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Research article

EFFECT OF *SACCHAROMYCES CEREVISIAE* ON SURVIVAL, GROWTH, BIOCHEMICAL CONSTITUENTS AND ENERGY UTILIZATION IN THE PRAWN *MACROBRACHIUM ROSENBERGII*

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ABSTRACT: A probiotic yeast, *Saccharomyces cerevisiae* was incorporated in basal diet prepared with fish meal, soybean meal, groundnut oil cake, corn flour, tapioca flour, egg albumin, cod liver oil and vitamin B-complex, at four different concentrations (10g, 20g, 30g and 40g kg^{-1}) and fed to *Macrobrachium rosenbergii* post larvae (PL) for 90 days. The effect of this probiotic incorporation on the growth and survival performances, concentration of protein, amino acid, carbohydrate and lipid, and energy utilization was found to be significantly ($P<0.05$) higher at 40g kg^{-1} followed by 30g, 20g and 10g kg^{-1}. 40g kg^{-1} *S. cerevisiae* incorporation was found to be established the highest rate of colony formation, $234x10^{-4}$ cfu (colony formation units). Actually, presence of *Bacillus* spp., *Bacillus cereus, Pseudomonas* spp., *Escherichia coli*, *Streptococcus* spp., and *Klebsiella pneumoniae* were deducted in water medium and the PL gut of control group. There is a general belief that *Pseudomonas* spp. and *K. pneumonia* are pathogenic to prawns. The establishment of *S. cerevisiae* colony in the gut of experimental PL has eradicated these pathogenic bacteria. Therefore, it is suggested that establishment of *S. cerevisiae* colony has led to better growth, survival and biochemical constituents in *M. rosenbergii* PL. Thus, *S. cerevisiae* could be taken as a useful probiotic in *M. rosenbergii* culture.

Key words: *M. rosenbergii*, *S. cerevisiae*, Survival, Growth, Protein

INTRODUCTION

The freshwater prawn, *Macrobrachium rosenbergii* is a commercially important species in India and south Asian countries. *M. rosenbergii* farming has the potential to revolutionize the rural employment and economy (Radheyshyam 2009). Probiotic organisms are live microorganisms that are thought to be beneficial to the host organism. According to FAO/WHO (2001), probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Probiotics (antonym of antibiotics) include certain bacteria, yeasts and fungi involved in growth of useful microbes, and suppress the growth of harmful ones (Kollath 1953; Parker 1974). Probiotics have been used to improve the growth performance and disease resistance in prawns (Intriago et al. 1998; Scholz et al. 1999; Suralikar & Sahu 2001; Venkat et al. 2004; Keysami et al. 2007; Deeseenthum et al. 2007; Shinde et al. 2008; Saad et al. 2009). Probiotic yeast, *Sacharomyces cerevisiae*, *Sacharomyces exiguous* and *Phaffia rhodozyma* have been reported to increase β-glucan level in penaeid prawns infected with *Vibriosis* (Scholz et al. 1999). Recently, it is reported that in *M. rosenbergii* PL probiotics, Binifit^{TM}, *Lactobacillus sporogenes*, *Bacillus subtilis* have enhanced the growth, survival, biochemical constituents and feed utilization performance (Seenivasan et al. 2011, 2012a, b, 2013). However, there is paucity of literature in relation to *S. cerevisiae* usage in this economically important species of prawn. Therefore, we aimed to study the effect of *S. cerevisiae* on growth, survival, biochemical constituents and energy utilization of *M. rosenbergii* PL.

MATERIALS AND METHODS

The post larvae of the freshwater prawn, *M. rosenbergii* (PL-15; 0.86±0.11cm; 0.014±0.005g) were purchased from a Happy Bay Annexe, Kanchipuram, Tamilnadu, India and acclimatised to ambient laboratory conditions for 15 days (up to PL 30) with ground water (pH, 7.10; total dissolved solids, 0.100 g l^{-1} dissolved oxygen, 7.0 mg l^{-1} BOD, 32.00 mg/L^{-1}; COD, 120.00 mg l^{-1} and ammonia, 0.024 mg l^{-1} in a cement tank (1000 L).

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During acclimatization the PL were fed with *Artemia* nauplii (approximately 10 nauplii/ individual/day) and egg albumin (approximately 10% of body weight) alternatively two times a day.

