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Improved growth rate in farmed *Haliotis midae* through probiotic treatment

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ABSTRACT

There is great potential for the use of probiotics in aquaculture to increase growth rates and improve the nutritional status of the cultured animal. In this study, *Haliotis midae* fed a kelp diet supplemented with *Pseudoalteromonas* sp. strain C4 exhibited an increased growth rate compared to abalone fed standard kelp feed both under laboratory and farm conditions. The growth rate of antibiotic-treated *H. midae* was extremely poor in comparison to abalone that had not been treated with antibiotics when fed an unsupplemented kelp diet, reflecting the importance of gastrointestinal microflora in abalone growth. Alginate lyase activity in the *H. midae* digestive tract was significantly higher in abalone fed kelp supplemented with strain C4 compared to abalone fed unsupplemented kelp. Antibiotic-treated abalone incorporated ^{14}C-labelled strain C4 proteins into their tissue. Although most of the radiolabelled bacterial protein was incorporated into the hepatopancreas, incorporation was also observed in the gills, foot, adductor muscle and intestine. Thus, the *Pseudoalteromonas* strain tested in this study shows tremendous potential for use as a probiotic in abalone aquaculture, particularly with regard to improving the nutritional status and digestibility of the feed and hence, increasing the growth rate of farmed *H. midae*.

1. Introduction

Abalone aquaculture is an economically important industry in South Africa in terms of generating foreign income and job creation. Abalone are a sought after delicacy, especially in eastern Asia, and fetch prices of approximately $34–$36/kg for cocktail sized abalone (Stanford, 2004). Land-based abalone aquaculture has expanded in South Africa over the last decade, and there are now at least twelve farms in commercial production.

A major problem facing abalone farmers is the slow growth rate of abalone. Although abalone growth under aquaculture conditions is much faster compared to natural populations, abalone still require about four to five years to reach a market size of 80 mm. Shortening this time period would reduce production costs and increase turnover. Recent research to improve the growth rate of farmed abalone has focused on the development of artificial diets (Simpson, 1994; Knauer et al., 1996; Britz and Hecht, 1997) as feed represents the major component of operational costs (Britz et al., 1997; Gómez-Montesa et al., 2003). However, it has been suggested that enteric bacteria may be involved in the nutrition of marine invertebrates (Prim and Lawrence, 1975, Muir et al., 1986, Vitalis et al., 1988, Erasmus et al., 1997) and numerous studies have demonstrated the ability of bacteria to improve the growth of cultured marine organisms (Douillet and Langdon, 1994; Ringø et al., 1996;; Riquelme et al., 1997; Jory, 1998; Rengpipat et al., 1998; Douillet, 2000a,b; Olafsen, 2001; Tovar et al., 2002). This is especially important where less than optimally nutritious seaweeds or other plants are eaten, where foods are particularly indigestible or where a variety of seaweeds are consumed (Vitalis et al., 1988).

The kelp *Ecklonia maxima* is widely used as feed on abalone farms as it is readily available on the west and south coasts of South Africa where most of the abalone farms are situated. However, farmed abalone fed solely on *E. maxima* do not exhibit high growth rates (Simpson, 1994). El-Shanshoury et al. (1994) suggested that bacteria may enhance the digestive efficiency of a host by supplying polysaccharolytic enzymes, and consequently, improve the growth rate of the host. Bacteria isolated from the gut of the sea hare, *Aplysia juliana* (Vitalis et al., 1988), sea urchins (Prim and Lawrence, 1975), the minke whale (Olsen et al., 1994) and abalone (Knauer et al., 1996; Erasmus et al., 1997) have been shown to produce enzymes capable of hydrolysing complex polysaccharides present in the host's food. Seaweed breakdown products generated by bacterial enzymatic activity, whether glucose or amino acids (Vitalis et al., 1988), would be expected to be readily absorbed and metabolised by the host animal. Erasmus et al. (1997) suggested that enteric bacteria play an integral role in abalone nutrition by hydrolysing complex polysaccharide components of macroalgae to simple polymers and smaller units which are rapidly assimilated by abalone. Erasmus et al. (1997) showed that abalone enteric bacteria produced enzymes able to degrade agar, carrageenan, laminarin and alginate and that 70–90% of enzyme activity was extracellular suggesting that bacterial enzymes are secreted into the lumen of the gut where they are able to hydrolyse complex algal polysaccharides. Macey and Coyne (2005) incorporated

