Antibacterial Activity of Peptides Derived From *ParasilurusAsotus* (CatFish) Against Bacterial Pathogens

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ABSTRACT: *Marine animals may constitute a huge reservoir of efficient antimicrobial compounds and of symbiotic association with microorganisms. Anti-Microbial Peptides (AMPs) have mainly been investigated in economically important species with the praiseworthy goal of achieving better understanding of the host's natural defense.These peptides are potentially effective alternative therapeutants.In the present research one such marine peptide from Parasilurusasotus (catfish) was selected to determine its synergistic antibacterial potential against bacterial pathogens.Parasilurusasotus(catfish) were injured by scratching the skin and AMP's were extracted and subjected to silica gel column-chromatography for separation of bioactive compounds. About 10 different fractions were collected and all the fractions were subjected to antibacterial assay. The anti-bacterial activity of the AMP's obtained from Parasilurusasotus showed significant zone of inhibition against both the test organisms (Staphylococcus aureus &Escherichia coli). The maximum zone of inhibition was obtained from F5 (21mm and 19mm). It denotes that the bioactive metabolites responsible for anti-bacterial activity were present at F5. Thus, from the present study, it is confirmed that AMP's obtained from Parasilurusasotus (Catfish) possess potent antimicrobial metabolites which could be used as an alternative in treating microbial infections.*

Key Words: *Anti-Microbial Peptides, Parasilurusasotus (catfish), parasin, silica gel column-chromatography, anti-bacterial activity*

Introduction

The marine environment presents one of the greatest biodiversities on earth at both the eukaryotic and prokaryotic levels. To survive and thrive in such a medium, aquatic animals have had no alternative than to associate with beneficial micro-organisms to occupy the ecological niche and to prevent pathogenic implantation and/or to produce potent chemical weapons to fight against the pathogenic microbes. In the light of such selection pressure, marine animals may constitute a huge reservoir of efficient antimicrobial compounds and of symbiotic association with microorganisms. Investigation of marine pharmacology literature for antimicrobial compounds from 1998 to 2010 resulted in the identification of four main structural families, polyketides (27%), terpenoids (20%), peptides (16%) and alkaloids (15%) (Mayer and Hamann., 2002). To date, various antimicrobial compounds from the sea have been isolated, characterized and investigated in clinical trials (Donia and Hamann, 2003, Blunt et al. 2004).

AMPs are the main component of biochemical defense systems in invertebrates while these peptides ensure a first line of defense in vertebrates, complementing adaptative immunity (Wiesner and Vilcinskas 2010). During the past twenty years, AMPs have been described in marine invertebrates (Arthropoda, Chordata, Mollusca and Gastropoda) and vertebrates mostly in teleost fish. Marine AMPs (mAMPs) have mainly been investigated in economically important species with the praiseworthy goal of achieving better understanding of the host's natural defense. Aquacultural species have therefore been extensively studied. These peptides are potentially effective alternative therapeutants. AMP activities against viruses, bacteria, fungi and parasitic cells have been described. AMPs are more effective on enveloped viruses than nonenveloped viruses, and may act at different stages during the course of viral infection to inhibit viral replication (Daher et al. 1986). Three mechanisms have been proposed to account for antiviral action: direct inactivation of viral particles by perturbing lipid bilayers of viral envelopes (Daher et al. 1986); prevention of viral penetration into the host cell by inhibiting viral-cellular membrane fusion; and inhibition of viral replication in infected cells by suppressing viral gene expression (Wachinger et al. 1998).

Parasin is the mAMP extracted from the *Parasilurusasotus*(catfish). It is a potent 19-residue antimicrobial peptide isolated from the skin mucus of wounded catfish (*Parasilurusasotus*). Parasin I show

strong antimicrobial activity, about 12-100 times as strong as magainin 2, against a wide spectrum of microorganisms, without any hemolytic activity. Furthermore, parasin I shows good antimicrobial activity against fish-specific bacterial pathogens (Zasloff., 1987).In the present research one such marine peptide from *Parasilurusasotus* (cat fish) was selected to determine its synergistic antibacterial potential against bacterial pathogens.