Diet preparation

The composition of the experimental diets is given in Table 1. *S. cerevisiae* was incorporated with this diet at four different concentrations, 10, 20, 30 and 40g kg^{-1} respectively (*S. cerevisiae*, Intercare Ltd, Gujarat, India, one gram of lyophilized powders contains $10x10^7$ colony formation units (cfu). Diet without *S. cerevisiae* incorporation was served as control. Diet formulation was done by following ''Pearson's square-method'' using determined values of 40% protein content (Table 1). The proportion of each ingredient required was calculated precisely providing allowance for the premix. The dough was steam cooked and cooled at room temperature (28°C). The desired concentrations of *S. cerevisiae* were mixed and the respective dough was pelletized separately. The pellets were dried in a thermostatic oven (M/s. Modern Industrial, Mumbai, India) at 40°C until it reached constant weight and stored in airtight jars at room temperature. The concentrations of total protein, amino acid, lipid and carbohydrate were determined by following the standard methods (Lowry et al. 1951, Moore & Stein 1948, Folch et al. 1957, and Roe 1955 respectively). The ash and moisture contents were determined by adopting the method of APHA (2005). These diets were freshly prepared after every 30 days to ensure high probiotic viability throughout the duration of feeding trail. In the control diet, no *S. cerevisiae* was added.

Table 1. Proportion of ingredients and concentrations of biochemical constituents in formulated feeds

Ingredients (g/kg^{-1})	Control	*S. cerevisiae* incorporated feed			
		1% SC	2% SC	3% SC	4% SC
Fish meal	33.84	33.84	33.84	34.84	35.84
Ground nut oil	25.00	25.00	25.00	25.00	24.00
Soybean meal	24.00	24.00	23.00	21.00	20.00
Corn flour	4.00	3.00	3.00	3.00	3.00
Egg albumin	5.06	5.06	5.06	5.06	5.06
Topica flour	5.10	5.10	5.10	5.10	5.10
Cod liver oil	2.00	2.00	2.00	2.00	2.00
B-complex Vitamin mix	1.00	1.00	1.00	1.00	1.00
S. cerevisiae (SC)	0	1	2	3	4
Total	100	100	100	100	100
Biochemical Constituents					
Protein (%)	40.10	40.00	39.63	39.52	39.40
Carbohydrate (%)	21.76	21.10	20.71	20.01	19.50
Lipid %)	9.28	9.24	9.17	9.08	8.90
Ash (%)	14.00	13.00	12.00	13.00	14.00
Moisture (%)	9.50	9.90	9.40	9.10	9.10
Digestible energy ($k.cal/kg^{-1}$)	3296.86	3262.52	3228.17	3193.83	3159.49

Feeding experiment

M. rosenbergii (PL-30; 1.60±0.02cm and 0.24±0.04g) were subjected to feeding trial for a period of 90 days. Five groups of 40 PL each were maintained in 20 L capacity plastic tanks in triplicate. One group served as control and fed with feed devoid of *S. cerevisiae*. The experimental groups were fed with the respective concentrations of *S. cerevisiae* incorporated feeds two times a day (6:00 am and 6:00 pm) at 10% of body weight. The water (25°C) was renewed daily and aerated. The unfed feed, faeces and moult if any were collected.

Determination of growth parameters

On the final day of feeding trial the growth parameters, such as survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), food conversion efficiency (FCE) and protein efficiency rate (PER) were calculated by following the formulae adopted by Tekinay & Davis (2001).

Energy utilization

The energy content of whole prawns, feeds, moult and faeces was measured using Oxygen Bomb Calorimeter (230 VAC; Sl. No. 26036; Advance Research Instrument Company, India). The energy utilization was calculated using the equation (C = (P+E) + R + F +U) derived by Petrusewicz & Macfadyen (1970); where, C is the energy consumed; P is the growth; R is the material lost as heat due to metabolism; F is the energy lost through faeces; U is the energy lost through NH_3 excretion and; E is the energy lost through exuvia.

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Estimation of food consumed (C)

Each group of PL was uniformly fed with respective feed two times a day (6:00 am and 6:00 pm) at 10% of body weight. Every day, the unconsumed food was separately collected into a filter by the siphoning method and dried in a hot air oven 90^{o}C for 48 h and weighed. To estimate the dry weight of food consumed, a sample of food was dried everyday and the dry weight of the unconsumed food was subtracted from the dry weight of the food offered.

