Summary of the thesis submitted by Hilke Honnens

Development of a nematode-based product as live food for marine fish and crustacean larvae in aquaculture

Free-living nematodes have potential to be used as live food for early life stages of several species in marine aquaculture. In the present work three aspects that determine the suitability for this application have been investigated in a *Panagrolaimus* strain.

One prerequisite for commercial application is the mass production at low costs. The present study proved feasibility of propagation in monoxenic liquid culture on *Saccharomyces cerevisiae*. In shaker flasks containing pregrown yeast cells the inoculated first stage juveniles developed to adults within 4 days after a lag phase of 4 days and then started to reproduce. Yields in terms of nematode number as well as biomass were highly variable: The maximum number of nematodes varied from 45,000-238,000 ml^{-1} and maximum biomass from 49-143 g l^{-1}. These values were attained after 13-14 days. A fed-batch mode of cultivation was tested in which yeast cells were added during the cultivation process, but it did not result in higher yields than batch cultivation. Moreover nematode cultivation was also carried out in lab-scale bioreactors. A maximum nematode density of 270 x 10^3 nematodes ml^{-1} was obtained after a cultivation time of 9 days, because the nematodes started to reproduce four days earlier than in flasks culture.

A second important aspect is the nematode biochemical composition, which is not optimal for marine larvae. For example nematodes lack the fatty acid docosahexaenoic acid that is essential for most marine larvae. An investigation was carried out to incorporate this fatty acid into the nematodes. Mass-produced nematodes were cleaned and then exposed to concentrations of 0.1 – 3 % of the self-emulsifying DHA enrichment product S.presso® (INVE Aquaculture, Belgium) for 24 h at 200.000 nematodes ml^{-1}. The DHA content increased with increasing percentage of the enrichment product reaching a maximum value of 5.8 % of total fatty acids.

One further constraint to commercialisation is the lack of a method that allows storage over a longer time span. Several experiments were carried out aiming to develop a procedure to transfer nematodes into a dormant state by desiccation. The nematodes were appplied at densities of 25, 50, 100 and 200 x 10^3 individials cm^{-2} on nylon net or cellulose paper, preconditioned for 72 h at 97.3 % relative humidity (rH) and then stored at 52.9 or 32.8 % rH for one week. Storage on cellulose at a density of maximum 100 x 10^3 individials cm^{-2} resulted in optimium survival at both humidities. Over 92 % of the nematodes survived a storage period of 10 weeks at 25 x 10^3 nematodes cm^{-2} at 52.9 and 32.8 % rH. During this period changes in body size distribution were recorded at 32.8 %, but not at 52.9 % rH.

In conclusion, procedures have been developed for propagation, adjustment of biochemical composition and storage of *Panagrolaimus* strain NFS- 24-5, which are important steps for a potential use of this nematode as live food in marine aquaculture.

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