Materials and methods

Procurement of *Parasilurusasotus* and microorganisms

Live *Parasilurusasotus* (cat fish) was obtained from a local fish market in Coimbatore, Tamilnadu, India (Figure 1). Microorganisms used in this study were procured from CMS College of Science and College, Tamilnadu, India.

Figure 1: Procurement of *Parasilurusasotus*(Catfish)

Extraction of Anti-microbial peptides from *Parasilurusasotus*(Park *et al., 1998*)

Catfishes were injured by scratching the skin (16 cm^2) with a sandpaper and 5 h after the wounding; the catfishes were stunned by electro-shock. The proteinaceous epithelial mucosal layer was scraped of from both the unwounded and wounded catfishes. The mucus (20 g) collected from the catfish skin was then homogenized using a Waring blender (Waring, New Hartford, CT, USA) in 200 ml of extraction medium (0.2 M sodium acetate, 0.2% Triton X-100, and 1 mMphenylmethylsulfonyl fluoride). The homogenate was centrifuged at 20 000Ug for 30 min in a Himac SCR20BR (Hitachi, Tokyo, Japan) and the supernatant was collected.

Separation of bioactive compounds using silica gel Column-chromatography method

A long cylindrical glass column (450mm X 20mm) should be stand firm on a column-chromatography stand was selected for the present research. Silica gel (60 - 120 mesh) was packed with the aid of hexane without any air bubbles. The extracts were distilled dried and finely powdered form for easy distribution of sample in already packed silica gel column. Sample powdered mass was placed on the top of the pre-packed silica column and sample was covered with a layer of cotton. Then solvents (100% hexane) were passed through column at uniform rate under gravity to fractionate the sample extract (Figure 2). Each fraction was collected separately in a test tube and numbered consecutively for further analysis and about 10 different fractions were collected (Figure 3).