Estimation of faeces (F)

Actually, the thin thread like faeces were siphoned out using filler then and there (once in hour) and dried on muslin cloth (blotting silk-filter). This was then dried in a hot air oven at 90^{o}C for 24 h, weighed and stored.

Estimation of nitrogenous excretory product (U*)*

The daily excretion of ammonia by the prawn was estimated after feeding as per the phenol hypochloride method of Solorzano (1969). The energy loss occurring by ammonia excretion was calculated using the ammonia calorific quotient 1 mg NH_3: 5.9 cal. (Elliot, 1976).

Estimation of exuvia (E)

During daily renewal of test medium the moults, if any were collected. After blotting the adhering water, the moults were weighed and dried overnight in a hot air oven (90^{o}C and the dry weight was then recorded. The number of moults per individual was also observed. Since exuvia (E) constitutes part of the converted energy in crustaceans, in the present study, the energy loss through exuvia was considered to be part of conversion. The production of new tissues (= conversion P) was calculated by adding the exuvial weight to the gain in total weight of the prawn and the actual growth was calculated subtracting the exuvial weight from the gain in total weight.

Estimation of growth (P)

The term conversion has been used to refer to growth, *i.e*., P of the IBP terminology. As already mentioned above, prior to the commencement of the experiment, the test prawns were starved for 24 h in order to evacuate the undigested food consumed the previous day. Subsequently, the wet (live) weight of the each individual was determined at the commencement of the experiment. To estimate the initial dry weight of the test individuals the 'sacrifice method' was adopted (Maynard & Loosli, 1962). A group of five juvenile prawns of similar live weight and experimental state served as control to determine the initial weight and energy content. These prawns were sacrificed and dried in a hot air oven at 90^{o}C till they attained constant weight. The dry weight and energy content of these prawns were considered to represent those of the test individual at the commencement of the experiments.

On the 90th days, the morphometric data, such as the final length and weight of all prawns in triplicate was measured; the prawns were then sacrificed and dried in a hot air oven as defined above to estimate the energy content.

The food energy consumed was measured as the difference between the energy content of food offered and that of the uneaten food. The quantity of absorbed food energy was calculated by subtracting F and U from C. Conversion of growth is the sum of energy channelled to somatic growth (P) and exuvia (E). The efficiency of absorption was calculated by relating the food absorbed to the food consumed. Feeding rate (FR), absorption rate (AR), conversion rate (CR) and metabolic rate (MR) were all calculated by dividing the respective amounts of energy by initial live weight of the prawn per unit time in days. Following the estimations of C, F, U, and P, the metabolism (R=Respiration, material lost as heat) was calculated.

Biochemical constituents of the experimental animals

On the initial and final days of the experiment, the biochemical constituents of PL were determined. The biochemical constituents, such as total protein, amino acid, lipid, carbohydrate, ash and moisture contents of individual diet fed prawns were analyzed as described previously.

Microbial studies

The control water and the gut of control and experimental PL fed with 40 g Kg^{-1} *S. cerevisiae* incorporated diet (the best concentration) were subjected to bacterial culture. The mobility of experimental PL was suppressed by keeping them in freezer at -20°C for 10 minutes. Then the surface was sterilized with 50 ppm formalin for 30 seconds in order to remove the external flora. Then the digestive tract was dissected out individually and homogenized with phosphate buffered saline (pH 7.2) under aseptic condition. Afterwards the homogenate were serially diluted up to 10^{-5} dilution individually. From this 0.5 ml of aliquots were taken and mixed with agar nutrient broth for 24 h at 35^{o}C. 0.1 broth culture was seeded over the surface of freshly prepared nutrient agar plates and incubated at 37°C for 24h.

