

## USE OF A MINIATURIZED BIOASSAY TO DETERMINE THE INNOCUITY OF ANTIMICROBIAL PEPTIDES ON LIVE FOOD IN AQUACULTURE

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### Introduction

Antimicrobial peptides, effectors of innate immunity, could be interesting molecules in the search for alternatives for antibiotics. These peptides are rather resistant to proteases, they are active against a broad range of micro-organisms and they are not toxic to eukaryotic cells (Bachère, 2003). This would make it interesting to develop such compounds as potentially new drugs for therapeutic use in aquaculture

In this research 10 antimicrobial peptides were screened for their toxicity towards live food (*Isochrysis aff galbana* and/or *Pavlova lutheri* and *Artemia* nauplii). Because only small amounts of the peptides were available, micro-assays had to be developed.

### Materials and methods

For the experiments with *Artemia*, a homogenous population of instar I and instar II-III nauplii was obtained 18h or 42 h respectively after decapsulation of EG-*Artemia* cysts following the procedure described in Sorgeloos et al. (1986) and subsequent hatching under sterile conditions. The nauplii were divided in 96-microcellular plates, at a density of 10 animals per well containing 200 µl of test volume (artificial seawater plus the peptides). The plates were incubated in the dark during 24h at a temperature of 25°C, without aeration. After 24h, the survival was recorded.

The microalgae bioassays were run in 96-microcellular plates. The wells were filled with 200 µl of phytoplankton (*Isochrysis aff galbana* or *Pavlova lutheri*), plus the peptide to be tested. The algae were cultured under controlled conditions. Optical densities (450 nm) were measured on day 0, day 4 and day 8, after homogenisation of the phytoplankton solution by shaking the microplate using an orbital shaker during 5 minutes at 1350 rpm. Regression lines between optical density and phytoplankton concentrations using Mallassez hematocytometer were previously established.

The following peptides were tested: penaeidin 3 (~~QG-man2~~ and ~~QG-manx~~), protegrin, tachyplesin, polymyxin, pyrrocorticin, MGD1-loop, protegrin loop, clavanin and magainin. The tested concentrations ranged from 0.1 to 100µM. Each concentration was tested in triplicate.

### Results and Discussion

The survival of the *Artemia* instar I nauplii was close to 100% in most of the treatments. Only protegrin at a concentration of 100µM had a negative effect on the survival of the *Artemia* nauplii, both in the instar I ( $29.7 \pm 7\%$ ) and in the instar II-III stage ( $21.1 \pm 4\%$ ). Polymyxin at a concentration of 100µM had a slightly negative influence on the survival of *Artemia* instar II-III nauplii ( $90 \pm 17\%$ ).

Table 1 shows the concentrations of the algae after 8 days of culture and exposed to different concentrations of the different peptides. Penaeidin QG-man2, Pen QG-manx and clavainin had no effect on microalgae growth. Protegrin, tachypleisin and polymyxin inhibited however growth. Pyrrhocoricin, MGD1 loop and protegrin loop might activate T. iso growth. Magainin also seemed to activate T. iso growth, but inhibited growth of *Pavlova lutheri* at a concentration higher than 50µM.

Table 1. Concentration of T. iso and of P. lutheri ( $10^3$  cells.ml<sup>-1</sup>) on day 8, exposed to different concentrations of antimicrobial peptides, mean  $\pm$  stdev

T. iso					
Peptide	100µM	10µM	1µM	0,1µM	0µM
Pen QG man2	25771 $\pm$ 4007	19739 $\pm$ 1693	19291 $\pm$ 855	18455 $\pm$ 2669	19775 $\pm$ 4608
Pen QG manx	19436 $\pm$ 4069	18527 $\pm$ 3060	20879 $\pm$ 2291	22048 $\pm$ 2068	21826 $\pm$ 4197
Protegrin	16261 $\pm$ 402	17791 $\pm$ 360	21081 $\pm$ 3050	20172 $\pm$ 4413	26285 $\pm$ 2192
Polymyxin	27820 $\pm$ 3534	27012 $\pm$ 1271	27603 $\pm$ 892	27646 $\pm$ 2451	31858 $\pm$ 1811
Pyrrhocoricin	33173 $\pm$ 15851	26781 $\pm$ 2201	27978 $\pm$ 2352	26781 $\pm$ 4501	32269 $\pm$ 1481
MGD1 (D)loop	27762 $\pm$ 527	15843 $\pm$ 4624	20994 $\pm$ 4267	22365 $\pm$ 2338	24564 $\pm$ 5384
Protegrin loop	25785 $\pm$ 10164	17430 $\pm$ 1965	22380 $\pm$ 4360	22481 $\pm$ 2516	25987 $\pm$ 3422
Clavainin	10107 $\pm$ 1911	9793 $\pm$ 1573	7734 $\pm$ 1806	10030 $\pm$ 1326	9487 $\pm$ 1316
Magainin	23248 $\pm$ 1963	12882 $\pm$ 853	10275 $\pm$ 710	10711 $\pm$ 404	9942 $\pm$ 583
Tachypleisin	3947 $\pm$ 521	12841 $\pm$ 698	10899 $\pm$ 343	10811 $\pm$ 797	8692 $\pm$ 1212
<i>Pavlova lutheri</i>					
Clavainin	38216 $\pm$ 3833	34163 $\pm$ 1608	32722 $\pm$ 1320	27814 $\pm$ 1550	36637 $\pm$ 1398
Magainin	27663 $\pm$ 4306	39699 $\pm$ 1709	36300 $\pm$ 1896	32356 $\pm$ 1701	38229 $\pm$ 1768
Tachypleisin	5842 $\pm$ 881	6520 $\pm$ 1584	33231 $\pm$ 2015	29158 $\pm$ 1769	37619 $\pm$ 924

## Conclusions

Until now, only a few examples of the use of antimicrobial peptides in aquaculture are known. The screening of antimicrobial peptides for their toxicity towards live food is an important first step in the evaluation of their potential for use as therapeutic agents in larviculture. Further study of their effect on larvae is **now undertaken in the frame of the Immunaqua project**.

## References

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