J. Mar. Biol. Ass. India, **51** (2) : 227 - 230, July - December 2009

Short Communication

Effect of treatment with β-1,3 glucan from the yeast *Candida sake* S165 on protection to *Fenneropenaeus indicus* postlarvae against WSSV infection

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Abstract

β1,3 glucan extracted from the cell wall of a marine yeast *Candida sake* S165 was tested for its potential as an immunostimulant in *Fenneropenaeus indicus* postlarvae through dip treatment. Glucan suspensions of different concentrations *viz.*, 0.1 mg/ml, 0.5mg/ml, 1mg/ml and 2mg/ml were prepared and postlarvae were subjected to dip treatment for 3 hours. Postlarvae dipped in solution without glucan addition was considered as control. To find out the extent and duration of protection conferred by glucan dip treatment White Spot Syndrome Virus (WSSV) challenge was conducted on 10th, 15th and 20th day of postdip treatment. Post-challenge survival showed that 0.5 mg/ml and 1 mg/ml glucan concentration impart better protection whereas, higher concentration, 2mg/ml gave adverse effect on post-challenge survival. Challenge on the 20th day post-glucan treatment resulted in considerable mortality in all the four treatment groups. The study showed that the protection from WSSV through β1,3 glucan is short lived and lasted for a period of only 10 to 15 days.

Keywords: *Fenneropenaeus indicus*, glucan, dip treatment, immunostimulant, short-term protection

Introduction

β-1,3 glucan has been successfully used as immunostimulant to enhance the defence resistance of fishes and shellfishes against bacterial and viral infection (Raa, 1996; Sakai, 1999; Chang *et al*., 2003). Sung *et al*. (1994) reported that glucan obtained from *Saccharomyces cerevisiae* enhanced vibriosis resistance in the postlarvae of *Penaeus monodon*. Using a mixture of 1,3 and 1,6 glucan extracted from cell wall of *S. cerevisiae,* Song *et al.* (1997) reported an enhanced disease resistance in *P. monodon* against induced infection of white spot syndrome virus. Chang *et al.* (2000) observed that feeding *P. monodon* with glucan derived from *Schizophyllum commune* at a concentration of 2 g/kg in diet showed an increase in phagocytic index and superoxide anion production by haemocytes. Apart from glucan, cell wall components like peptidoglycan and lipopolysaccharides (LPS) of certain bacteria showed considerable immunostimulation in shrimps (Sakai, 1999). Itami *et al.* (1998) reported enhancement of disease resistance in kuruma shrimp, *Penaeus japonicus*, fed with peptidoglycan derived from *Bifidobacterium thermophilum* against penaeid rod-shaped DNA virus. Takahashi *et al.* (2000) showed a higher phagocytic index and phenoloxidase activity in kuruma shrimp *P. japonicus* fed with lipopolysaccharides extracted from *Pantoea agglomerans.* Song and Hsieh (1994) reported that zymosan, a protein-carbohydrate (mannan and glucose) complex extracted from *S. cerevisiae* and β-glucan extracted from *S. cerevisiae* increased the overall defence system of tiger shrimp *P. monodon*. We previously reported the immunostimulatory effect of whole cell marine yeast isolate *Candida sake* against white spot syndrome virus infection in *F. indicus* (Sajeevan *et al*., 2006). In this paper we made an attempt to study the immunostimulatory potential of the extract of the cell wall component

β-glucans from *C. sake* in the postlarvae of *Fenneropenaeus indicus* by dip treatment.

Material and Methods

***Marine yeast and glucan extraction**: C. sake* S165 isolated from the coastal waters off Cochin and maintained in the Microbiology Laboratory of the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences was used for the study. A pure lawn culture of *C. sake* was prepared using Malt Extract Agar (malt extract, 20 g; mycological peptone, 5 g; agar, 20 g; 20 ppt seawater, 1 L; pH: 6) and the biomass was harvested at exponential phase into sterile seawater (20 ppt). The harvested cells were separated by centrifugation at 7500x g for 10 min at 4° C and dried at 80° C for 24 h. Glucan was extracted from the dried yeast biomass following the method of Williams *et al.* (1991) with modification. Briefly, 1 g dried yeast biomass suspended in 20 ml of 3% (w/v) aqueous sodium hydroxide was maintained at 100° C for 6 h in a serological water bath. Filtering through muslin silk and re-extracting with aqueous sodium hydroxide resulted in separation of alkali insoluble material. The insoluble material was separated and extracted with 20 ml of 0.5 N acetic acid at 75° C for 6 h. The insoluble material was recovered by filtration through muslin silk and refluxed repeatedly with ethanol until the filtrate became colourless. The glucan residue obtained was washed extensively with distilled water and vacuum dried over silica gel at 28±1° C. The dried glucan was subjected to sonication by using an ultrasonicator (Soinc Vibra, USA). For this, particulate glucan was suspended in distilled water (1% w/v) and sonicated at 20 Khz at 100 W for 15 min and solutions with different glucan strength (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 2 mg/ml) were prepared in 15 ppt seawater.

