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Effects of Salinity on Acid Production and Growth of Three Probiotic Microbes with Potential for Application in Intensive Shrimp Aquaculture

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Abstract

Shrimp has been among the top value-added products targeted for production by the aquaculture industry. The increasing demand for shrimp has led to a massive increase in production in several countries across the world. Intensive and super-intensive production systems are facing great challenges handling newly emerging shrimp diseases. The use of antibiotics was one of the first approaches when dealing with such diseases, but the effects of misusing antibiotics and the appearance of antibiotic resistant bacteria are of public concern. As an alternative, probiotics have been applied in aquaculture systems to increase disease resistance, improve feed efficiency, maintain water quality and enhance the growth of aquatic organisms. In this study, the ability of three probiotic microbes to tolerate salinity levels commonly found in intensive shrimp production systems were evaluated. *Lactobacillus casei*, *Saccharomyces cerevisiae* and *Rhodopseudomonas palustris* were cultured in MRS broth, yeast and mold broth, and Van Neil's broth, respectively, enriched with 1 and 2% NaCl. Microbial survival between treatments were compared as well as the metabolic activity in terms of acidity levels. Additionally, cell morphology was compared using scanning electron microscopy. *L. casei* and *S. cerevisiae* showed no significant differences ($P>0.05$) in media with 1% and 2% NaCl in terms of microbial survival and media acidity levels at 24 h. *R. palustris* showed a prolonged lag phase extending up to 12 h in 1% and 48 h in 2% NaCl media, and acidity of the media did not vary significantly. Cell morphology of all microbes did not change significantly across all treatments. From these results, it was concluded that *L. casei*, *S. cerevisiae* and *R. palustris* are suitable for application in aquaculture ponds with up to 2% salinity.

Keywords: Probiotics; Lactic acid bacteria; Yeast; Photosynthetic bacteria; Salinity; Aquaculture

Introduction

In the last decade, the aquaculture industry has experienced a steady increase in the demand for seafood products, whereas the wild fishery practice has been stagnant [1]. A significant proportion of the total fishery trade consists of high-value products, among which are shrimp, salmon, tuna, bass, and bream. According to the State of World Fishery and Aquaculture report [1], farmed shrimp production has increased in recent years. However, the ranking of major producing countries has shifted mainly due to the emergence of devastating diseases, which caused Thailand to be removed from the list of the top three world producers. Newly emerging diseases are attributed not only to the high density of microbial communities and intensive production practices [2], but also to the excessive use of antibiotics as the first mitigation strategy against opportunistic pathogens [3].

The use of antibiotics in the animal industry began as a necessity to lessen the economic impact of diseases that originated due to high population densities in production units. Even though the contribution of antibiotics used in animal production towards the evolution of resistant bacteria is unknown, the overuse of these is considered a problem [4], resulting in more rigorous norms and regulations regarding their application in aquaculture systems [5].

It has been well documented that the increasing use of antibiotics in high density shrimp ponds [6,7] has contributed to a higher pool of resistance genes. Bacteria isolated from water and sediments collected from shrimp farms in the mangrove areas of Vietnam showed high incidences of antibiotic resistance, specifically to trimethoprim and sulfamethoxazole [8,9]. In April 2015, 342 packages of wild and farmed (cultured), raw and cooked shrimp were evaluated in the US, and methicillin-resistant *Staphylococcus aureus* (MRSA) was found in 60% of the samples [10]. Also, 5% of the farmed shrimp samples contained residues of antibiotics such as oxytetracycline, enrofloxacin, and sulfa antibiotics [10].

