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The effects of dietary administration with chemical treated *Saccharomyces cerevisiae* strain *YG3-1* on the growth of aquatic invertebrates in *Artemia*

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ABSTRACT

Objective: To investigate the biological effects of β-glucans in cell wall of new identified strain *Saccharomyces cerevisiae* strain *YG3-1* on the growth of aquatic invertebrates, in *Artemia* as model organism.

Methods: All yeasts used in the present study were isolated from *Rainbow trout* intestine and then cultured in yeast extract-peptone-glycerol medium. Activation of β-glucan in yeasts was performed by chemical treatment with 2-mercaptoethanol (2ME) (3.5% v/v). Then nauplii and larvae individuals of *Artemia urmiana* and *Artemia franciscana* (two different species of *Artemia* as test organisms) were fed with 2ME-treated yeasts during the culture. At the end of experiment, after feeding individual length (total length and growth rate) in adult individuals of *Artemia* was measured.

Results: Following this administration, growth in both species of *Artemia* was improved ($P < 0.05$). So, the results showed that *Artemia urmiana* adults individuals that fed with 2ME-treated yeasts had the highest growth and total length. These results were confirmed with growth measurement in adult individuals of *Artemia*.

Conclusions: This study suggested that 2ME-treated *Saccharomyces cerevisiae* strain *YG3-1* yeasts can be used for enhancing the growth of other aquatic invertebrates like shrimps as probiotic supplement and growth promoter.

1. Introduction

Artemia is one of the most important live foods in aquaculture[1] and found favor as a model organism for using in aquaculture biotechnology[2]. For example, *Artemia* is an excellent model organism to study the mode of action of probiotic bacteria[3]. Function of growth factors and immune system agents in *Artemia* is similar to other aquatic invertebrates performance[4]. The phenoloxidase system (pro-PO system) has been detected in a wide range of invertebrates including *Artemia*[1].

Probiotics are live microbial feed supplements with beneficial effects on host by some actions such as producing inhibitory components and improving the microbial balance[5]. They were used in aquaculture in order to control disease and improve growth and survival[6].

Saccharomyces cerevisiae (*S. cerevisiae*) is one of the most important yeasts that had been used as probiotic in aquaculture and used in *Artemia* culture as a well-known food supplement[1,7]. Many studies have shown that the protective effects of cell wall deficient *S. cerevisiae* strains as microbial diet for *Artemia* larvae against vibriosis[1,8].

β-Glucans are glucose polymers that naturally occur in yeasts[9]. Yeast is a well-known micro-organism that is used in biotechnology since ancient times, therefore it is a good source of β-glucan. β-Glucans in yeast cell wall are branch-on-branch molecules containing linear (1,3)-β-glucosyl chains that are joined through (1,6)-linkages[10]. Cell wall of yeast *S. cerevisiae*

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is one of the most common sources of β-1,3/1,6-glucan[11]. Yeast β-glucan is located under the manno-protein layer of yeast cell wall. So, applying the chemical treatment for activation and increasing the bioavailability of β-glucan is necessary. Yeast β-glucan can stimulate the immune system of animals including the aquatic invertebrates. *S. cerevisiae* β-glucan could improve the immune system function and growth in aquatic invertebrates such as *Artemia* by affecting on some immune system particles and growth factors along with chitin, another polysaccharide structure in the yeast cell wall[8]. Also, physiological, nutritional and immunological role of dietary β-1-3 glucan had been studied in *Litopenaeus vannamei* (Whiteleg shrimp) juveniles[12]. However, the effect of 2-mercaptoethanol (2ME)-treated *S. cerevisiae YG3-1* on the growth of *Artemia* and other aquatic invertebrates has not been studied by now.

The purpose of this study was to investigate the biological effects of β-glucans in cell wall of new identified strain *S. cerevisiae* strain *YG3-1* on the growth of aquatic invertebrates, in *Artemia* as model organism.

2. Materials and methods

2.1. Preparation of S. cerevisiae YG3-1 as probiotic yeast

All of yeasts used in the present study were isolated from intestine of farmed endemic *Rainbow trout* belonging to the Western Azerbaijan State of Iran according to the procedure described by Andlid *et al.*[13] and then identified by molecular methods.

