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Cloning, expression and antimicrobial activity of an antimicrobial peptide, epinecidin-1, from the orange-spotted grouper, *Epinephelus coioides*

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Abstract

Outbreaks of infectious diseases have caused huge losses in the fish culture industry. The production of antimicrobial peptides has been identified as a major defense mechanism against infections. A cDNA encoding an antimicrobial peptide was isolated from the leukocyte cDNA library of orange-spotted grouper, *Epinephelus coioides*. The predicted 67-amino acid prepropeptide, named epinecidin-1, consists of three domains: a signal peptide of 22 amino acids, a mature peptide of 25 amino acids, and a carboxy-terminal prodomain of 20 amino acids. The epinecidin-1 gene consisted of three introns and four exons. A TATA box and several consensus-binding motifs for transcription factors were found in the proximal region 5′ to the transcription initiation site. A synthetic, amidated mature peptide of epinecidin-1 exhibited high antimicrobial activity against *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Pasturella multocida*, *Morganella morganii*, *Aeromonas sobrio*, *Aeromonas hydrophila*, *Flavobacterium meningosepticum* and *Escherichia coli* DH5α (minimal bactericidal concentration (MBC) < 5 μM). Most of these bacteria are known pathogens in aquaculture. Some fungi, such as *Candida albicans* and *Microsporosis sanis*, were also sensitive to this synthetic peptide (MBC < 20 μM). In conclusion, epinecidin-1 may be effective in the treatment or prevention of bacterial infections in aquaculture, and it may be a candidate gene for the generation of transgenic fish to improve disease resistance in aquaculture.

Keywords: Antimicrobial peptide; Orange-spotted grouper; Gene organization; Minimal bactericidal concentration

1. Introduction

Fish culture is a world economic activity especially important for tropical and sub-tropical countries. The recent improvement of fish culture using intensive culture techniques has increased the production of fish from aquaculture. However, the intensification of fish farming has also been accompanied by the outbreak of many infectious diseases in fish farms. Most of these diseases are caused by pathogens of bacterial and viral origin. The outbreak of infectious diseases has caused huge losses in fish production in the last few years. To prevent the outbreak of these diseases, large amounts of antibiotics have been used in the fish farms (Lalumera et al., 2004). However, bacterial resistance to conventional antibiotics is becoming more

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prevalent. Therefore, there have been many recent attempts to find effective replacements for antibiotic use. The production of antimicrobial peptides (AMPs) has been identified as a major defense mechanism against infections in lower organisms as well as an important component of the innate immune response of mammals, including humans. In the last decade, many species-specific AMPs have been isolated from fish, and some of them showed a broad spectrum of activity against Gram-positive and Gram-negative bacteria (Cole et al., 1997; Lauth et al., 2002; Patrzykat et al., 2003).

The orange-spotted grouper, *Epinephelus coioides*, is an important marine fish cultured in southern China, the production reaches 10,000 tons annually. Recently, bacteria and virus have caused high mortality (50% to 70%) in *E. coioides* cultures (Zhu et al., 2000). This paper describes the isolation of an AMP cDNA from the orange-spotted grouper, and the bactericidal activity of this peptide.

2. Materials and methods

2.1. Isolation of orange-spotted grouper antimicrobial peptide cDNA

An orange-spotted grouper leukocyte cDNA library was constructed using the SMART cDNA Library Construction Kit (Clontech, USA), and more than 300 expressed sequence tags (ESTs) were sequenced (Yin et al., 2003). The ESTs were compared with GenBank Database using the BLASTX program (www.ncbi.nlm.nih.gov), and an antimicrobial peptide cDNA was identified that we named "epinecidin-1", after the genus *Epinephelus*.

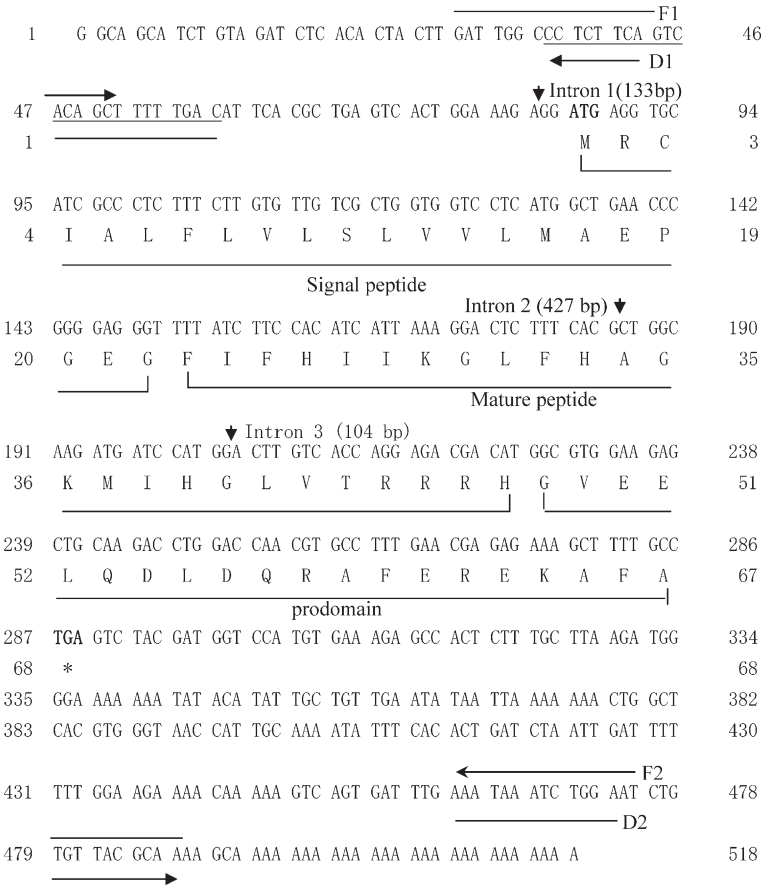

Fig. 1. Nucleotide and predicted amino acid sequence of epinecidin-1 cDNA from the orange-spotted grouper (GenBank accession no. AY294407). Amino acid sequences are shown by one capital letter below the nucleotide sequences. The organization of the peptide domains (signal peptide, mature peptide, and prodomain) is shown by the bar. Binding sites for primers are shown with arrows (5′ to 3′). Primer F1 and F2 are for the amplification of genomic DNA. Primers D1 and D2 are for reverse PCR. The start and stop codons are shown in bold letters.