LARVI '95 - FISH & SHELLFISH LARVICULTURE SYMPOSIUM
P. Lavens, E. Jaspers, and I. Roelants (Eds)
European Aquaculture Society, Special Publication No. 24, Gent, Belgium. 1995.

BIOENCAPSULATION OF ANTIBACTERIAL DRUGS IN NAUPLII AND ADULT BRINE SHRIMP, *ARTEMIA FRANCISCANA*

B.A. Dixon[1], S.O. Van Poucke[2], D. Kawahigashi[4], M. Chair[1], M. Dehasque[1], H.J. Nelis[2], P. Sorgeloos[1], and A.P. De Leenheer[3]

[1] Laboratory of Aquaculture and Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Gent, Belgium
[2] Laboratory for Pharmaceutical Microbiology, University of Ghent, Harelbekestraat 72, B-9000 Gent, Belgium
[3] Laboratory for Medical Biochemistry, University of Ghent, Harelbekestraat 72, B-9000 Gent, Belgium
[4] San Francisco Bay Brand Inc., 8239 Enterprise Dr. Newark, CA 94560, USA

Introduction

A promising new method for the prevention and treatment of bacterial diseases in fish is the administration of live food supplemented with therapeutic agents by the technique of bioencapsulation. This study was initiated to determine if effective levels of antibacterial drugs would accumulate in brine shrimp nauplii and adults through bioencapsulation.

Materials and methods

Nauplii (*Artemia franciscana*) hatched from Great Salt Lake cysts were enriched with 0.6 $g.l^{-1}$ of the commercial live food enrichment diet Super Selco (INVE Aquaculture NV, Belgium) and concentrations of sarafloxacin hydrochloride provided by Abbott Laboratories, North Chicago, Illinois (Léger et al., 1986; 1987). Controls consisted of nauplii in the enrichment medium without sarafloxacin. At 2h intervals samples were concentrated, resuspended in 2ml of sterile saline, macerated and filter sterilized. The sterile filtrate was used in microbial assays.

Three strains of *Vibrio anguillarum* and one strain of *Vibrio vulnificus* were used in a modification of the Kirby-Bauer disk diffusion method. A colonial suspension was adjusted to match a 0.5 McFarland turbidity standard and swabbed onto a Mueller-Hinton agar plate supplemented with 2% sodium chloride. A disk containing 5mg of sarafloxacin hydrochloride (Abbott Laboratories, North Chicago, Illinois) was used

as the standard control. Blank sterile disks inoculated with 0.2ml of each sample suspension were used to determine efficacy of the test samples. Following incubation for 24h, and the zones of inhibition were measured (Dixon et al., 1995).

SXT was used in this research to determine efficacy against pathogenic strains of *Vibrio* in comparison to sarafloxacin. Nauplii were enriched in Super Selco as described. A mixture of trimethoprim (TMP): sulfamethoxazole (SMX) (1:5) was used for enrichment (Verpraet et al., 1992). All samples were handled in a manner similar to that described for sarafloxacin samples. A standard SXT disk (23.75mg sulfamethoxazole/1.25mg trimethoprim) was used as the control for sensitivity assays (Dixon et al., 1995).

Adult *Artemia* were enriched with Romet 30 and Terramycin through the manipulation of bioencapsulation parameters. For Terramycin, liposome encapsulation was accomplished by mixing the drug with an emulsified fish oil in a high speed blender. Binding agents with high capillary ratios were used to absorb the drug prior to *Artemia* feeding. Enriched adults were prepared for microbial assays as described for nauplii. Sensitivity assays were performed using strains of *Aeromonas hydrophila* and *A. sobria*.

Results and discussion

The optimum nauplii enrichment time, for efficacy in the sensitivity assay against all four strains of pathogenic *Vibrio*, was determined as 6h at a concentration of 15% sarafloxacin. At these parameters zones of inhibition were obtained that equalled or exceeded 19mm, the minimum zone size indicating sensitivity to sarafloxacin. Control samples enriched with Super Selco alone did not produce zones. Enrichment with 15% SXT was not efficacious for any of the four bacterial isolates.

Bioencapsulation of adults was achieved within 2h at a stocking density of 5000 adults.l^{-1}. Preliminary results from bacterial sensitivity testing demonstrated an increased concentration of both Romet and Terramycin using liposome encapsulation over absorption in other fibers.

Conclusions

The results of this study and others indicate that the oral delivery of antimicrobial drugs can be facilitated through bioencapsulation in brine shrimp nauplii and adults. This methodology represents a potentially useful new tool for both the prophylactic and therapeutic treatment of larval fish and shrimp. However, more research is needed to better delineate dose concentrations particularly in regard to antibiotic resistant bacteria.

Acknowledgements

The authors thank Abbott Laboratories for providing the sarafloxacin hydrochloride. The research was funded by a grant from the Belgian National Science Foundation and the European Union (FAR AQ194 GB, UK).

References

Dixon B.A., S.O. Van Poucke, M. Chair, M. Dehasque, H.J. Nelis, P. Sorgeloos, and A.P. De Leenheer. 1995. Bioencapsulation of the antibacterial drug sarafloxacin in nauplii of brine shrimp, *Artemia franciscana*. J. of Aquatic Animal Health. (in press).

Léger P., D.A. Bengtson, K.L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanography and Marine Biology Annual Review 24: 521-623.

Léger P., E. Naessens-Foucquaert, and P. Sorgeloos. 1987. International study on *Artemia*. XXXV. Techniques to manipulate the fatty acid profile in *Artemia* nauplii, and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.). p.411-424. In: *Artemia* research and its applications 3. Ecology, culturing, use in aquaculture. Sorgeloos P., D.A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 556p.

Verpraet R., M. Chair, P. Léger, H. Nelis, P. Sorgeloos, and A.. De Leenheer. 1992. Live-food mediated drug delivery as a tool for disease treatments in larviculture. The enrichment of therapeutics in rotifers and *Artemia* nauplii. Aquacultural Engineering 11:133-139.