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the proteolytic bacterium *Vibrio midae* SY9 and two yeast strains into artificially produced abalone feed. Abalone fed the supplemented diet exhibited an increased growth of between 8 and 34% and increased intestinal proteolytic activity compared to abalone fed the standard feed.

The objectives of this study were to determine whether the alginolytic bacterium *Pseudoalteromonas* sp. strain C4 can improve the growth rate of kelp-fed, farmed abalone and, if so, elucidate a possible mechanism for increased growth rates exhibited by farmed abalone fed a diet supplemented with *Pseudoalteromonas* sp. strain C4.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Pseudoalteromonas sp. strain C4 was previously isolated from the gastrointestinal tract of *H. midae* (Erasmus et al., 1997). Strain C4 was cultured in basal marine broth (BMB) ((w/v) 3% NaCl, 0.23%, $MgCl_2$·6-H_2O, 0.03% KCl, 0.5% casamino acids, 0.1% yeast extract) with shaking at 100 rpm at 22 °C and maintained on basal marine agar (BMA) (BMB supplemented with 2% bacteriological agar (w/v)).

2.2. Preparation of feed

Kelp cakes were prepared by allowing dried kelp (obtained from Kelpak, Simonstown, South Africa) to rehydrate overnight in synthetic sea salts (SSS) ((w/v) 3% NaCl, 0.23% $MgCl_2$·$6H_2O$, 0.03% KCl). The rehydrated kelp (30% w/v) was mixed with bacteriological agar (1.5% w/v) in SSS and sterilised by autoclaving. Strain C4 was added to autoclaved feed at a concentration of approximately 3×10^{10} cfu/mL to a final concentration of approximately 2.4×10^{10} cfu/g. Chloramphenicol (250 µg/mL), ampicillin (100 µg/mL) and cefotaxine (Claforan®, Roussel) (500 µg/mL) were added to the feed to reduce the number of enteric bacteria present in the abalone digestive tract.

2.3. Animals

Abalone were kindly donated by two commercial abalone farms in South Africa, HIK Abalone and Irvin and Johnson, and kept at the Marine and Coastal Management Research Aquarium in Cape Town, South Africa. Animals were maintained in large polyethylene tanks containing 98 L of aerated and continuously flowing (330 L/h) natural seawater at 15–18 °C. Abalone were acclimatized for at least three weeks prior to each experiment during which time they were fed unsupplemented kelp cakes.

2.4. Growth trials

2.4.1. Laboratory growth trials

Laboratory based growth trials were carried out at the Marine and Coastal Management Research Aquarium, Sea Point, Cape Town. Four 24 L glass tanks were used with a constant water flow rate of 860 mL/min. Two of the tanks were kept relatively sterile by circulating 0.2 µm filtered seawater and air, as well as ethanol-cleaning all equipment used to collect samples. The remaining two tanks were supplied with 10 µm filtered seawater and unfiltered air. Forty abalone, measuring approximately 25 mm each, were placed in each tank. The animals were initially starved for two days after which abalone in the two sterile tanks were fed kelp cakes containing antibiotics over a four day period in order to reduce enteric bacterial numbers. After the first two days, the water supply was turned off for 24 h and antibiotics were added to the tank water (ampicillin, 1 mL/L and chloramphenicol, 1.25 mL/L). This was repeated for a second 24 h period during which time the abalone were fed kelp cakes containing antibiotics. After each 24 h treatment, the tank water was replaced with fresh 0.2 µm filtered seawater and allowed to circulate again after the second antibiotic treatment. The remaining two tanks were untreated. A set of antibiotic-treated and untreated abalone, stocked in separate tanks, were fed kelp supplemented with strain C4, while the abalone in the remaining two tanks were fed standard kelp cakes. The tanks were cleaned every three days by siphoning out unconsumed feed and abalone faeces using equipment surface sterilised with 70% ethanol. The abalone were fed 50 g of kelp cake per tank every second day and abalone shell length was measured monthly over a seven month period.