The presence allochthonous and autochthonous microbiota belongs to *Bacillus* spp., *Bacillus cereus, Pseudomonas* sp, *Escherichia coli*, *Streptococcus* spp., and *Klebsiella pneumoniae* colonies were identified and they were confirmed through routine bacteriological tests (Holt et al. 1996). The following tests, such as gram's staining, motility test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, starch hydrolases, gelatin hydrolases, nitrate reduction test, oxidase test, catalase test, glucose test, lactose test, sucrose test and manitol test were conducted. Yeasts are a heterogenous group of fungi that superficially appear to be homogeneous. Yeasts grow in a conspicuous unicellular form that reproduces by fission, budding, or a combination of both. True yeasts reproduce sexually, developing ascospores or basidiospores under favorable conditions. The majority of ascomycetous and basidiomycetous yeasts isolated were unrecognized because most of them are heterothallic. In most instances, only one of the mating types isolated and therefore no asci or basidia are produced (Bowman and Ahearan 1975).

Colony isolation on SAB agar

A small portion of the yeast to be decontaminated was suspended in sterile distilled water. A loopful of the suspension was streaked onto a plate of SAB agar and incubated at 30^{o}C for 48 hours. The isolated colonies were examined. Colony isolation on SAB agar was also done with chloramphenicol, penicillin and streptomycin. Direct mounts were made in order to study yeast morphology microscopically and to determine the purity of the isolates.

Lactophenol Mount

This is the most widely used method of staining and observing fungi. A small drop of lactophenol (LP) was placed on a clean glass microscope slide. A small portion of the yeast colony was placed on the drop of LP, suspended the cells, covered with a clean cover glass and sealed the edges with fingernail polish to temporarily preserve the mount.

Ascospore induction and detection

One step in indentifying yeast involves determining whether or not the isolate has the ability to form ascospores. Some yeast will readily form ascospores on primary isolation medium, whereas others require special media. The ability to form ascospores varies from isolate to isolate and may be lost in old laboratory strains. If only one mating type of heterothallic yeast is present, no ascospores will be formed. Ascospore media contain small amount of carbohydrates; this restricts vegetative growth while enhancing ascospore formation.

The yeast was inoculated to yeast malt agar for enrichment, incubated for 3 days. The yeast from the yeast malt agar was inoculated to a V-8 juice agar slant. Incubated aerobically at 20-25^{o}C. Most freshly isolated strains begin forming ascospores in 1-2 days. Older stock cultures usually require a longer period of time. The culture was examined in 3-5 days and weekly thereafter for 3 weeks.

A wet mounts of the yeast in distilled water was prepared and examined under the oil immersion lense. Ascospore form, surface topography, size, colour, brims, number of ascospores per ascus, and the presence or absence of inclusion bodies were characteristics used in part to identify the various species.

The yeast colony was enumerated with the following formula,

$$\text{Colony formation unit (CFU/ g)} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume of sample (g)}}$$

Data are reported as means ± standard deviations. One way analysis of variance (ANOVA) using SPSS (version 13.0) was applied to determine whether significant variations between the treatments existed. Difference between means were determined and compared by DMRT test and the significances are expressed.

RESULTS AND DISCUSSION

Morphometric analysis

The morphometric measurement of *S. cerevisiae* incorporated feed fed PL is presented in Table 2. The initial body length and weight of PL was 1.60±0.02 cm and 0.24±0.04 g respectively. The final length and weight were significantly higher (F=11.28 length; 28.44 weight, P<0.05) in 40 g Kg^{-1} *S. cerevisiae* incorporated feed fed PL followed by the 30 g, 20 g and 10 g Kg^{-1} Similar increase in morphometric values has been reported in *M. rosenbergii* PL fed with commercial diets containing 10^{7} cfu ml^{-1} of *Baciilus* spp., KKU02 and *Baciilus* spp., KKU03 (Deeseenthum et al. 2007). *Bacillus* spp., enhanced growth in the tiger shrimp, *Penaeus monodon* has also been reported (Boonthai et al. 2011). It has been reported that a concentration of $5x10^{6}$ g^{-1} of Lactic acid bacteria (*Lactobacillus acidophilus, Streptococcus cremoris*, *Lactobacillus bulgaricus*-56 and *L. bulgaricus*-57) supplemented diets influences the growth enhancement in white prawn, *Penaeus indicus* (Fernandez et al. 2011).

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Growth and survival

In this study, *S. cerevisiae* supplemented diets produced growth promotion in *M. rosenbergii* PL (Table 2) when compared with control diet (P<0.05). The WG and SGR were found to be increased in 40 g Kg^{-1} *S. cerevisiae* incorporated diet fed PL followed by the 30 g, 20 g and 10 g Kg^{-1} (F=29.54, WG & 82.44, SGR; P<0.05). Similar enhancement in WG and SGR has been reported in *M. rosenbergii* fed with *Lactobacillus cremoris*, *L. sporogenes* and *L. acidophillus* incorporated diets (Suralikar & Sahu 2001; Venkat et al. 2004).

