lysozymes has long been an interest, and the first report on lysozymes appeared as early as in 1960s (Canfield and McMurry, 1967). Two different lysozymes have been found in vertebrates, i.e. chicken (*c*-) and goose (*g*-) types, which differ in molecular weight, amino acid composition and enzymatic properties (Prager and Jollès, 1996; Irwin and Gong, 2003). Goose-type lysozyme (*g*-lysozyme) was initially identified as an anti-bacterial enzyme in egg whites of several avian species, and in recent years genes of its homologues have been sequenced in mammals and fish (Irwin and Gong, 2003). In fish, the cDNA of *c*-type lysozyme has been reported from Japanese flounder *Paralichthys olivaceus* (Hikima et al.,

Abbreviations: ORF, open reading frame; nt, nucleotides; aa, amino acid(s); UTR, untranslated region; RT-PCR, reverse transcription-PCR.

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2000), turbot *Scophthalmus maximus*, rainbow trout *Oncorhynchus mykiss* (Dautigny et al., 1991), zebrafish *Danio rerio* and common carp *Cyprinus carpio*, and the *g*-type lysozyme in Japanese flounder (Hikima et al., 2001), common carp and orange-spotted grouper *Epinephelus coioides* (Yin et al., 2003). But, the gene structure of *g*-lysozyme in fish has only been just discussed in Japanese flounder, and much more information is needed to understand the existence of such lysozyme in fish.

In China, the mandarin fish or the so-called Chinese perch, *Siniperca chuatsi* (Basilewsky) (Perciformes) has a relatively high market value, and is widely cultured throughout the country with importance also in stocking fishery in lakes and reservoirs (Liu et al., 1998). However, outbreaks of diseases caused by parasites, bacteria and viruses have caused severe economic losses to the aquaculture industry, and in some cases the mortality can reach as high as 100% (He et al., 2002). Despite the economical importance of the fish and severe economic losses caused by diseases, little research has been carried out on the mandarin fish immune factors. The only reported literature has been related to genes of immunoglobulin (Ig) and a virus induced protein (Zhang et al., 2003; Sun and Nie, 2004). The present study was designed to identify the *g*-lysozyme gene of the mandarin fish, and the activity of its recombinant protein in *E. coli*.

2. Materials and methods

2.1. Cloning of g-type lysozyme cDNA by RACE-PCR

Degenerate primers were designed from a conserved region obtained by comparing all known *g*-type lysozyme sequences, and all primers used in this paper are listed in Table 1. Two mandarin fish, weighing about 200 g each were injected with 400 μg LPS (Sigma, USA), respectively, and 3 days later the RNA was isolated using Trizol reagent (Invitrogen, USA) from the head kidney and reverse transcribed into cDNA by Powerscript II reverse transcriptase with CDS primer (SMART RACE cDNA Amplification kit, Clontech, USA). The PCR cycling conditions were 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, and then a final elongation step at 72 °C for 5 min. PCR products were cloned into pGEM-T vector (Promega, USA) and sequenced.

To recover the full-length cDNA sequence, 3′ RACE and 5′ RACE were performed by using the gene specific primers and adaptor primers. The PCR cycling conditions were 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 1 min, and then a final elongation step at 72 °C for 5 min. The BLAST program from the National Center for Biotechnology Information was used to identify similar sequences. The multiple sequence alignments were performed using the CLUSTAL W 1.8 program, and the putative signal peptide was analyzed using the SignalIP software (Nielsen et al., 1997).

2.2. Cloning of g-type lysozyme genomic sequence and 5′ flanking region

The 5′ flanking region was obtained using a genome walking approach, by constructing genomic libraries with a Universal Genome Walker™ kit (Clontech). The sequence of the 5′ flanking region was analyzed by TRANSFAC software for potential transcriptional factor binding sites (Wingender et al., 2000).

2.3. Production and purification of mandarin fish recombinant g-lysozyme in E. coli

The PCR amplified *g*-type lysozyme gene fragment encoding the open reading frame was digested with

Table 1
Primers used for the mandarin fish *g*-lysozyme cloning and expression

Name	Sequence (5′–3′)	Application
LF	GCATCACACA(CA)(CA)ATGGCA	Conserved region cloning
LR	TTTGTACCACTGAGCTCTGGC	Conserved region cloning
SclysF	GGAGGTACCATGGGTTATGGAAACATC	Expression in *E. coli*
SclysR	CCAGATATCTTAAAAGCCTTCGTTG	Expression in *E. coli*
L51	CCCTGGACTCTCTGGAGATGATGG	cDNA 5′ RACE, 1st round
L52	TGATGGCAGCGATTAGAGCTGGA	cDNA 5′ RACE, 2nd round
L31	TCCTGGCTGGAGCACGGAGCAGC	cDNA 3′ RACE, 1st round and genomic walking
L32	AGCAGCAGCTGAAAGGAGGGATAG	cDNA 3′ RACE, 2nd round and genomic walking
DNAF	CGTTGCTGCTCTCGCATTCCAG	Genomic DNA cloning
DNAD	GGATTTGCTTTGCATTATGTTT	Genomic DNA cloning