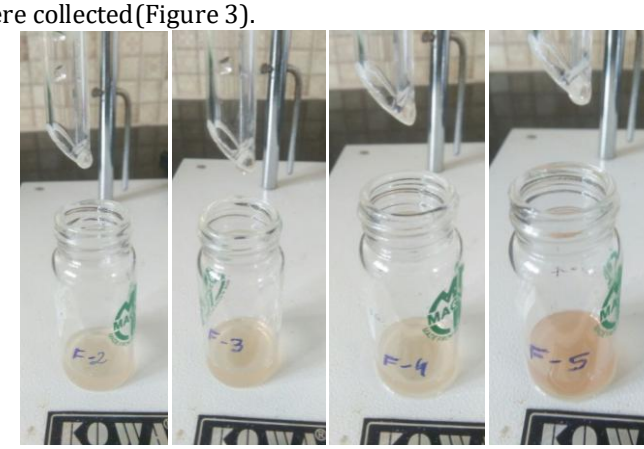


Figure 2: Purification by Ion exchange chromatography

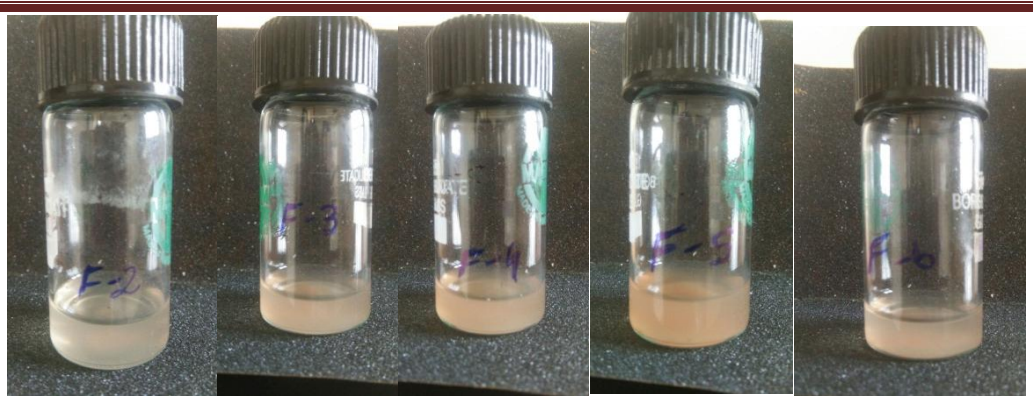


Figure 3: Collected Fractions

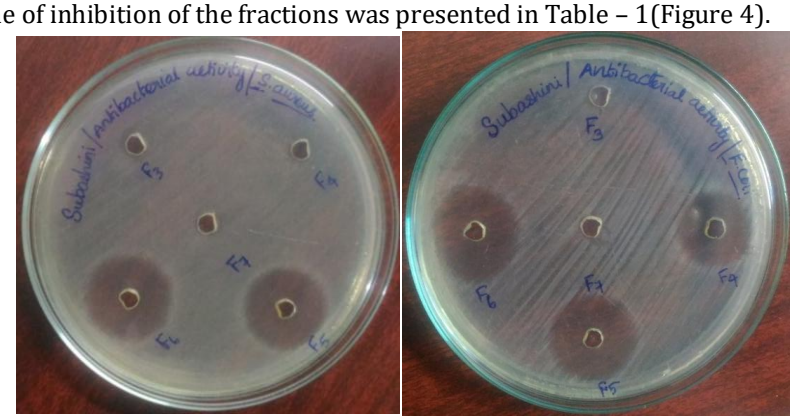
Anti-bacterial activity of the AMP's by well diffusion method

The antibacterial activity of AMP's obtained from *Parasilurusasotus* were evaluated against the test organisms by well diffusion method. Sterile Nutrient Agar (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g, Agar 15 g; Final pH (7.0 ± 0.2) plates were prepared and allowed to solidify. About 0.1% inoculum suspensions of the test organism one gram positive (*Staphylococcus aureus*) and one gram negativeand(*Escherichia coli*) were swabbed uniformly over the agar surface. Under sterile conditions, 6mm wells were cut on the agar surface of each NA plates. About 50µl each of each fractions were loaded into the well and the plates were incubated at 37ºC for 24 - 48h. The antibacterial activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimeter.

Results and Discussion

Anti-bacterial activity of the purified fractions

The anti-bacterial activity of the AMP's obtained from *Parasilurusasotus* showed significant zone of inhibition against both the test organisms (*Staphylococcus aureus* &*Escherichia coli*). No zone of inhibition was recorded in F3, F4and F7. Surprisingly inhibitory zones against *E. coli* were observed in F4 (15mm). About 21mm and 19mm of inhibition zoneswere obtained from F5. About 19mm, 16mm; and 14mm, 13mm of inhibitory zones were obtained for Fraction 6. Thus, maximum zone of inhibition was obtained from fraction 5. It denotes that the bioactive metabolites responsible for anti-bacterial activity were present at Fraction 5. The zone of inhibition of the fractions was presented in Table – 1(Figure 4).



Staphylococcus aureus *Escherichia coli*

Figure 4: Antibacterial activity

Table- 1: Zone of inhibition of the fractions obtained from *Parasilurusasotus*

S. No	Fractions	Zone of inhibition (mm)	
		Staphylococcus aureus	*Escherichia coli*
1	F3	Nil	Nil
2	F4	Nil	15
3	F5	21	19
4	F6	19	16
5	F7	Nil	Nil