The lower FCR recorded in experimental PL reflects the superior quality of the feeds formulated with *S. cerevisiae* incorporation (F=21.98, P<0.05). Similar result in FCR has been reported in *M. rosenbergii* PL fed with different probioitics incorporated diets (Shinde et al. 2008). In this study, the FCE and PER were found to be increased in 40 g Kg^{-1} *S. cerevisiae* incorporated diet fed PL followed by the 30 g, 20 g and 10 g kg^{-1} (F=14.04 & 32.18, P<0.05) when compared with control (Table 2). It has been reported that brewers' yeast supplemented diets improved the FCE in striped bass, *Menticirrhus saxatilis* (Li et al. 2005). Similarly *S. cerevisiae* supplemented diets improved the PER in *M. rosenbergii* (Venkat et al. 2004). The survival rate was found to be significantly increased (F=3.32, P<0.05) in PL fed with 40 g Kg^{-1} SC incorporated diet (87%) followed by 30 g, 20 g and 10 g Kg^{-1} when compared with control, 80% (Table 2). An increase in survival have also been reported in Indian white shrimp, *Fenneropenaus indicus* and in *M. rosenbergii* fed with Biogen® and other probiotics incorporated diets (Ziaei-Nejad et al. 2006; Shinde et al. 2008; Saad et al. 2009).

Biochemical constituents

The content of total protein, amino acid, carbohydrate, lipid and ash were found to be increased in 40 g kg^{-1} *S. cerevisiae* incorporated diet fed PL followed by 30 g, 20 g and 10 g kg^{-1} (P<0.05) when compared with control (Table 2). In the case of moisture content just the reverse was recorded. A similar elevation in biochemical constituents was previously reported by us in *M. rosenbergii* PL fed with *L. sporogenes* bio-encapsulated *Artemia* (Seenivasan et al. 2012a). An increase in the carcasses biochemical proximate composition has been reported in *M. rosenbergii* PL fed with *L. sporogenes* and *L. acidophilus* supplemented diets (Venkat et al. 2004) and Biogen® supplemented diets (Saad et al. 2009). Increase in biochemical composition has also been reported in marine prawn, *Litopenaeus vannamei* fed with *Bacillus spp.*, supplemented diet (Yu et al. 2009).

Table 2. Morphometeric data, growth performance, biochemical constituents and energy utilization in *M. rosenbergii* PL fed with *S. cerevisiae* incorporated diets