One of the alternative strategies to the use of antibiotics is the use of probiotics. Probiotics could provide the following benefits to an aquaculture ecosystem: modification of microbial communities, efficient feed absorption, enhanced response to pathogens, and improvement in the quality of the environment [11]. Probiotics applied in aquaculture ponds could help maintain health and enhance performance of aquatic animals [12,13]. For instance, administering a mixture of *Bacillus* spp. and non-pathogenic *Vibrio* spp. in shrimp feed had a positive effect on the growth and survival of Pacific white shrimp larvae, and provided protection against the pathogen *V. harveyi* and the white spot syndrome virus (WSSV) [14]. Moreover, a study on the effect of a probiotic (used for human consumption), *L. rhamnosus* against *Aeromonas salmonicida*, the causative agent of furunculosis in rainbow trout, concluded that *L. rhamnosus* significantly reduced mortality to one third compared to the mortality in the control group [15]. Nonetheless, not all probiotics have the same properties. Suitable candidates must not only be safe for human and animal consumption, but also be able to thrive in the conditions of salinity, typical of shrimp ponds. Potential candidates must also be tested in a systematic manner

[16] *in vitro* and in field conditions. The purpose of the present study was to evaluate the growth and/or survival of three microbes (with potential to be used as probiotics) in two different salinity conditions, in order to be further evaluated for application in shrimp aquaculture.

Materials and methods

Probiotic microbes and culture conditions

The three microorganisms used in this study included: a lactic acid bacterium (LAB) (*Lactobacillus casei* NBRC 15883), a yeast (Y) (*Saccharomyces cerevisiae* NBRC 0333) and a photosynthetic bacterium (PB) (*Rhodopseudomonas palustris* NBRC 100419). Certified pure freeze-dried cultures were purchased from Nite Biological Resource Center (NBRC, Chiva, Japan). Lactic acid bacteria were revived by preparing three consecutive overnight cultures in Criterion™ *Lactobacilli* deMan, Rogosa and Sharpe broth (MRS Broth) (Hardy Diagnostics, Springboro, OH) and incubating at 37°C for 24 h. Subsequently, the final culture was plated on MRS Agar and incubated under the same conditions. Then, colonies were randomly selected for identification and further confirmation using Gram staining and DNA sequencing. The permanent stock culture was prepared according to Li et al. [17] with the modification of the percentage of glycerol. The bacterial culture was suspended in 2 ml tubes containing MRS broth supplemented with 40% (v/v) glycerol and stored at -80°C (TSX Ultra-Low Freezer, Thermo Fisher Scientific, Inc, Asheville, NC).

Yeast was cultured on Difco™ yeast mold (YM) broth (Becton, Dickinson and Company, Sparks, MD) and incubated at 30°C for 24 h, followed by plating on YM broth with 1.5% agar [18]. Colonies were randomly selected for identification and further confirmation. Colonies were stained with crystal violet and observed under a compound light microscope (TMS-F, Nikon, Japan). In addition, DNA was extracted from the culture and sequenced for confirmation. The permanent stock culture was suspended in 2 ml tubes containing YM broth supplemented with 40% (v/v) glycerol and stored at -80°C.

Photosynthetic bacteria were cultured on Van Neil's medium (VN broth) prepared using the following composition: 0.1% K_2HPO_4 (Spectrum Quality Products, Inc., Gardena, CA), 0.05% $MgSO_4$ (Fisher Scientific, Fair Lawn, NJ), 1% yeast extract (Hardy Diagnostics, Springboro, OH) and supplemented with 0.20% of Bacto™ peptone (Becton, Dickinson and Company, Sparks, MD). Photosynthetic bacteria were cultured in transparent transport tubes previously filled with VN broth and placed under full spectrum light at an intensity of approximately 4,000 lux for 48 h [19]. Successively, culture was plated on VN broth with 1.5% agar and incubated for 48 to 72 h under the same conditions. Bacteria were Gram-stained and sequenced for confirmation. The permanent stock culture was suspended in 2 ml tubes containing VN broth supplemented with 40% (v/v) glycerol and stored at -80°C.

