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Characterisation of bacterial communities associated with early stages of intensively reared cod (*Gadus morhua*) using Denaturing Gradient Gel Electrophoresis (DGGE)

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Abstract

High mortality is often observed during the early life stages of intensively reared cod, and believed to be at least partly caused by opportunistic bacteria. The aim of the present study was to use Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified 16S rDNA to characterise the bacterial populations associated with early life stages of cod larvae in intensive hatcheries. At one hatchery the analysis was carried out during a period of approximately 4 weeks post hatch, and confirmed that cod larvae are associated with bacteria before and after the onset of exogenous feeding. A change in the number of bands and banding positions indicate that new bacteria or bacterial community were introduced between the samples taken at day 5 and 13, probably as a result of the onset of exogenous feeding. The post-feeding analyses were dominated by α-proteobacteria. An additional study from two other hatcheries of moribund fry, were dominated by *Vibrio* spp., including *V. xuii* and *V. logei*. It is concluded that DGGE is a suitable method for characterising bacterial communities in hatcheries. However, other genes than 16S rDNA might be more suitable for the discrimination of closely related taxa, particularly different *Vibrio* spp.

Keywords: Cod larvae; Bacteria; DGGE; *Vibrio* spp.; *Vibrio xuii*; *Vibrio logei*; α-Proteobacteria

1. Introduction

Aquaculture of Atlantic cod, *Gadus morhua*, is currently on the verge of commercial breakthrough in Norway (Svåsand et al., 2004, Samuelsen et al., 2006). As with several other marine fish species, a major obstacle facing a stable aquaculture industry has been to ensure reliable intensive production of juvenile fish for ongrowing. Although extensive or semi-intensive protocols for cod juvenile production have existed for more than a century (reviewed by Meeren and Naas 1997), such methods have never become economically feasible,

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and the substantial investments that have been made since 2000 have been almost exclusively based on intensive production techniques (Engelsen et al., 2004).

In intensive aquaculture systems, high concentrations of nutrients and high densities of fish larvae provide favourable conditions for opportunistic pathogenic bacteria (Vadstein et al., 2004). The bacterial concentration in cod incubators may also increase a thousand-fold during hatching (Hansen and Olafsen, 1989), in response to the addition of live feed (Nicolas et al., 1989, Skjermo and Vadstein, 1993, Makridis et al., 2000). In a recent study by Korsnes et al., 2006, the pathogen *Listonella anguillarum* was detected in four different commercial media used to enrich live feed (Korsnes et al., 2006). As cod larvae are known to ingest bacteria even before active feeding commences (Olafsen 1984, Olafsen and Hansen, 1992), intestinal colonisation and uptake of bacteria probably influence larval survival. High mortalities are frequently observed during the early life stages of cod (Samuelsen et al., 2006) and may be due to suboptimal rearing conditions in addition to the generally immature or primitive immune system of fish larvae (Vadstein et al., 2004).

In Norway, from 2002 to 2004, most intensive cod hatcheries have independently reported mortality during live feeding on rotifers and *Artemia*. The larvae displayed signs of bacterial enteritis in the intestinal epithelium and there were reports of larvae losing their ability to osmoregulate. These findings point to opportunistic bacteria as causative agents, although it cannot be outruled that nutritional conditions may be the primary cause, or add to the problem.

Challenge experiments with yolk-sac larvae of cod (Bergh 2000), halibut (Bergh et al., 1992), and turbot (Bergh et al., 1997, Hjelm et al., 2004a) suggest that exposure of larvae to certain bacterial species causes mortality (Sandlund et al. unpubl.), while other bacteria may actually enhance survival. Although knowledge of the modes of action of beneficial bacteria is still limited, reviews suggest that the addition of such bacteria as probiotics may enhance larval development and survival (Gatesoupe 1999, Ringø and Birkbeck 1999, Hansen and Olafsen, 1999). Recently, a *Roseobacter* sp. was shown in two of three experiments with turbot yolk-sac larvae to significantly decrease larval mortality when added to culture water (Hjelm et al., 2004a). Although the mechanisms by which bacteria interact with fish larvae are poorly understood, a substantial body of information suggests that the addition of beneficial strains may increase survival (Gatesoupe 1999, Verschuere et al., 2000), emphasizing the importance of the composition of the microbiota associated with fish larvae.

Denaturing Gradient Gel Electrophoresis (DGGE), a "genetic fingerprint" method based on PCR amplification of 16S rDNA, has been employed to describe bacterial populations associated with haddock juveniles fed different diets (Griffiths et al., 2001) and rotifers (Rombaut et al., 2001). In our laboratories, we have employed such a methodology to describe bacterial populations associated with the early life stages of scallop, *Pecten maximus*, different algal cultures used as live feed (Sandaa et al., 2003), and larvae and juveniles of halibut, *Hippoglossus hippoglossus* (Jensen et al., 2004). This method circumvents bacterial cultivation and in principle provides information about all the dominant bacteria present, regardless of their ability to grow on cultivation media. By excising bands from the DGGE gel, followed by reamplification and sequencing, it is also possible to identify the bacteria present in the population.

The purpose of the present study was to use PCR-DGGE to characterise the bacterial populations associated with early life stages of cod larvae in intensive hatcheries.

2. Materials and methods

2.1. Sampling

Sampling was carried out in three Norwegian cod hatcheries, A, B and C, all following their standard intensive protocols.

2.2. Hatchery A

Samples from hatchery A were taken weekly from four larval tanks during April and May 2003 (Table 1), results from two of these tanks are included in this study. The larvae were removed from the tanks before feeding and starved for 4 h at 4 °C in filtered seawater. They were then killed with an overdose of benzocain (0.05%), surface disinfected with benzalkonium chloride (0.1%) for 30 s and rinsed three times in 30 ml sterile distilled water before being transferred to 100% ethanol. The samples were kept at −20 °C until analysis.

Table 1
Sampling and feeding regime at hatchery A

Date	Days post hatch	Feed (start of feeding)
09.04.2003	0	None
14.04.2003	5	None
22.04.2003	13	Rotifers (14.04.2003)
28.04.2003	19	Rotifers
05.05.2003	26	Rotifers

The larvae were fed algal culture (*Nanochloropsis oculata*) on day 2 post hatch.