Parameters	Control diet	Experimental diet (*S. cerevisiae* incorporated)				F-Value
		1% SC	2% SC	3% SC	4% SC	
Initial length (cm)	1.60±0.02	1.60±0.02	1.60±0.02	1.60±0.02	1.60±0.02	-
Final length (cm)	4.00^{c}±0.20	4.72^{b}±0.28	4.76^{b}±0.25	4.80^{b}±0.21	5.22^{a}±0.18	11.28
Initial weight (g)	0.24±0.04	0.24±0.04	0.24±0.04	0.24±0.04	0.24±0.04	-
Final weight (g)	1.00^{c}±0.08	1.48^{b}±0.15	1.80^{a}±0.18	1.92^{a}±0.13	2.00^{a}±0.10	28.44
SR (%)	80.00^{b}±2.50	82.50^{ab}±3.00	85.00^{ab}±3.00	87.50^{a}±2.50	82.50^{b}±2.50	3.32
WG (g)	0.76^{c}±0.08	1.24^{b}±0.11	1.56^{a}±0.14	1.68^{a}±0.18	1.76^{a}±0.12	29.54
SGR (%)	0.688^{d}±0.031	0.877^{c}±0.023	0.972^{b}±0.032	1.003^{ab}±0.027	1.02^{a}±0.014	82.44
FCR (g)	3.47^{a}±0.20	2.67^{b}±0.15	2.40^{bc}±0.18	2.30^{c}±0.19	2.20^{c}±0.22	21.98
FCE (%)	0.78^{c}±0.07	1.21^{b}±0.10	1.50^{ab}±0.20	1.58^{a}±0.18	1.62^{a}±0.21	14.04
PER (g)	0.63^{c}±0.04	0.82^{b}±0.03	0.92^{a}±0.05	0.96^{a}±0.04	1.00^{a}±0.06	32.18
Protein (%)	56.40^{b}±2.84	58.60^{ab}±2.60	59.40^{ab}±2.32	62.10^{a}±2.80	63.40^{a}±2.67	3.33
Amino acid (%)	22.80^{b}±2.51	24.20^{b}±2.82	25.90^{ab}±2.94	27.30^{ab}±2.70	29.90^{a}±2.86	2.97
Carbohydrate (%)	11.0^{c}±1.20	13.04^{bc}±1.71	15.10^{ab}±1.84	16.66^{a}±1.52	17.04^{a}±1.18	8.44
Lipid (%)	7.00^{c}±0.33	8.40^{bc}±1.04	9.63^{b}±1.08	11.99^{a}±1.00	13.07^{a}±1.67	15.30
Ash (%)	16.30^{b}±1.19	17.80^{ab}±1.30	17.00^{ab}±1.36	18.00^{ab}±1.28	19.00^{a}±1.40	1.84
Moisture (%)	76.10^{a}±3.00	76.40^{a}±3.12	76.80^{a}±3.28	76.40^{a}±3.05	75.60^{a}±3.14	<1
FR (k.cal.g.d^{-1})	0.350^{b}±0.019	0.383^{c}±0.085	0.430^{d}±0.092	0.452^{a}±0.061	0.477^{b}±0.048	12.32
AR (k.cal.g.d^{-1})	0.303^{b}±0.054	0.342^{bc}±0.042	0.392^{cd}±0.038	0.418^{a}±0.099	0.447^{b}±0.023	11.43
CR (k.cal.g.d^{-1})	0.208^{a}±0.086	0.237^{ab}±0.048	0.262^{bc}±0.072	0.288^{a}±0.067	0.307^{b}±0.031	13.02
AE (k.cal.g.d^{-1})	0.01^{a}±0.005	0.013^{a}±0.006	0.015^{ab}±0.004	0.017^{a}±0.009	0.019^{b}±0.011	1.67
MR (k.cal.g.d^{-1})	0.106^{a}±0.019	0.118^{a}±0.030	0.145^{a}±0.028	0.147^{a}±0.031	0.189^{a}±0.022	5.93

Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).

SC: *S. cerevisiae*; SR: Survival; WG: Weight gain; SGR: Specific growth rate; FCR: Feed conversion ratio; FCE: Feed conversion efficiency; PER: Protein efficiency rate; FR: Feeding rate; AR: Absorption rate; CR: Conversion rate; AE: NH_3 Excretory rate; MR: Metabolic rate.

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Energy utilization performance

In this study, there were significant increases in the feeding rate, absorption rate, conversion rate, NH_3 execratory rate and metabolic rate in PL fed with 40 g kg^{-1} *S. cerevisiae* incorporated diet followed by 30 g, 20 g and 10 g kg^{-1} ($P<0.05$) when compared with control (Table 2). Similar result has been reported in pearl spot, *Etroplus suratensis* fed with *Lactobacillus* and yeast supplemented diets (Immanuel et al., 2003). Probiotics influences the digestive process by enhancing the beneficial gut microflora, which in turn ultimately enhances absorption of food and food utilization (Bomba et al., 2002).

Microbial study

The qualitative bacterial study showed presence of *Bacillus* spp., *Bacillus cereus, Pseudomonas* spp., *Escherichia coli, Streptococcus* spp., and *Klebsiella pneumonia* (*Bacillus* spp., *Pseudomonas* spp., and *Streptococcus* spp., were identified up to genus level only) in the control water medium and control PL gut (Table 3 & 4). In the experimental PL (fed with 40 g kg^{-1} *S. cerevisiae* incorporated diet, the best concentration), the presence of *K. pneumonia* was replaced by establishment of *S. cerevisiae* ($234x10^{-4}$ cfu cells) colonies (Table 5). The presence of above said six isolates were identified as bacterial colonies and confirmed through routine biochemical tests (Tables 3-6). In *M. rosenbergii* PL the establishment of the probiotic bacteria, *L. sporogenes* and *L. acidophilus* have been reported (Venkat et al. 2004; Seenivasan et al. 2012a). The replacement of *Vibrio spp*., by *Bacillus* S11 in the shrimp, *P. monodon* increases survival rate (Rengpipat et al. 1998).