Experimental design: Postlarvae (PL18) of *F. indicus* (PCR–negative for WSSV) brought from a hatchery located at Kannamali, Cochin were used for the study. After acclimatization for seven days, the animals were divided into 5 groups of 500 PL each. Group 1 was treated as control and dipped in seawater without glucan. Group II and Group III were dipped in glucan solution of 0.1 mg/ml and 0.5mg/ml concentration respectively and Group IV and Group V were dipped in glucan solution of 1mg/ml and 2mg/ml, respectively. After 3 hours, dip treated larvae were transferred to fibre reinforced plastic (FRP) tanks with aerated seawater of 15 ppt and were maintained on commercial shrimp diet with 48.5% protein (Higashimaru, India). Feeding was performed twice daily (8 am and 7 pm) at a ration of 8-10% body weight. Physico-chemical parameters of water such as salinity, NH_3–N, NO_2–N, NO_3–N and dissolved oxygen were monitored regularly (APHA, 1995) and maintained at optimal levels by water exchange.

To find out the extent and duration of protection conferred by glucan, dip treatment challenge experiment was conducted with white spot syndrome virus (WSSV). For this, after ten days of dip treatment, 60 postlarvae each from the treatment groups were transferred and distributed into 3 FRP tanks with 20 animals each. Viral challenge was done by feeding WSSV infected prawn gill tissue at the rate of 1 g per 20 postlarvae. Similarly on 15th and 20th day of post dip treatment, the postlarvae were challenged with WSSV *via* diet. The percentage survival in each group was recorded for a period of seven days.

The results were analyzed using one way analysis of variance (ANOVA) and Duncan's multiple comparison of the means using SPSS 10.0 for Windows. Differences were considered significant when $p < 0.05$.

Results and Discussion

The WSSV challenge performed on 10th day of glucan dip treatment gave significant post challenge survival in Group III (0.5 mg/ml) and Group IV (1 mg/ml) animals (36±5% each) when compared to control ($p < 0.05$) (Fig. 1). Animals dipped in 0.1 mg/ml glucan showed 16±5% survival, whereas, the lowest survival (8±2%) was recorded in postlarvae subjected to 2 mg/ml glucan solution dip treatment. In case of viral challenge on 15th day of glucan dip treatment (Fig. 2), Group IV showed the maximum survival (31±3%) followed by Group III (29±2%). However, Group II showed 18±2% survival and Group V gave a survival of 13±1% only. WSSV challenge on 20th day post-glucan treatment exhibited considerable mortality in all the

four treatment groups and control (Fig. 3). The post-challenge survival in Group I and Group II were 12±1%, and 14±2 respectively, while that of Group III and Group IV were 11±2% and 14±1%. Even though no significant difference in the post-challenge survival was seen among the treatment groups, Group V (2mg/ml) exhibited higher post-challenge survival (15±1%).

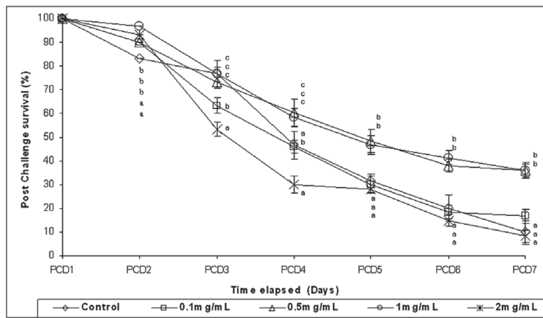

Fig. 1. Post-challenge survival (mean ± S.D.) of *F. indicus* postlarvae dip treated with different concentrations of yeast glucan solution after 10 days of treatment; values with the same superscript do not differ significantly ($p < 0.05$). PCD = post-challenge day

