Modification of gut microflora in rainbow trout (O. mykiss) using live yeast

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Introduction

- The conditions for farming aquatic animals are changing
  - Increase of disease
  - Pressure to decrease the use of antibiotics
  - Increasing use of protein from plant origin in aquaculture feeds
- Among different solutions studied, probiotics seem to represent a viable option
  - Improvement of digestibility
  - Stimulation of immune system
  - Regulation of gut microflora (pathogens)
  - Anti-microbial properties

Yeast as a probiotic in Fish

- Most of the published studies use bacterial probiotics
- Few studies with yeast to date, some examples

Yeast	Fish species	Effect	Author
Debaryomyces Hansenii	European seabass	Gut maturation Antioxidant	Tovar-Ramirez et al
Debaryomyces Hansenii, Candida tropicalis	Indian white Prawn	Immune stimulant	Sarlin and Philip 2011
Saccharomyces cerevisiae	Rainbow trout	Gut maturation	Waché et al 2006
		Immunestimulant	Panigrahi et al
	Tilapia	Growth promoter	Lara-Flores et al 2003
	Whiteleg shrimp		Sholtz et al 1999

Live yeast (*Saccharomyces cerevisiae*)

Baker's yeast , or Instant yeast

- Dry yeast
- Small granulometry, easily dissolved
- Low stability to moisture and heat

Usage in aquaculture

- Feed for rotifers and artemia (shrimp and fish larvae)
- Sometimes used to treat ponds in shrimp farming
- Fermentation of on-farm made feeds.

Yeast for animal nutrition .

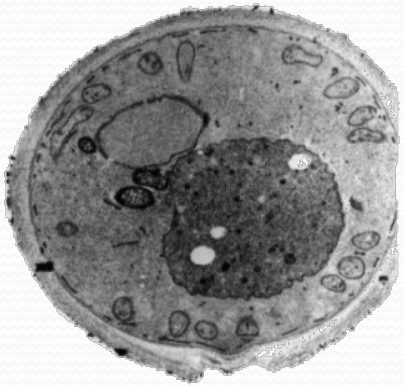
- Dry yeast
- Low porosity microgranule
- Bigger granulometry
- High stability to moisture and heat

Potential usage in aquaculture

- Probiotics
- Gut modifiers
- Regulation of gut microflora

Objectives of the study

- Feasibility study to further explore the effects of live yeast as a probiotic in fish.
- Proof of concept of live yeast as gut modifier in fish.



Material and Methods

- 4 tanks of 750 L in a flow through system (9°C) – 2 control tanks + 2 tanks for the treatment feed.
- 100 rainbow trout of 150g per tank in order to have standard farming conditions (15 kg of biomass in 750L).
- One week acclimatisation period before a 8 week feeding trial
- Feeding with Skretting LA30 3mm fish feed pellets supplemented or not with 0.1% Live yeast (Actisaf pwd, Lesaffre Feed Additives, France)
- Weigh and length measurement at day 0, and at 4 and 8 weeks
  - (100 individual fish at T0, 30 at T4, 50 at T8)
- At 8 weeks, a sample of gut tissue was sampled and stored for further bacterial analysis. (16 fish from each group)

