IN VITRO ACTIVITIES AND PROPERTIES OF SOME ANTIMICROBIAL PEPTIDES AGAINST MARINE *VIBRIO* AND OTHER MICROORGANISMS

S.E. Löfgren[1], A. Smânia Jr.[2], S.M. Souza[2], M. Steindel[2], M.C. Carriel[2], C.M.O. Simões[2], J. Fievet[3], E. Bachère[3], and M.A. Barracco[1]

[1]Dept. Cell Biology, Embryology and Genetics, Federal University of Santa Catarina (UFSC), P.O.B. 476, 88.040-900 Florianópolis, SC, Brazil. E-mail: barracco@mbox1.ufsc.br
[2]Dept. Microbiology and Parasitology, UFSC.
[3]UMR 5171, Ifremer- CNRS - Université de Montpellier2, CC 80, Place E. Bataillon, F-34095 Montpellier cedex 5, France

Introduction

Infectious disease is considered to be the most devastating problem in aquaculture. The development of alternative therapeutic agents, less ecologically harmful than usual antibiotics and that do not lead to bacteria resistance is an essential need to a sustainable Aquaculture. In the present study, we investigated the *in vitro* activities of some antimicrobial peptides (AMP) produced by different aquatic animals in order to evaluate their potential use as therapeutic agents in shrimp and mollusk farming (Bachère, 2003).

Material and Methods

The activity of the following purified AMPs was examined: penaeidin (Pen) from *Litopenaeus vannamei,* tachyplesin (Tach) from *Tachypleus tridentatus,* clavanin A (Cla) from *Styela clava* and magainin (Mag) from *Xenopus laevis.* The AMPs were assayed against marine *Vibrio* (*V. anguillarum, V. alginolyticus* and *V. harveyi),* filamentous fungi (*Microsporum canis* and *Trycophyton mentagrophites),* protozoan parasites (epimastigote forms of *Trypanosoma cruzi* and promastigote forms of *Leishmania brasiliensis*) and viruses (Herpes-virus – HSV-1- KOS, rotavirus – SA-11 and adenovirus type 5). The growth inhibition (MIC) of the bacteria and fungi was determined through optical density (OD = 630nm), after incubation with the different AMPs. The parasite viability, antiviral and cytotoxic effect (Vero, MA-104 and Hep-2) were assayed through the MTT method. In addition, the stability of the peptide activity was investigated under different saline conditions.

Results

Vibrio - Tach was the most potent peptide against standard and marine bacteria. Its activity against all tested *Vibrio* was similar (MIC=0.78μM). Mag had an activity against *V. anguillarum* and *V. alginolyticus* (MIC=12.5μM) but was less potent than Tach especially against *V. harveyi* (MIC=50μM). Cla and Pen were completely ineffective.

Fungi - Only Clav and Tach were used in the experiments, since the other AMPs were previously tested against several filamentous fungi. Clav had no effect on both fungi, whereas Tach was active against *T. mentagrophytes* (MIC=6.25μM)

Parasites – Clav was not used in the assays, since it was inactivated by fetal bovine serum, which is a necessary requirement for the parasite cultivation. The other PAM did not have any effect on both parasites after 5h incubation (lytic effect), except Tach that had an activity against *L. brasiliensis* at 100μM. After 24h (one parasite cell cycle), all AMPs had a slight effect on the viability of both parasites at high concentrations (>50μM) and Tach had a potent effect against *L. brasiliensis* (from 12.5μM). After 72h (three parasite cell cycles), Tach had a potent effect on both parasites (from 12.5μM), whereas the other AMPs maintain the same pattern as for 24h incubation.

Viruses - Among the tested viruses, only Pen exhibited a promising antiviral effect against the DNA enveloped virus, HSV-1 (CE_{50}=0.625μM; SI=64).

Cytotoxic effect on eukaryotic cells – The tested peptides did not exhibit any cytotoxic effect (up to 40μM) on the tested cells.

Stability under saline conditions - *Micrococcus luteus* and *V. anguillarum* could efficiently grow from 0-450mM NaCl and 28-450mM NaCl respectively and were thus selected for the assays. Cla, Tach and Pen augmented their activity against *M. luteus* at low salinity. Tach and Mag maintained a potent activity at high salinity, whereas Cla and Pen completely lost their effect. The latter were inactive against *V. anguillarum* under any saline concentration. Tach slightly decreased its activity (2x) at high salinity and Mag lost it completely. Differently from Tach, whose activity was rather stable at any saline concentration, Mag increased its activity against *V. anguillarum* (8x) at low salinity. Curiously, all peptides including Tach were ineffective (up to 25μM) towards *V. anguillarum* under seawater conditions.

Discussion and Conclusions

From the above results, Tach appears to be the most promising AMP for potential use in Aquaculture, since it was non-cytotoxic, very stable under saline conditions and potent against marine bacteria, filamentous fungi and protozoan parasites. In addition it is a small peptide (2.26kDa) that can be easily synthesized. Mag was also rather effective and Clav was the least active AMP. On the other hand, Pen, which is mainly active against Gram positive bacteria and fungi, exhibited an interesting antiviral effect against the enveloped virus, HSV-1. This antiviral activity is currently under confirmation.

References

Bachère, E., 2003. Anti-infectious immune effectors in marine invertebrates: potential tools for disease control in larviculture. Aquaculture 227: 427-438.

Acknowledgments

This work was supported by the European Community – INCO-DEV (IMMUNAQUA). ICA4-CT-2001-10023. http://www.ifremer.fr/Immunaqua