2.4.2. Farm based growth trials

Farm based growth trials were conducted at HIK Abalone Farm in Hermanus, Western Cape. Approximately 1500 abalone, measuring between 15 and 17.5 mm and weighing between 0.85 and 1.1 g, were placed in each of four tanks with a volume of 144 L. Abalone in two of the tanks were fed kelp supplemented with strain C4, while abalone in the remaining two tanks were fed standard kelp cakes. Abalone were fed approximately 600 g of kelp cake/tank every second day. Thirty abalone were randomly selected for weight and shell length measurements taken monthly over a six month period. After six months, the abalone were transferred to 8 raceway tanks and fed regular *E. maxima*. Shell length and weight measurements were recorded monthly for an additional two months.

2.5. In situ alginate lyase activity

Thirty abalone were placed into each of four aerated, 98 L tanks with a constant water supply at a flow rate of 5.5 L/min. Abalone in two of the tanks were fed standard kelp cakes, whereas abalone in the remaining two tanks were fed kelp cakes supplemented with strain C4 over a period of 14 days. Eight abalone were sacrificed from each tank, the digestive tracts removed and the crop, stomach and intestine were separated. Each of the tissue samples were weighed and homogenised in 0.1 M citric acid/0.2 M phosphate buffer, pH 5.2 at a volume of 2 mL/g tissue. The samples were dialysed for 48 h against 0.1 M citric acid/0.2 M phosphate buffer, pH 5.2 at 4 °C with two buffer changes. The protein concentration in each sample was determined using the Bradford Assay (Ausubel et al., 1989) and alginate lyase activity was measured using the TBA assay (Weissbach and Hurwitz,

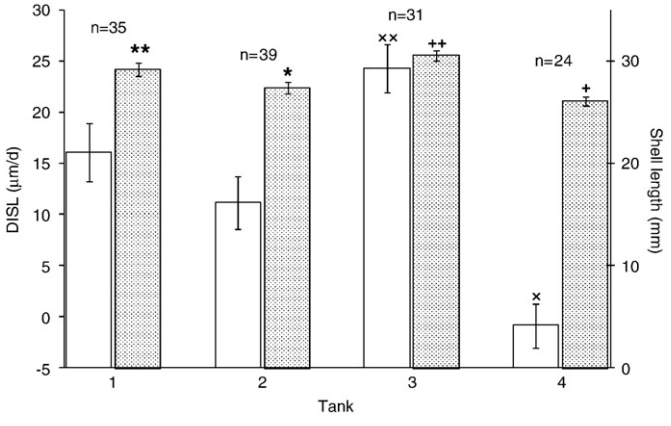

Fig. 1. Daily increase in shell length (DISL) (□) and final shell length (■) of abalone fed either kelp supplemented with strain C4 (Tank 1) or a standard kelp diet (Tank 2), or antibiotic-treated abalone fed either kelp supplemented with strain C4 (Tank 3) or a standard kelp diet (Tank 4). Bars represent mean shell length growth rate/day over a seven month period and the mean final shell length of the animals (animals had an initial mean shell length of 25.5 mm). Error bars depict standard error calculated from three different sets of measurements. The number of individuals measured in each tank (n) is indicated above each set of bars. Different number of (*), (×) or (+) indicate a significant difference (ANOVA, Tukey post-hoc, $P<0.05$) between data obtained from abalone fed the strain C4 supplemented kelp diet and the corresponding parameter obtained from abalone fed the unsupplemented kelp diet (Tank 1 vs Tank 2 or Tank 3 vs Tank 4).