ORIGINAL ARTICLE

Microbial Pack as Probiotic Supplements to Improve Gut Immunity for Homeostasis

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Abstract

Introduction: Probiotics are live microorganisms that, when administered or present in sufficient quantities, have beneficial effects on host health in increased lifespan. Probiotic supplements must be tested to determine their efficacy as a non-chemical approach to promoting health and well-being. **Objective:** Our research work focuses on determining the molecular impact of probiotic administration on homeostasis and immunity. **Methods:** Shrimpswere administered with recommended doses of microbial pack containing *Bacillus* species and *Rhodococcus* species and the fermentation was carried at 37°C for 72 h under microaerophilic condition. The parameters such as pH, microbial load, acidity, and concentration of reducing sugar had been measured. **Results:** This probiotic pack when used either alone or in combination with traditional dairy starter, significantly improved the nutritional properties and the shelf life of the product. **Conclusion:** The current work will be useful in developing novel functional foods based on these unique probiotic properties.

Keywords: *Bacillus*, *Rhodococcus*, probiotics, microbial pack, immunity, homeostasis

INTRODUCTION

The gastrointestinal tract is one of the most microbiologically active ecosystems containing a mass of bacteria crucial for the maturation of immune cells.[1] The human gut microbiome consists of more than 100 trillion microorganisms and thousands of bacterial species, exerting vital functions in host immunomodulation, nutrient metabolism, maintenance of structural integrity of intestinal barrier, and defense against pathogens.[2] However, archaea, protists, and viruses have received little attention, but several studies have revealed diverse communities of bacteria and yeast.[3] Microbes have a significant impact on host health, development, welfare, and nutrition, significant efforts have been made over the last two decades to fortify these communities and maintain microbial balance,[4] Probiotics[5] and prebiotics[6] applications have been at the forefront of such efforts. Numerous external factors, such as diet, antibiotics, and pathogen invasion,[7] can alter microbial ecology and lead to compositional and functional variations of the gut microbiome.[8] Probiotic strains isolated from human microflora are well characterized by high adhesive levels to the human intestinal epithelial barrier than others and more likely to be safe.[9] This body of evidence has helped to create a market and drive demand for commercial probiotics and prebiotics for use in aquaculture operations globally.[10] As a result, many feed manufacturers, both multinationals and small domestic operations, routinely incorporate probiotics and prebiotics into their feed formulations.[11] The extent of their economic benefits is not yet clear, as such information is not often openly discussed by farmers, but the increasing demand and increasing volumes of probiotic/prebiotic aquafeeds produced are positive indicators for industrial level applications.[12] Future research efforts should focus on a better understanding of the modes of action, which must include a better understanding of the composition and activity of indigenous microbiomes, as well as the effects on the host itself, so that optimization of probiotic/prebiotic selection, dosage and application strategies can occur.[13]

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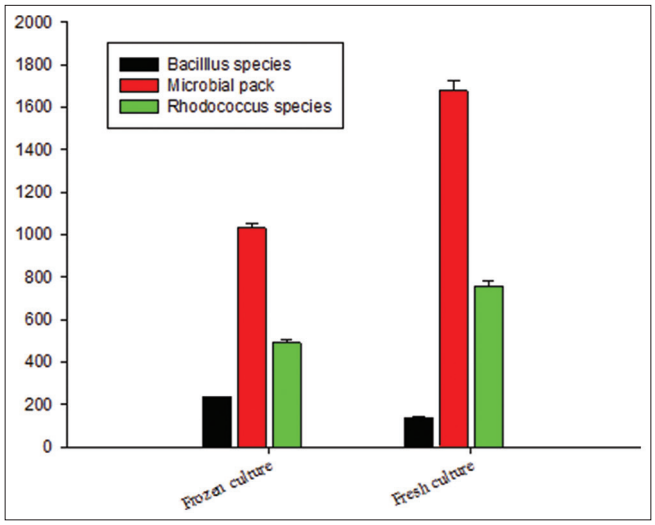

Figure 1: Viable cell count of microbial pack

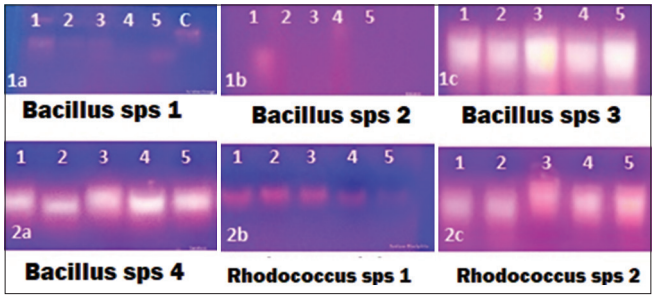

Figure 2: High-copy number plasmid DNA was isolated from overnight *Bacillus* and *Rhodococcus* culture and purified plasmid DNA was analysed by agarose (1%) electrophoresis

MATERIALS AND METHODS

All chemicals were procured from Merck Laboratories, Mumbai. Microbial pack was procured from Microbial Type Culture Collection and Gene Bank, Chandigarh. All solvents and reagents were of analytical grade, and all experiments were performed with deionized water.

Preparation of bacterial culture

Stock culture of bacterial pack was sub-cultured on Lysogeny broth (LB) agar at 37°C for 24 h. A total of 45 sterile falcon tubes were taken and grouped into three categories, every five tubes were added having a density of $2.25*10^7$ cells/ml, inoculated a flask containing 250 ml of sterile culture medium and labeled inoculated aseptically, incubated for overnight at 37°C for 120 rpm to obtain a concentration of 1.5 × 10 cells/ml.[14]

Bacterial colony-forming units

The main culture was inoculated with preculture, approximately generating a start OD_{600} of 0.1. After growing at 37°C for several hours to a final OD_{600} of 1, the culture was transferred into a sterile, precooled centrifuge tube and put on ice bath for 10 min. Aliquots of 100 µL of bacteria cultures (10^6 cells/mL) were grown in 10 mL of LB broth for 6 h and were spread over LB-agar plates supplemented with the respective drugs, chemicals, and radiation. After overnight period, the growth of each sample was documented and compared to those of wild organisms to verify any synergistic effect among the mutant organism. Bacterial colony-forming units of each plated was enumerated by manual counting and or by automated plate counter.[15]

RESULTS

Determination of colony-forming units from *in vitro* cultures

Culture viability was determined by plating **100** µl **of** 10^6 dilutions of the appropriate culture grown in LB broth, layered on LB agar plates, and counting the colonies after an overnight aerobic incubation at 37°C. To test the effect of bacterial cell count, values were taken every frequent interval. Among cultures that had been stored for several months, fresh cultures yielded the highest yield in 10^6 cells as shown in Figure 1.

Plasmid stability studies

Plasmid stability has historically been a problem in bacterial research, and antibiotics have been used to ensure plasmid stability for bacterial growth. We used various starter culture combinations in this study to maintain microbial growth stability while avoiding the use of antibiotics. The samples were then run on a 1 percentage agarose gel with a 1 kb ladder DNA for reference and the purity was checked and depicted in Figure 2.

CONCLUSION

Comparative studies between microbial packs have elucidated the viability and stability of strains, as well as their activity. However, additional research, such as 16s rRNA sequencing and knockout technology, will be required to confirm the strains' stability. Our study demonstrated that a microbial pack system as a fresh starter culture has a higher yield of plasmids than an ancient culture that can maintain stability over a large number of generations in not only *in vitro* but also *in vivo* without any antibiotic selection. As a result, our method can be used as a potent feed supplement to maximize yield in aquaculture.

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