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Effect of dietary probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress status of shrimp *Litopenaeus stylirostris*

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ABSTRACT

In this study we evaluated the effects of the probiotic *Pediococcus acidilactici* MA18/5M on shrimp, *Litopenaeus stylirostris* (also called *Penaeus stylirostris*), first on antioxidant defences and secondly on the oxidative stress status in the shrimps' haemolymph and digestive gland.

We conducted two experiments with the same protocol in which shrimps were fed two diets for three weeks: a control diet and a probiotic diet containing 1 g of live *P. acidilactici* MA18/5M kg^{-1}. In the first experiment, the shrimps were found to be healthy over the trial period; no mortalities and non-detectable signs of infection were recorded. These resulted in high final survival rates (above 90% in both treatments). On the other hand, during the second trial, carried out at a period of increased risk for an outbreak of the summer syndrome, higher mortalities were recorded, associated with high *V. nigripulchritudo* prevalence and loads in the shrimp haemolymph. In healthy shrimps (trial 1), no detectable response of the antioxidant defence system and of oxidative stress bio-indicators were observed. However, feeding the probiotic significantly increased the total antioxidant status (TAS) and glutathione peroxidase activity, while all other parameters remained significantly unchanged.

In the shrimps exposed to *V. nigripulchritudo* (trial 2), the antioxidant response was characterized by higher antioxidant enzyme activities (superoxide dismutase and catalase) and higher oxidative stress level in the digestive gland (higher oxidized/reduced glutathione ratio, higher malondialdehyde and carbonyl protein contents) compared to levels found in trial 1. However, shrimps fed the probiotic diet exhibited (i) significantly higher final survival rates ($67 \pm 3\%$) compared to the control ($47 \pm 4\%$) and (ii) a lower prevalence of *V. nigripulchritudo* throughout the trial. Moreover, the antioxidant response and the oxidative stress level recorded in the digestive gland with shrimps submitted to the probiotic diet were lower.

In the light of these results, we confirm that *P. acidilactici* enrichment in shrimps' diet seems an effective way of reducing the susceptibility of shrimps to bacterial pathogens.

1. Introduction

Marine shrimps, like other aquatic animals, are constantly threatened by micro-organisms such as fungi, bacteria and protozoa, which can greatly affect their health and well-being. Under farming conditions, pathogen pressure can be higher leading, in some cases, to dramatic crop failures, as observed over the past three decades with marine shrimp cultures affected by vibriosis (Lightner, 1988; Lin, 1995).

Shrimp farming in New Caledonia today faces two main challenges in terms of diseases implicating bacterial pathogens: the "Syndrome 93" (Mermoud et al., 1998) and the "summer syndrome" (Goarant et al., 2006). The latter phenomenon is causal linked to infectious identified to be due to strains of *V. nigripulchritudo* (Goarant et al., 2006). In recent years, biological control of these diseases, affecting aqua-cultured species especially bacteriological disorders including environmentally friendlier methods such as the use of probiotics, has become an important subject of investigations (Gatesoupe, 1999; Vershuere et al., 2000; Irianto and Austin, 2002; Vine et al., 2006). Recent works carried out in farm-scaled studies have demonstrated the beneficial effects of feeding the probiotic strain, *Pediococcus acidilactici* MA18/5M, to shrimps *Litopenaeus stylirostris* (also called *Penaeus stylirostris*) naturally infected with *Vibrio nigripulchritudo* (Castex et al., 2008).

Apart from pathogen pressure, reared shrimps are also subject to temperature changes and other environmental perturbations which can severely affect their physiological state (Le Moullac and Haffner, 2000; Wabete et al., 2008). An emerging field of study in physiology of aquatic species is therefore increasingly focusing on "oxidative stress", especially since many authors have reported the effect of environmental perturbations on oxidative stress. Such studies include elucidation of the presence of a wide range of contaminants (xenobiotics) (Winston and Di Giulio, 1991; Livingstone, 2001; Manduzio et al., 2005; Ferreira et al., 2005), UV radiation, hypoxia and hyperoxia (Halliwell and Gutteridge, 1999; Zenteno-Savín et al., 2006), and other environmental physico-chemical

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parameters (Le Moullac and Haffner, 2000; Abele et al., 2002; Lesser, 2006) being linked to changes to physiological states of shrimps.

Oxidative stress results from either increased exposure or production by the organism of reactive oxygen species (ROS) or from a decrease in the antioxidant defences due to exposure, resulting in oxidative damage to lipids, protein and nucleic acid. ROS include several reactive oxygen intermediates (ROIs) such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($O_2^{\bullet}$). ROS are continually produced in animals during normal aerobic metabolism (Livingstone, 2001). However, the rate or amount of ROS production depends on the metabolic rate of the species under consideration and can be increased by environmental stress, as shown for penaeid shrimps (Zenteno-Savín et al., 2006; Wang and Chen, 2006; Cheng et al., 2007; Lemaire and Chim, 2007). The reported exceptionally high oxygen consumption capacity of the shrimp, *L. stylirostris* (Wabete et al., 2006) makes this species particularly sensitive to oxidative stress.

ROS production is also implicated in the immune response mechanism to both prokaryotic and eukaryotic pathogens (Adema et al., 1991). In crustaceans, once a pathogen enters the haemolymph, NADPH-oxidase is activated in the hemocyte of the host, which in turn reduces oxygen and subsequently produces several reactive oxygen intermediates. This process, known as respiratory burst, is well documented for aquatic species and recently demonstrated in penaeid shrimps (Muñoz et al., 2001). The immune system and the antioxidant defence system are closely linked to responses due pathogens and other stress-related issues that might lead to respiratory burst (Holmblad and Söderhäll, 1999). It has even been suggested that the antioxidant and oxidative responses could become useful parameters for evaluating the *in vivo* immune response(s) in cultured organisms exposed to given environmental perturbations due to biotic parameters or pathogenic micro-organisms (Campa-Cordova et al., 2005).