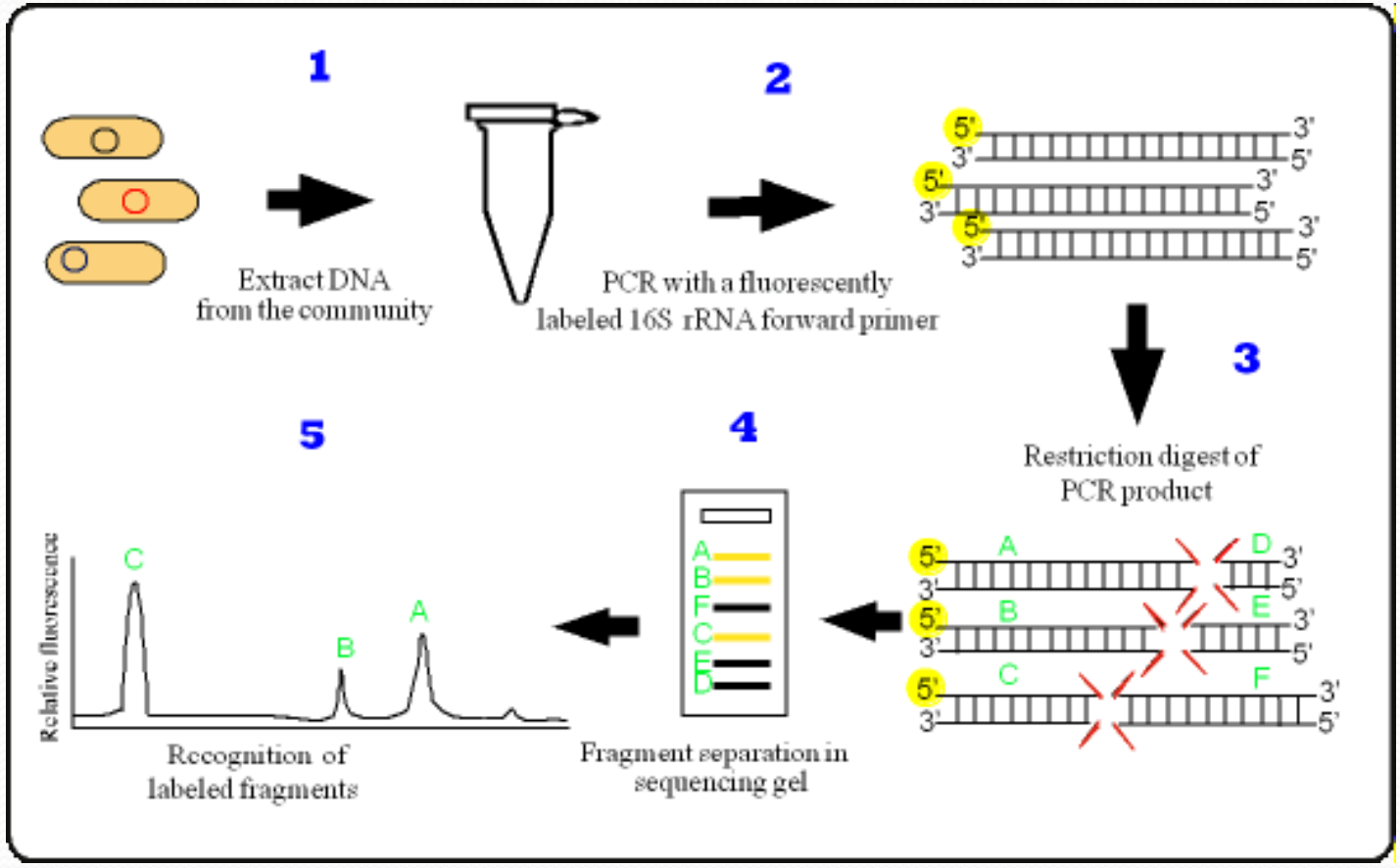
Method used for live yeast feed coating

1. Weigh out 200g of fish feed and 0.2g live yeast.
2. Dissolve the live yeast in 15mL of distilled water.
3. Add the yeast solution to the fish feed gradually whilst mixing.
4. Continue mixing until all of the solution is absorbed and the feed is evenly coated.
5. Rinse any residual yeast solution from the container with 2mL of distilled water and add this to the feed whilst mixing.

Gut analysis

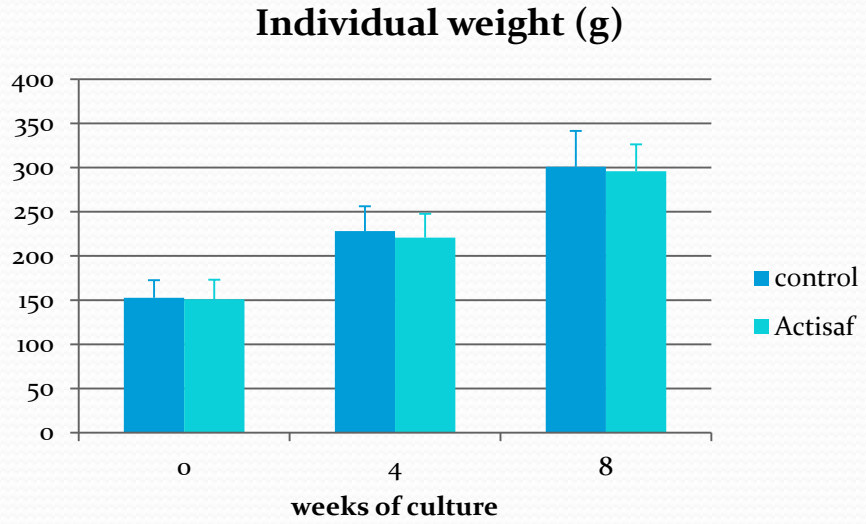
- The gut samples were washed from faeces and stored at -20°C and then -80°C until TRFLP analysis
- TRFLP Procedure
  - After DNA extraction, PCR were performed using 0.5µL of DNA (100-200ng/reaction) and the 27F (labelled with Cy5)-1389R primers combination (~full length 16s rDNA).
  - The PCR product was digested with Hae3 and Msp1 and run on a sequencer (capillary electrophoresis).
  - T-RFS were assigned to a bin size with the Beckman fragment analysis software using a 600bp internal standard.
  - The profiles of T-RFs were analysed with R (vegan package).