Confirmation of probiotic microbes

Microbial DNA was extracted using the Mo Bio PowerWater® DNA isolation kit (Mo Bio Laboratories, Inc, Carlsbad, CA) following the manufacturer's protocol for water samples. Test microbes were confirmed using primers 515F and 806R [20,21] for lactic acid bacteria and photosynthetic bacteria, and ITS1 and ITS4 [22] for yeast. The bacterial primers amplified a segment of approximately 250 base pairs, whereas yeast primers amplified a segment of 800 base pairs. PCR products were sequenced by conventional Sanger sequencing at the University of Arizona Genetics Core where a 3730 Automated DNA Analyzer (Applied Biosystems, Foster City, CA) was used to generate sequences. Identification was based on best matches obtained in the Basic Local Alignment Search Tool (BLAST) [23] with an e-value cutoff of 0.0 and maximum identity of 95% or greater.

Preparation of microbial working stock

Frozen cultures of the bacteria and yeast were revived two consecutive times. The incubation conditions for each microbe were as described previously. After the second revival, microbes were cultured one additional time under the same conditions. However, the liquid nutrient media (MRS broth, YM broth and VN broth) were modified to evaluate salt tolerance by adding either 1% or 2% NaCl. All treatments and the control were cultured in duplicates for each of the three repeats.

Determining the salt tolerance of probiotic microbes

Salt tolerance was determined following the protocol described by Succi et al. [24] with modifications. Each bacterium and yeast were grown in liquid broth containing 1% or 2% NaCl. Controls were grown without adding salt to the nutrient media. For the lactic acid bacteria and yeast, a 50 µl aliquot of working stock at 15 hours of growth was dispensed into a 50 ml sterilized transport tube previously filled with lactobacilli MRS broth containing 1% or 2% salt (for lactic acid bacteria) or YM broth containing 1% or 2% salt (for yeast), and incubated under the conditions previously described. Samples were taken at 3 h time intervals for 24 h and two additional samples taken at 48 and 72 h, respectively, for enumeration of survivors. Samples were serially diluted using 0.1% peptone water containing 0, 1, or 2% NaCl. Consequently, dilutions were plated in the respective agar for each microbe and incubated for 24 to 48 h under the conditions previously described.

For the photosynthetic bacteria, a 250 µl aliquot of working stock at mid-exponential phase was dispensed into a 250 ml glass flask to allow maximum light exposure. The flask was prefilled with VN broth containing 0, 1 and 2% salt and incubated under the conditions previously described. Samples were taken every 12 h for the first 24 h and at 24 h intervals thereafter for 5 days, for enumeration of survivors. Serial dilutions were made in 0.1% peptone water with 0, 1 or 2% salt, and plated on VN agar for photosynthetic bacteria. Incubation conditions were as described previously.

Enumeration and pH measurements

Every sample was spread plated in duplicates and all experiments were repeated three times for statistical significance. Furthermore, pH values of each culture were measured using a pH meter Beckman 300 (Beckman Instruments, Inc., Fullerton, CA) at each sampling time point to correlate media acidity levels with cell growth. Additionally, the data obtained for each growth curve were graphed in a scatter plot using the surviving microbial population in $\log_{10}$ CFU ml^{-1} over time.

Visualization of probiotic microbes using scanning electron microscopy

The effects of adding NaCl to the liquid broth media on probiotic microbes were observed using field emission scanning electron

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microscopy (SEM). Samples consisted of lactic acid bacteria and yeast grown in MRS broth and YM broth, respectively, containing 2% NaCl, and photosynthetic bacteria grown in VN broth containing 1% NaCl. The controls consisted of the three microorganisms grown in their respective broths without NaCl. An aliquot of 200 µl of microbial cultures at 20 hours of growth were transferred onto poly-L lysine coated glass slides (Sigma-Aldrich, Saint Louis, MO), and incubated for 2 h at room temperature. Samples were fixed following the protocol described by Lv et al. [25] and McQuade et al. [26] with modifications. Samples were fixed by adding 25 µl of 8% glutaraldehyde solution (Electron Microscopy Sciences, Hatfield, PA). After 40 min of incubation at room temperature, samples were washed three times with 1X phosphate buffered saline (PBS, pH 7.0) (Fisher Scientific, Fair Lawn, NJ). Subsequently, samples were washed with water and stained with 1% osmium tetroxide (Ted Pella, Inc., Redding, CA). Finally, samples were washed in water, and dehydrated in a series of ethanol (Fisher Scientific, Fair Lawn, NJ) solutions with concentrations ranging from 30% to 90% (v/v). The slides with fixed samples were mounted, sputter-coated in platinum under vaccum, and examined using a Hitachi S-4800 field emission scanning electron microscope (Hitachi High-Technologies Corporation, Japan).