2.2. Activation and enhancement of the bioavailability of β-glucan in S. cerevisiae cell wall

For this purpose, yeasts were cultured and grown using by yeast extract-peptone-glycerol medium. In the stationary growth phase (after 3 days) yeast cells were harvested by centrifugation (5 000 r/min for 10 min)[14]. Then, harvested yeasts were divided into two groups and stored at -20 ˚C. After that chemical (2ME) treatment was applied on the one group of yeast cells. In this way, yeast cells were suspended at a concentration of 200 mg/mL wet weight in a sterilized medium containing Na_2EDTA (0.05 mol/L) and Tris-buffer (0.2 mol/L) with pH = 8. After addition of 2ME (3.5% v/v), the yeast cells were incubated for 60 min at 30 ˚C on a shaker. Pretreated yeasts were collected and washed with protoplasting medium comprising a phosphate-citrate buffer (KH_2PO_4 0.08 mol/L, Na_3citrate 0.016 mol/L; pH = 5.8) and KCl (0.6 mol/L). Finally, yeast cells were washed twice with NaCl 9% and then stored at 4 ˚C until the end of experiment[5,15].

2.3. Artemia culture, feeding and growth measurement

For optimal hatching, 1.5 g cysts of the each population were incubated in artificial 0.45 μm filtered medium with salinity at 35 g/L. After hatching, 500 individuals of instar-I nauplii were transferred directly into the 1 cylindroconical vials at an initial density of 2 nauplii/mL of 80 g/L culture medium. Finally, based on the standard protocol, culture was performed in two treatments and four replicates for each treatment[16,17]. During the culture, both species of *Artemia* [*Artemia urmiana* (*A. urmiana*) and *Artemia franciscana* (*A. franciscana*)] fed with two forms of yeast *S. cerevisiae YG3-1*: 1) Whole cell yeast without any treatment (as control treatment); 2) 2ME-treated yeast (as β-glucan treatment), plus *Dunaliella tertiolecta* algae, for evaluation of biological effects of β-glucan in cell wall of new strain yeast (*S. cerevisiae* strain *YG3-1*) on the growth of *Artemia*. Feeding was performed according to the Coutteau *et al.*[15] feeding table. Air during culturing was passed through a 0.22 μm filter. At the end of the expreiment, after feeding to determine the effect of β-glucan on the growth of *Artemia*, their individual length was measured according to the procedure described by Marques *et al.*[3] with using a dissecting microscope equipped with a drawing mirror on Days 3, 7, 11 and 15 of experiment. Values of individual length and total length were logarithmic transformed or square root transformed to satisfy normal distribution and homocedasticity requirements. All treatments were applied based on the standard protocols that adapted from previous literatures. However, considering the lack of pain feel system in crustaceans, sampling for chemical treatment was applied with minimal samples. Individual length and total length of *Artemia* fed with different feeds were investigated with ANOVA and with multiple comparisons Tukey's range. One-way ANOVA and Duncan's test of SPSS 16 software were used to identify differences among means. Significances were accepted at $P < 0.05$, according to the procedure of Baxevanis *et al.*[18].

3. Results

Results of growth measurement indicated that at the parameter of total length, feeding with 2ME-treated *S. cerevisiae YG3-1* (without the mannoprotein and contains bioactive β-glucan) resulted in the maximum rate of growth in both species of *Artemia*. The maximum total length of *Artemia* was significantly obtained from the *A. urmiana* (Tables 1 and 2) ($P < 0.05$).

Table 1
Total length of *A. franciscana*.

Treatment	Day 3	Day 7	Day 11	Day 15
Control	1.30 ± 0.21^a	2.77 ± 0.76^a	3.87 ± 0.47^a	7.55 ± 0.84^a
β-Glucan	1.46 ± 0.17^b	0.63 ± 2.97^c	0.59 ± 3.67^c	1.14 ± 7.69^b

Different letters in each column indicate significant differentiation.

Table 2
Total length of *A. urmiana*.

Treatment	Day 3	Day 7	Day 11	Day 15
Control	1.29 ± 0.17^a	2.65 ± 0.52^a	3.73 ± 0.63^a	8.12 ± 1.70^a
β-Glucan	1.35 ± 0.19^c	0.74 ± 2.85^b	0.57 ± 4.93^c	1.84 ± 8.37^c

Different letters in each column indicate significant differentiation.

4. Discussion

In the present study the dietary administration with *S. cerevisiae* strain *YG3-1* in two forms improved the growth in *A. urmiana* and *A. franciscana*. As shown in this study, the highest growth rate was shown in 2ME-treated yeast group (without the mannoprotein and contains bioactive β-glucan). Treatment with 2ME results to the possibility facilitating the action of the digestive enzymes on the yeast biomass by breaking of disulphide linkages between the mannoprotein molecules of the yeast cell wall and giving rise to a more open structure in the cell wall. Furthermore, this treatment resulted in more efficient affecting of β-glucan. Before this, dietary administration of ME treated *S. cerevisiae* has been used for enhancing the growth, function of immune system, survival and resistance of aquatic animals in stress conditions[5]. But the effect of 2ME-treated *S. cerevisiae YG3-1* on the performance of aquatic animals including aquatic invertebrates like *Artemia* has not been studied by now.