TRFLP : Characterisation of the microflora

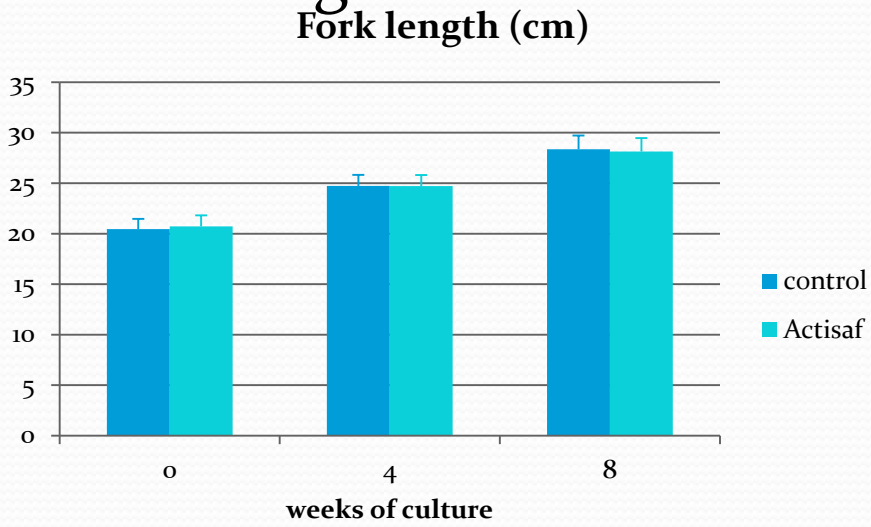


Results

- Weight



- Fork length



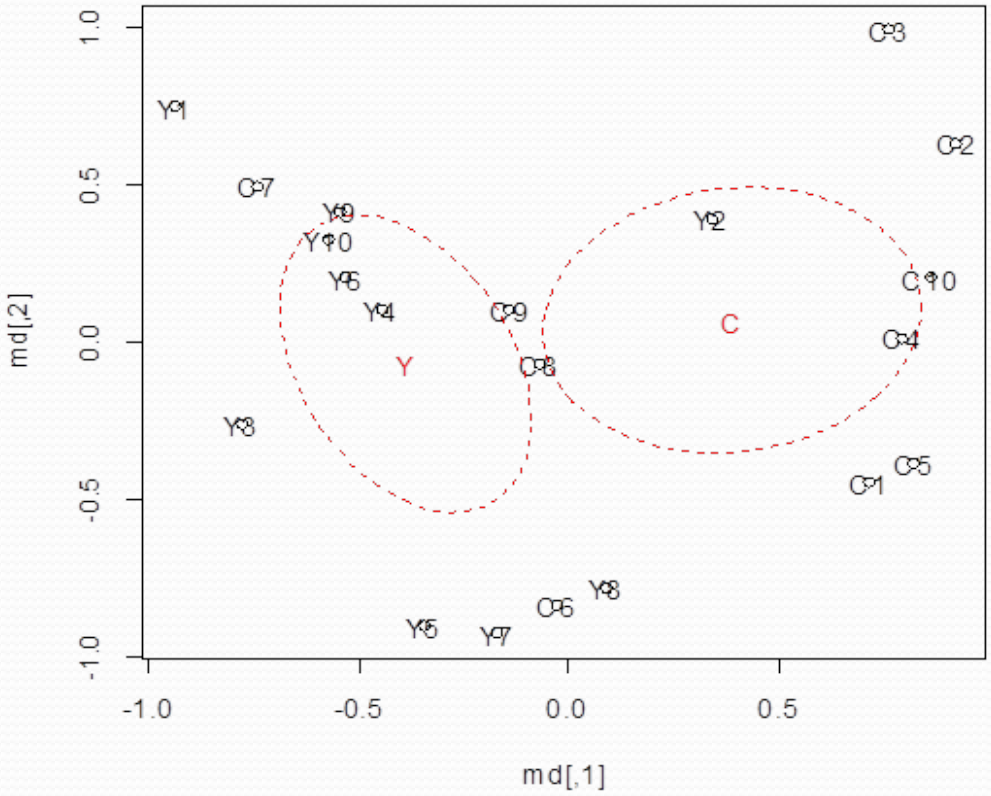
No differences in weight ,fork length or survival after 8 weeks

Gut Microbiota Analysis

- The number of peaks (~number of species) is much lower than in other animals such as pig or rumen: ~ 20 peaks for fish gut compared to 50-60 for rumen/pig colon.
- It could be that the bacterial population is indeed less diverse or it could be due to the high contamination with the host DNA.

Gut Microbiota Analysis

- Analysis of the 16s rDNA profiles of the 20 samples that could be successfully amplified using Principal Coordinates analysis. Md 1 and 2 are the multidimensional scales.
- The Manova analysis confirmed that there was indeed a **significant difference between the control and the treated group bacterial population.**
- P≥0.001



C=control and Y = yeast.
Red circle= confidence interval (95%) as calculated by the standard deviations of their (weighted) averages.

Conclusions

- **Significant differences were shown in gut microbial populations**
- The study did not show differences in growth and length probably due to the high performing feed used and low temperature
  - Work in more challenging production conditions for future studies
- This suggests that that current tests for yeast efficacy as a probiotic in the absence of stress, pathogen challenge, etc cannot determine the true effect of probiotics in fish

Future work –Action of yeast

- Define challenging conditions for yeast/probiotics in fish to further quantify performance.
  - Nutrition, stress, pathogens etc
- Optimise the inclusion of probiotics in fish feed
- Identification of the specific microbial population modification by pyrosequencing.
- Need to harness the power of new molecular and immunological techniques to fully evaluate feed additives

Thank you for your attention