Data processing and statistical analyses

Data processing and statistical analyses were conducted using R (R Core Team 2016) and statistical packages PMCMR [27] and STATS [28]. The Shapiro-Wilk normality test was used to determine the normality of the data [29]. For data following normal distribution, the Analysis of Variance (ANOVA) at a 5% significance level was followed by post-hoc analysis Tukey's Honest Significance Test. If data were not normally distributed, the nonparametric analysis of variance Kruskal Wallis test was utilized with pairwise comparisons using Tukey's and Kramer's Nemenyi test with Tukey-Distribution approximation for independent samples [21,27,30].

Results

Salt tolerance of the lactic acid bacteria

In general, growth curves of lactic acid bacteria in MRS broth with 1% and 2% NaCl depicted similar growth phases as the control with no significant difference (P>0.05). The bacteria reached logarithmic phase in all treatments within 6 h after incubation and this phase lasted 15 h. Control and treatment with 1% NaCl reached 9 $\log_{10}$ CFU ml^{-1} at 21 h. While there was no significant difference in bacterial survival between treatments and the control, treatment with 2% NaCl reached 9 $\log_{10}$ CFU ml^{-1} 3 h after the control. In addition, stationary phase was reached simultaneously in all treatments (Figure 1a). Boxplot analysis of surviving bacterial populations showed a similar data distribution in all treatments (Figure 2a), exhibiting median values with no significant difference between treatments and the control (P>0.05) (Figure 2b).

There was no significant difference (P>0.05) in the media acidity levels between the control and treatments. However, the results showed higher pH values between 12 and 21 h in treatments with 1 and 2% NaCl when compared to the control, indicating a potential delay in producing organic acids. After 24 h, the pH was similar in all treatments reaching levels of less than 4.0 (Figure 1b).

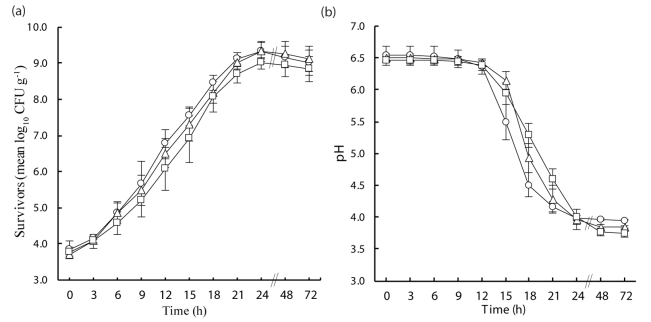
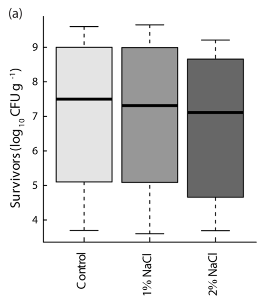


Figure 1: (a) Growth curves of *L. casei* and (b) pH values of MRS broth at different salt concentrations. Data shown are mean ± SD. (O) Control, (Δ) 1% NaCl and (□) 2% NaCl.



(b)

LAB	Control	1% NaCl
1% NaCl	0.98 *	-
2% NaCl	0.65 *	0.78 *

Figure 2: (a) Boxplot graph showing survival of *L. casei* in MRS broth with different salt concentrations and (b) pairwise comparisons between growth of *L. casei* in MRS broth with and without NaCl using Tukey and Kramer test. P values with (*) denote no significant difference.