Many studies have also examined antioxidants pressures in aquatic invertebrates, but few have focused on crustaceans (Holmblad and Söderhäll, 1999). With respect to shrimps, most of these studies have evaluated the effect of pollutant or environmental parameters on "oxidative stress" (Gonzalez-Rey et al., 2006; Zenteno-Savín et al., 2006; Li et al., 2008). However, very few have reported the effect of pathogen infection on antioxidant defences in penaeid shrimps (Rameshthangam and Ramasamy, 2006; Liu et al., 2007a; Zhang et al., 2008; Hsieh et al., 2008). Most of the scientific studies carried out to evaluate the effect of probiotics on aquatic farmed animals, apart from demonstrating improvement in survival and growth of the host species have generally focused on nutrition, antagonism toward pathogens, and immunity of the host (Rengpipat et al., 2000; Alavandi et al., 2004; Li et al., 2007; Rodríguez et al., 2007). However, there are very few articles reporting on the effects of dietary additives on oxidative status of cultured shrimps (Liu et al., 2007b; Chiu et al., 2007).

The aim of the present study was to evaluate, firstly, the possible effect of dietary *P. acidilactici* supplementation on the antioxidant defences and oxidative stress status in *L. stylirostris*. This effect was evaluated in two different situations: healthy shrimps and shrimps infected by *V. nigripulchritudo*. We also looked at oxidative damage to lipids and proteins to evaluate oxidative stress status by measuring respectively malondialdehyde (MDA), which is a commonly used indicator to evaluate lipid peroxydation, and carbonyl proteins which is recently being used as biomarker of oxidative damage to protein in fish (Parvez and Raisuddin, 2005).

2. Materials and methods

2.1. Shrimps

The shrimps *L. stylirostris* used in our experiments were reared semi-intensively (without aeration) in earthen ponds (1000 m^2) at the Saint-Vincent station (Ifremer). The ponds were stocked with 20-day post-larvae (PL20), at a density of 20 animals m^{-2}. It usually takes 3 to 4 weeks for the animals to reach 1 g. Thereafter the growth rate is estimated to be approximately 1 to 1.4 g per week. The experiments in this study were carried out with shrimps of 12.79 ± 2.72 g and 6.2 ± 0.7 g for the first and second trials respectively.

2.2. Feeds tested

Shrimps were fed an experimental feed produced in the laboratory (Table 1): the ingredients were ground up in a laboratory grinder (Retsch®) with a 1 mm screen. The meal obtained was mixed with oil and water (30%) in a horizontal mixer (Mainca®) until the consistency was suitable for pelleting. The mixture was then extruded in a meat grinder through a 3 mm die. Next, the entire mixture was dried in a drying oven (Venticell® 222) until a residual humidity less than 10% was obtained, and then broken up into pellets of 4–5 mm in length. The commercial probiotic diet preparation tested was Bactocell® PA 10 (Lallemand Animal Nutrition S.A., Blagnac, France) formulated with live *P. acidilactici* MA18/5M (Institut Pasteur, Paris, France).

For the treated group, 1 g kg^{-1} of the probiotic (powder form) was top-coated on the laboratory produced feed pellets using 2% of fish oil as a carrier, giving a final concentration of 1.8 ± 10^7 CFU and 9.5 ± 10^6 CFU of *P. acidilactici* per gram of diet for trials 1 and 2 respectively. The probiotic concentration in the feed was checked after diet formulation by counting *P. acidilactici* strains on MRS plates using serial dilution. The control diet was also top-coated with 2% fish oil and, prior to use, checked for possible contamination by the probiotic strain. The feed was then stored in 5-litre boxes at 20 °C until use.

2.3. Experimental design

In this study, two trials were conducted. Both trials (trial 1 and trial 2) were carried out using the same protocol. *L. stylirostris* obtained from the earthen ponds were transferred into 8 circular polyester tanks (capacity 1600 l). The animals were caught in the ponds using a cast net and transferred to the tanks in plastic containers (50 l) filled with seawater. One hundred individuals were put into each tank and acclimatized for one week prior to the beginning of the experiment using a previously described protocol, to reduce stress of transfer and

Table 1
Composition of the experimental diet.

Ingredients	%
LT Fish meal[a]	30
Soy bean meal[b]	20
Wheat meal[c]	37
Wheat gluten	7
Fish oil	0
Soy oil	2
Soy lecithin[d]	2
Shrimp vitamin premix[e]	0.05
Shrimp trace mineral premix[f]	0.1
Stay C (330 mg/kg)[g]	0.04
Compostion (analysed, dry matter basis)	
Protein[h] (%)	43.8
Fat[i] (%)	10
Fiber[j] (%)	2
Ash[k] (%)	6.9
Gross energy (kcal kg^{-1})	4502

[a] Chilean low temperature fish meal from anchovy and Jack Mackerel.
[b] Dehulled soybean meal solvent extracted.
[c] Whole wheat grain for animal feed.
[d] Ultrales® lecitin from ADM lecithin, Decatur, IL, USA.
[e] Vitamin premix SICA Cie.
[f] Mineral premix SICA Cie.
[g] ISO5983 standard.
[i] NF V18-117/B standard.
[j] NF V03-040 standard.
[k] NF V18-101standard.