In the present study, establishment of *S. cerevisiae* colony might have improved the general intestinal health, thereby increases digestion and absorption of nutrients and ultimately enhances the accumulation of biochemical constituents. Which in turn facilitate better growth and survival of *M. rosenbergii*. Therefore, the result of the present study recommends inclusion of *S. cerevisiae* in feed formulation for sustainable development of freshwater prawn culture.

Table 3. Biochemical characterization of isolates identified in control water

Tests	*Bacillus* spp.,	*B. cereus*	*Pseudomonas* spp.,	*E. coli*	*Streptococcus* spp.,	*K. pneumonia*
Gram's Staining	+	+	-	-	+	-
Motility test	+	+	+	+	+	-
Indole Test	-	-	-	+	-	-
Methyl red Test	-	-	-	+	-	-
VP Test	-	-	+	-	+	+
Citrate Utilization Test	+	+	+	-	+	+
Starch hydrolases	+	+	-	+	+	+
Gelatin Hydrolases	+	+	+	+	+	+
Nitrate reduction Test	+	+	-	+	+	+
Oxidase Test	-	-	+	+	-	+
Catalase Test	+	+	+	-	-	+
Glucose Test	A	A	A	A	A	A
Lactose Test	A	A	NA	A	A	A
Sucrose Test	A	A	A	A	A	A
Manitol Test	A	A	A	A	A	A

+, Positive; - Negative; A, Acid production; NA, No acid production

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Table 4. Biochemical characterization of isolates identified in control PL gut

Tests	*Bacillus* spp.,	*B. cereus*	*Pseudomonas* spp.,	*E. coli*	*Streptococcus* spp.,	*K. pneumonia*
Gram's Staining	+	+	-	-	+	-
Motility test	+	+	+	+	+	-
Indole Test	-	-	-	+	-	-
Methyl red Test	-	-	-	+	-	-
VP Test	-	-	+	-	+	+
Citrate Utilization Test	+	+	+	-	+	+
Starch hydrolases	+	+	-	+	+	+
Gelatin Hydrolases	+	+	+	+	+	+
Nitrate reduction Test	+	+	-	+	+	+
Oxidase Test	-	-	+	+	-	+
Catalase Test	+	+	+	-	-	+
Glucose Test	A	A	A	A	A	A
Lactose Test	A	A	NA	A	A	A
Sucrose Test	A	A	A	A	A	A
Manitol Test	A	A	A	A	A	A

+, Positive; - Negative; A, Acid production; NA, No acid production

Table 5. Biochemical characterization of isolates identified in Experimental PL gut

Tests	*Bacillus* spp.,	*B. cereus*	*Pseudomonas* spp.,	*E. coli*	*Streptococcus* spp.,	*K. pneumonia*
Gram's Staining	+	+	-	-	+	-
Motility test	+	+	-	+	+	-
Indole Test	-	-	-	+	-	-
Methyl red Test	-	-	-	+	-	-
VP Test	-	-	-	-	+	-
Citrate Utilization Test	+	+	-	-	+	-
Starch hydrolases	+	+	-	+	+	-
Gelatin Hydrolases	+	+	-	+	+	-
Nitrate reduction Test	+	+	-	+	+	-
Oxidase Test	-	-	-	+	-	-
Catalase Test	+	+	-	-	-	-
Glucose Test	A	A	-	A	A	-
Lactose Test	A	A	-	A	A	-
Sucrose Test	A	A	-	A	A	-
Manitol Test	A	A	-	A	A	-

+, Positive; - Negative; A, Acid production; NA, No acid production

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Table 6. Overall result of microbial load in control water, control PL and experimental PL

Isolates	Control water (10^{-5})	Control PL gut	Experimental PL gut (40g kg^{-1} SC)
Bacillus spp.,	P	P	P
B. cereus	P	P	P
Pseudomonas spp.,	P	P	A
E. coli	P	P	P
Streptococcus spp.,	P	P	P
K. pneumoniae	P	P	A
S. cerevisiae	A	A	P 234x10^{-4}cfu cells

P, present; A, absent; SC, *S. cerevisiae.*

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