Salt tolerance of the yeast

The results obtained during yeast growth at different salt concentrations (Figure 3a) showed no significant differences in yeast population among treatments and the control (P>0.05). Boxplot analysis of surviving yeast populations showed different data distributions between treatments (Figure 4a); nevertheless, the analysis of variance of median values of these results showed no significant difference (P>0.05) (Figure 4b). Yeast grown in YM broth with 2% NaCl showed a difference of 0.6 pH units higher than the control at 15, 18, and 21 h. However, all treatments reached an average pH of 4.5 at 24 h after inoculation. At 72 h, treatments with 1 and 2% NaCl reached an average pH of 4.0, while the control remained at a pH of 4.5 (Figure 3b).

Salt tolerance of the photosynthetic bacteria

Photosynthetic bacterial populations showed significant variation among the two treatments and the control (P<0.05). Control and treatment with 1% NaCl showed an initial phase characterized by slow growth that lasted 12 h. Treatment with 2% NaCl presented a lag phase that lasted 48 h. In addition, stationary phase in the control was reached at 24 h with a population of 9.05 ± 0.22 $\log_{10}$ CFU ml^{-1}, and treatment with 1% NaCl reached the stationary phase at 48 h after inoculation with a population of 9.01 ± 0.11 log_{10} CFU ml^{-1} (Figure 5a). Although the culture treated with 2% NaCl did not reach stationary phase during the length of the experiments, the average surviving population at 120 h was 7.23 ± 0.22 log_{10} CFU ml^{-1}.

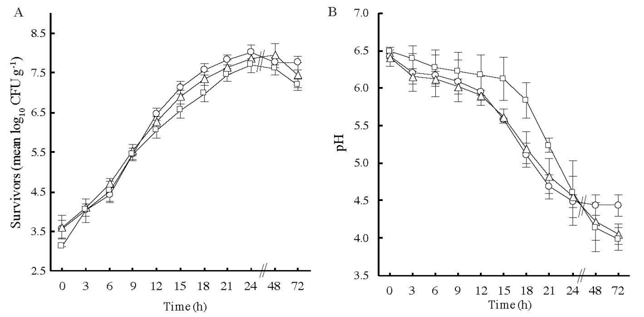
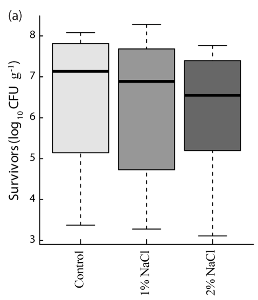


Figure 3: (a) Growth curves of *S. cerevisiae* and (b) pH values of YM broth at different salt concentrations. Data shown are mean ± SD. (○) Control, (Δ) 1% NaCl and (□) 2% NaCl.



(b)

Yeast	Control	1% NaCl
1% NaCl	0.98 *	-
2% NaCl	0.62 *	0.74 *

Figure 4: (a) Boxplot graph showing survival of *S. cerevisiae* in YM broth with different salt concentrations and (b) pairwise comparison between growth of *S. cerevisiae* in YM broth with and without NaCl using Tukey and Kramer test. P values with (*) denote no significant difference.

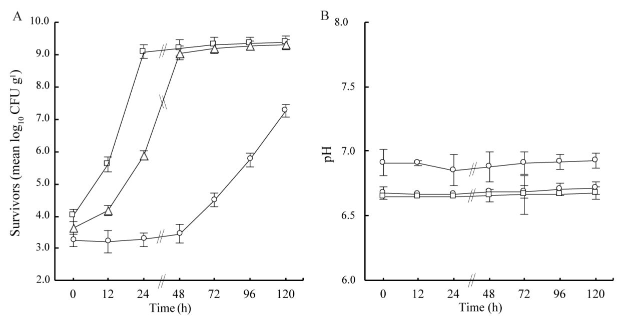
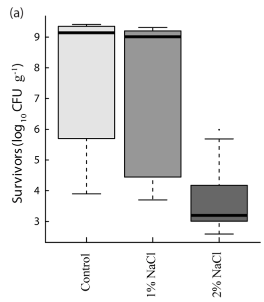


Figure 5: (a) Growth curves of *R. palustris* and (b) pH values of VN broth at different salt concentrations. Data shown are mean ± SD. (□) Control, (Δ) 1% NaCl and (○) 2% NaCl.