Parasin I was found only in the skin mucous extracts of the injured catfish and not in the uninjured catfish. The detection of parasin I only in the wounded catfish skin indicated that injuring the catfish skin stimulated the production or secretion of parasin I into the mucous layer. When threatened or injured, catfish secretes a thick gel-like layer of proteinaceous materials, which includes antibodies and lysozyme, to its skin surface mainly from the unicellular glands of the epidermis (Al-Hassan *et al.,* 1987). It is also known that amphibians, such as *X. laevis*, *Bombina* sp., and *Phyllomedusa* sp., produce and store antimicrobial peptides in the granular glands and release their contents onto the epithelia upon adrenergic stimulation or injury. A similar secretory immune system may also be responsible for the release of the antimicrobial peptide parasin I in the catfish skin. Parasin I showed a strong antimicrobial activity towards Gram-negative bacteria, Gram-positive bacteria. The MICs of parasin I was in the range of 1-4 µg/ml. The most potent antimicrobial peptides have been reported to kill susceptible bacteria in vitro at concentrations in the range of 0.25-4 µg/ml (Hancet., 1997), which indicates that parasin I is one of the most potent antimicrobial peptides found so far. The antimicrobial property of most antimicrobial peptides is generally attributed to their amphipathic secondary structures with a net positive charge. The primary target is the plasmatic membrane. They act on the sensitive cells by disrupting the plasmatic membrane (Yeaman and Yount 2003).

Conclusion

Catfishes were injured by scratching the skin and AMP's were extracted and subjected to silica gel column-chromatography for separation of bioactive compounds. About 10 different fractions were collected and all the fractions were subjected to antibacterial assay. The anti-bacterial activity of the AMP's obtained from *Parasilurusasotus* showed significant zone of inhibition against both the test organisms (*Staphylococcus aureus* &*Escherichia coli*). The maximum zone of inhibition was obtained from F5. It denotes that the bioactive metabolites responsible for anti-bacterial activity were present at F5. Thus, from the present study, it is confirmed that AMP's obtained from *Parasilurusasotus* (Catfish) possess potent antimicrobial metabolites which could be used as an alternative in treating microbial infections.

References

1. Al-Hassan, J.M., Thomson, M., Summers, B. and Criddle, R.S. (1987) Comp. Biochem. Physiol. B 88, 813-822.
2. Blunt, J.W., B.R. Copp, M.H.G. Munro, P.T. Northcote, and M.R. Prinsep. 2004. Marine Natural Products. Nat. Prod. Rep. 21: 1–49.
3. Daher, K.A., M.E. Selsted, and R.I. Lehrer. 1986. Direct Inactivation of Viruses by Human Granulocyte Defensins. J. Virol. 60(3): 1068–1074.
4. Donia, M., and M.T. Hamann. 2003. Marine Natural Products and Their Potential Applications as Anti-infective Agents. Lancet Infect. Dis. 3(6): 338.
5. Hancock, R.E. (1997). Peptide antibiotics. Lancet 349, 418-422.
6. In Yup Park, Chan Bae Park, Mi Sun Kim, Sun Chang Kim.Parasin I, an antimicrobial peptide derived from histone H2A in the catfish, Parasilurusasotus. FEBS Letters 437 (1998) 258-262.
7. Mayer, A.M.S., and M.T. Hamann. 2002. Marine Pharmacology in 1999: Compounds with Antibacterial, Anticoagulant, Antifungal, Anthelmintic, Anti-inflammatory, Antiplatelet, Antiprotozoal and Antiviral Activities Affecting the Cardiovascular, Endocrine, Immune and Nervous Systems, and Other Miscellaneous Mechanisms of Action. Comp. Biochem. Physiol., C. 132(3): 315–339.
8. Wachinger, M., A. Kleinschmidt, D. Winder, N. von Pechmann, A. Ludvigsen, M. Neumann, R. Holle, B. Salmons, V. Erfle, and R. Brack-Werner. 1998. Antimicrobial Peptides Melittin and Cecropin Inhibit Replication of Human Immunodeficiency Virus 1 by Suppressing Viral Gene Expression. J. Gen. Virol. 79(4): 731–740.
9. Wiesner, J., and A. Vilcinskas. 2010. Antimicrobial Peptides: The Ancient Arm of the Human Immune System. Virulence. 1(2150-5594): 440–464.
10. Yeaman, M.R., and N.Y. Yount. 2003. Mechanisms of Antimicrobial Peptide Action and Resistance. Pharmacol Rev. 55(1): 27–55.
11. Zasloff, M. (1987).Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. USA 84, 5449-5453.