In many studies, a wide range of micro-organisms have been used as probiotics for enhancing the growth of aquatic animals[19,20]. Among them, yeasts are particularly interesting because they provide β-glucan and nucleotides that stimulate the immune system of aquatic animals[6]. In this study, new identified yeast *S. cerevisiae YG3-1* was used. Also, deficient cell wall *S. cerevisiae* strains have been reported to have promoting effects on the growth of aquatic invertebrates including *Artemia*. Soltanian *et al*.[8] showed that feeding *Artemia* with isogenic mutant strains of baker's yeast has a big influence on the growth of *Artemia*. Results of their study were showed that compared with wild type yeast, total biomass production of nauplii was significantly improved when the isogenic yeast mutant strains were used as feed, due to both significant higher survival and/or individual length. In the present study, as earlier mentioned dietary administration with 2ME-treated *S. cerevisiae* strain *YG3-1* improved the growth of *Artemia*. This result maybe was obtained due to a considerable increase in survival of *Artemia*. Moreover, influence of yeast quality on performance of gnotobiotically grown *Artemia* was studied by Marques *et al*.[3]. In their study, only in five out of eight experiments, higher total biomass production was obtained when exponentially grown yeast cells were fed to *Artemia* compared to nauplii fed stationary phase grown cells. At the present study, feeding with 2ME-treated stationary phase grown *S. cerevisiae YG3-1* (without the mannoprotein and contains bioactive β-glucan) resulted in the maximum rate of growth in both species of *Artemia*. It is worth mentioning that isogenic mutant strains (deficient cell wall yeasts with genetic background) of baker's yeast (*S. cerevisiae*) were used in both study mentioned above. While, all of yeasts that were used in the present study were wild type yeasts that were treated with chemical treatment.

Many carbohydrates were reported to growth promotion in aquatic invertebrates. Effect of dietary carbohydrate on the growth of prawn was reported by Abdel-Rahman *et al*.[21]. Also, influence of dietary carbohydrate on the metabolism of juvenile *Litopenaeus stylirostris* was studied by Rosas *et al*.[22]. Effects of dietary mannan oligosaccharide on growth performance of juvenile Pacific white shrimp, *Litopenaeus vannamei*, were also determined by Zhang *et al*.[23]. In addition, Andrino *et al*.[24] showed that shrimps fed with mannan oligosaccharide and β-glucan supplemented diets have significantly higher % weight gain than those fed the control diet. Besides, the synergistic effects of β-glucan and mannan oligosaccharide on growth performance of sea cucumber were reported by Gu *et al*.[25]. However, some authors claim that the β-(1→3),(1→6)-glucan derived from yeast *S. cerevisiae* produce the highest biological effects[9].

β-Glucans were reported to growth promotion, enhance non-specific immune responses and disease resistance. According to the Soltanian *et al*.[8], β-glucan can improve the immune system function and growth in aquatic invertebrates such as *Artemia* by affecting on some immune system particles and growth factors. Due to biological effects of β-glucan and chemical treatment with 2ME, feeding with 2ME-treated *S. cerevisiae YG3-1* yeasts (without the mannoprotein and contains bioactive β-glucan) could enhance the growth in *Artemia*. In this study, bioactive β-glucans of cell wall of *S. cerevisiae* strain *YG3-1* might enhance the function of immune system particles and growth factors in *Artemia*.

The purpose of this study was to determine the biological effects of β-glucans of cell wall of *S. cerevisiae* strain *YG3-1* on the growth of aquaqtic invertebrates. So, these claimed effects were evaluated in *Artemia* as model organism with using by a dietary administration. At the present study, the effects of *S. cerevisiae YG3-1* β-glucans on the growth promotion were agreed. This study indicates that 2ME-treated *S. cerevisiae YG3-1* yeast has probiotic properties and can promote the growth of aquatic invertebrates. Also, obtained results of this study suggest that *S. cerevisiae YG3-1* yeasts (specially 2ME-treated yeasts) can be used for enhancing the growth of other aquatic invertebrates such as shrimps as probiotic supplement.

Conflict of interest statement

We declare that we have no conflict of interest.

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