Growth behavior of photosynthetic bacteria in media with 2% NaCl showed a prolonged lag phase and a less pronounced slope. Samples were taken up to 120 h after inoculation, during which time, the growth curve showed a continuing exponential phase (Figure 5a). Furthermore, boxplots showed similarities between 1% NaCl treatment and the control in terms of distribution and median, whereas the values of 2% NaCl treatment were significantly different (P<0.05) from that of the control (Figure 6a). Analysis of variance indicated that there was no significant difference between 1% NaCl treatment and the control (P>0.05); however, a significant difference (P<0.05) was found between treatments with 2% NaCl and 1% NaCl as well as the control (Figure 6b). In terms of pH levels, there was no change throughout the experiment, regardless of the NaCl concentration (Figure 5b).



(b)

PB	Control	1% NaCl
1% NaCl	0.43 *	-
2% NaCl	8.1x10^{-7}	2.9x10^{-4}

Figure 6: (a) Boxplot graph showing survival of *R. palustris* in VN broth with different salt concentrations and (b) pairwise comparison between growth of *R. palustris* in VN broth with and without NaCl using Tukey and Kramer test. P values with (*) denote no significant difference.

Effects of NaCl on microbial cells

The high-resolution images of lactic acid bacteria obtained using SEM of the microbial cultures grown in the presence of 2% NaCl (Figure 7c and 7b) did not show morphological differences in comparison to the control (Figure 7a and 7b). In both cases, smooth rod-shaped cells were evident, with a slightly higher amount of exopolysaccharide clumped around cell clusters observed in 2% NaCl exposed cells in comparison to the control. In addition, there were no significant differences (P>0.05) between the length and width of bacterial cells cultured in media with 2% NaCl in comparison to the control (Table 1).

Similarly, there were no significant morphological changes between yeast cells grown in YM broth with 2% NaCl (Figure 8b) and the control (Figure 8a). In both cases, smooth surfaces were evident as well as a similar occurrence of budding.

In addition, the morphology of photosynthetic bacteria cultured in VN broth with 1% NaCl (Figure 9c and 9d) and the controls (Figure 9a and 9b) did not present significant differences. In both cases, bacteria seemed to form tight clusters. The amount of exopolysaccharide was similar in the control and the bacterial culture grown in media with 1% NaCl.

Microorganisms	Treatment	Average Length (mm ± S.D.)	P values	Average Width (mm ± S.D.)	P values
L. casei	Control	2.815 ± 1.1	0.914	0.751 ± 0.03	0.767
L. casei	2% NaCl	2.831 ± 1.05		0.753 ± 0.02	
S. cerevisiae	Control	4.516 ± 0.67	0.618	3.477 ± 0.56	0.54
S. cerevisiae	2% NaCl	4.674 ± 0.92		3.665 ± 0.92	
R. palustris	Control	1.462 ± 0.44	0.091	0.466 ± 0.07	0.491
R. palustris	1% NaCl	1.726 ± 0.55		0.485 ± 0.05	

Table 1: Length and width (μm) of microbial cells exposed to various treatments measured using scanning electron micrographs.