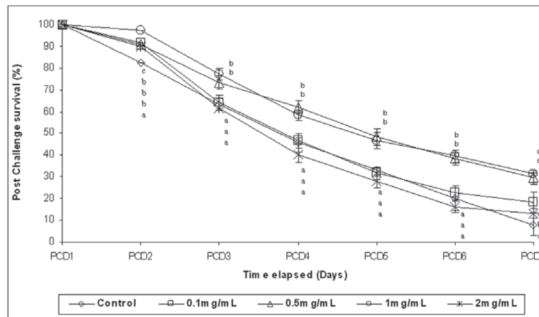

Fig. 2. Post-challenge survival (mean ± S.D.) of *F. indicus* postlarvae dip treated with different concentrations of yeast glucan solution after 15 days of treatment; values with the same superscript do not differ significantly ($p < 0.05$). PCD = post-challenge day

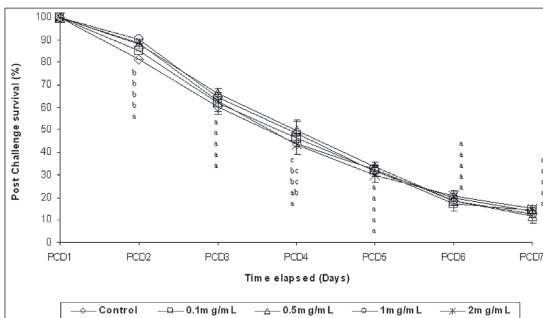

Fig. 3. Post-challenge survival (mean ± S.D.) of *F. indicus* postlarvae dip treated with different concentrations of yeast glucan solution after 20 days of treatment; values with the same superscript do not differ significantly ($p < 0.05$). PCD = post-challenge day

The present experiment revealed that over the course of time, the defense potential imparted by glucan gradually reduced and at the end of 20 days of dip treatment, the protective effect was nearly absent. This shows that the protective effect by â-1,3 glucan is short-lived and lasted for a period of only 10 to 15 days which coincide with the life span of shrimp haemocytes (van de Braak *et al*., 2002). It was observed that high concentration of glucan, *i.e.*, 2mg/ml, resulted in less protection to the animals. This shows that animals in this group became stressed due to high concentrations of glucan in dip treatment. In this group considerable death was also observed 3 hours after dip treatment which confirms the adverse effect of high concentration of glucan. Sajeevan *et al.* (2009) reported that higher concentrations of glucan can cause excessive degranulation of both granular and semigranular haemocytes resulting in the release of prophenoloxidase and exhaustion of immune system leading to an "immune fatigue condition". It is more likely that such animals may not survive an infection by potent pathogens and succumb to death following pathogenic invasion. The present study confirms the finding of Chang *et al.* (2000) who reported that a higher dose of immunostimulant β-glucan reduces the non-specific immunity and disease resistance to pathogens in shrimps. Apart from these high concentration of glucan in solution would have caused physical damage to the gill tissues and subsequent stress to the animals. A similar observation was made by Sung *et al.* (1994) in *P. monodon* postlarvae treated with 2 mg/ml glucan suspension where considerable damage was caused to gill tissue due to high concentration of glucan in suspension. Unlike many chemotherapeutics, immunostimulants do not show a linear dose–effect relationship (Bliznakov and Adler, 1972). In fact they often show a distinct maximum at a certain intermediate concentration and even a complete absence of effect or an adverse toxic effect at higher

concentrations (Floch *et al.,* 1987). In conclusion, if infection is more frequent in hatcheries, the use of optimal dose of glucan as immunostimulant through dip treatment appears to be a reliable and practical way of enhancing the defense status of larvae and fingerlings during early days of larval rearing. Also at the time of transportation from hatcheries to stocking in aquaculture ponds, application of optimal dose of immunostimulants would benefit the postlarvae to tide over the associated stress. However, the duration of protection offered trough dip treatment is short. Subsequent application of immunostimulants through feed in grow-out ponds at a optimal dose and frequency is essential for ensuring effective protection throughout the culture period.

Acknowledgments

The authors are grateful to the Department of Ocean Development, Govt. of India for a research grant (F.No.DOD/II-MRDF/4/3/UNI/97-(P-6)) with which the work was carried out.

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Received : 27/03/09
Accepted : 06/10/09