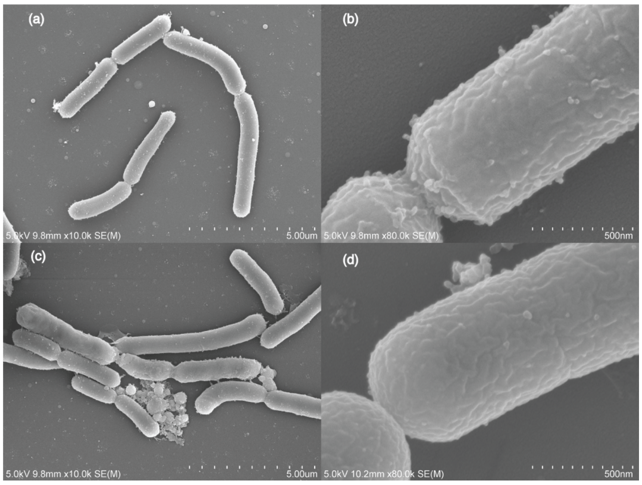


Figure 7: Scanning electron micrographs of *L. casei*: (a) Control (magnification X 10,000), (b) control (magnification X 80,000), (c) treatment with 2% NaCl (magnification X 10,000), and (d) treatment with 2% NaCl (magnification X 80,000).

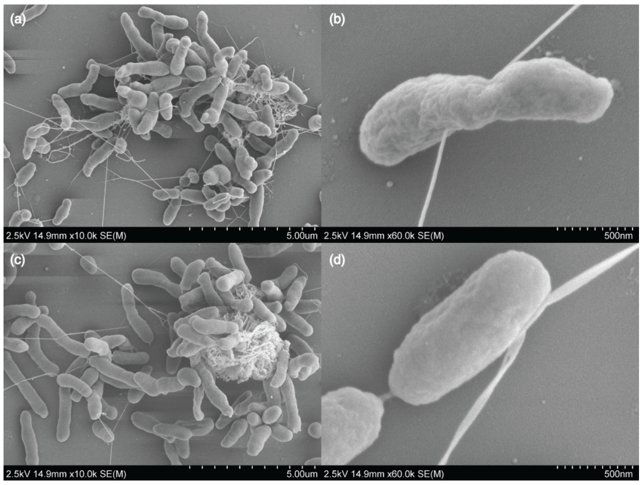


Figure 9: Scanning electron micrographs of *R. palustris*: (a) Control (magnification X 10,000), (b) control (magnification X 60,000), (c) treatment with 1% NaCl (magnification X 10,000), and (d) treatment with 1% NaCl (magnification X 60,000).

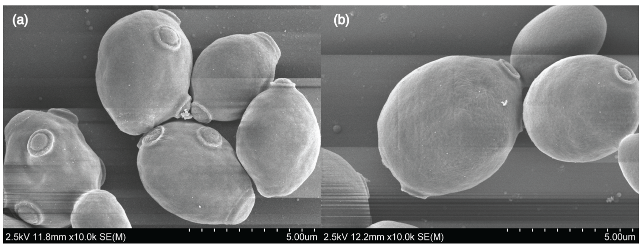


Figure 8: Scanning electron micrographs of *S. cerevisiae*: (a) Control (magnification X 10,000), and (b) treatment with 2% NaCl (magnification X 10,000).

Discussion

Probiotics such as lactic acid bacteria, have been applied in aquaculture for decades. Notably, shrimp has been among the top value products targeted for production by the aquaculture industry. This has led to a massive increase in the production of shrimp in several countries across the world [1]. However, the major producing countries are facing a great challenge in managing emerging diseases caused by pathogens that mainly thrive in high density production systems and hence, once a shrimp farm is affected, the production decreases [31]. It is noteworthy that probiotic microbes have been applied to confer disease resistance, increase feed efficiency, and enhance the growth of aquatic organisms, such as fish and crustaceans. A study showed that probiotic species of the genus *Bacillus* can enhance tolerance to stress due to low salinity levels in lobsters. In addition, the probiotic properties of marine bacteria have been evaluated in aquaculture species of commercial importance [32]. For instance, *Aeromonas media* was found to increase the tolerance of Pacific oysters to disease caused by *V. tubiashii* [33]. Moreover, the growth of South African abalone increased when fed with a diet supplemented with *V. midae* SY9, a beneficial species of *Vibrio* [34].

From the results obtained in the present study, it can be inferred that a specific degree of salinity can affect the growth of certain microbes positively or adversely, which makes it imperative for any aquaculture industry to ensure that appropriate probiotic bacteria are applied to maximize the benefits [35]. The results obtained showed that the amount of NaCl used did not affect the growth of lactic acid bacteria and yeast since there was no significant difference between the control and cultures grown in media containing salt. In addition, the growth curves of these microbes in media with 1% and 2% NaCl were very similar to that of the controls. Based on the statistical analysis of the data on salt tolerance of lactic acid bacteria and yeast, it could be concluded that salinity may not affect the growth of these microorganisms. The results obtained for *S. cerevisiae* are consistent with those of Hounsa et al. [36] who showed that *S. cerevisiae* can tolerate osmotic stress due to aw of 0.866 which is equivalent to 22% NaCl. Even though salinity tolerance of *L. casei* in terms of NaCl concentration has not been explored, it has been reported that *L. casei* can withstand bile salts at concentrations of up to 0.3% [37]. A similar study was performed on *L. rhamnosus*, demonstrating the ability of this bacterium to grow in MRS broth with up to 2% bile salt [24]. Within the scope of the present study, salinity levels in shrimp ponds up to 2% may not have any adverse effect on the probiotics evaluated.

Some lactic acid bacteria have metabolic mechanisms that allow them to resist salt levels and maintain their normal metabolic activity [38,39]. The mechanism associated with salt tolerance in lactic acid bacteria of the genus *Lactobacillus* [40] and yeast of the genus *Saccharomyces* [36] is believed to be the synthesis and mobilization of trehalose within the cell. When an osmotic stress response is triggered, trehalose is metabolized and/or mobilized to maintain the turgor pressure and decrease permeability of the cell membrane. The similarity in media pH levels at different time intervals among treatments and the controls supports the possible existence of osmotic regulation mechanisms. The results showed the potential of lactic acid bacteria and yeast to be applied in salt water conditions of shrimp ponds where the salinity is up to 2%.

Regarding the salt tolerance of photosynthetic bacteria, it is evident from the results that salinity could have a delaying effect on their growth. This is observed in the growth curves obtained from cultures treated with 2% salt that had a lag phase longer than that of the cultures treated with 1% NaCl and the control. Therefore, salinity levels can increase the time needed for photosynthetic bacteria to adapt to the salt concentration in the media. This could be interpreted as an adverse effect on the potential for use of these bacteria in shrimp production [38]. However, after 5 days of incubation, the growth curve showed the beginning of exponential phase, which suggests that if given sufficient time to grow, the photosynthetic bacteria can adapt to the environment and continue with their metabolic processes. Although investigations on the salinity tolerance of *R. palustris* in terms of NaCl concentration are limited, a previous study reported that *R. palustris* can withstand media with bile salt concentrations of up to 1.5% [41]. Additionally, Zhou et al. [42] reported a significant increase in cell viability of *R. palustris* when exposed to simulated small intestinal juices with 0.3% bile salts. The salinity tolerance of photosynthetic bacteria as well as tolerances to other environmental stresses, such as heat or freezing, have been attributed to the production of hopanoids, which are a class of pentacyclic triterpenoids considered to be the bacterial counterparts of eukaryotic sterols [41]. Hopanoids maintain the integrity and stability of bacterial cell membrane while downregulating permeability [43].

In summary, yeast and lactic acid bacteria demonstrated tolerance to the salinity levels evaluated, whereas photosynthetic bacteria showed a prolonged adaptation period to salinity reaching exponential phase 24 to 72 h later than the control. The high-resolution images obtained via scanning electron microscopy suggested that there are no significant changes in the microbial cellular morphology in comparison to the controls. Therefore, it can be concluded that the lactic acid bacteria and yeast evaluated may have mechanisms that provide tolerance to salinity conditions, thereby allowing them to grow and maintain production of organic acids. These results suggest that the three probiotic microbes evaluated have the potential to be used in shrimp production systems with salinity levels of up to 2%. The suitability of using these probiotics in aquaculture waters with higher than 2% salinity needs to be evaluated in the future.

Conflict of Interests

No conflict of interest declared.

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