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*RESEARCH ARTICLE*

# BENEFICIAL EFFECTS OF PROBIOTICS BASED THERAPY FOR IMMUNE HOMEOSTASIS

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## *Abstract*

Several approaches have been used to demonstrate that signals derived from gut microbiota are critical for the development of the immune system. Probiotics are live microorganisms that have beneficial effects on host health, including extended lifespan, when they are administered or present in adequate quantities. However, the mechanisms by which probiotics stimulate host longevity remain unclear and very poorly understood. Probiotic supplements are needed to determine the effectiveness as a non-chemical approach to promote health and welfare. Our studies focus on the assessment of the molecular impact of probiotic administration involved in homeostasis and immunity. Shrimps received the recommended doses of microbial pack containing Bacillus species andRhodococcus species. The fermentation was carried at 37°C for 72 hours under microaerophilic condition. The parameters like pH, microbial load, acidity and concentration of reducing sugar had been measured.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. The present work will be valuable to elaborate novel functional food based on these original probiotic properties.



## Introduction:-

Since the initial investigations on the gut microbiota of aquaculture some five decades ago, considerable information has been presented on their composition, abundance, diversity and activity[1].Numerous studies have demonstrated that these communities are complex and generally of lowcultivability, containing Bacteria, Archaea, viruses, yeasts and protists[2]. However, little attention has been paid to the Archaea, protists or viruses but several studies have revealed diversecommunities of bacteria and yeast[3]. These microbes have major implications on host health,development, welfare and nutrition and therefore great efforts have been made in the past twodecades to fortify these communities and maintain microbial balance[4]. Among such efforts theapplications of probiotics and prebiotics have been at the forefront[5-7]. The scientific evidencewhich underpins the efficacy, and to some extent elucidates their modes of action, has beencomprehensive, although not always reproducible[8]. This body of evidence has helped to create amarket and drivedemand forcommercial probiotics and prebiotics for use in aquaculture operations globally[9,10]. As such, many feed manufacturers, multi-nationals and small domestic operations, routinely add pro- and prebiotic products to their feed formulations[11]. The extent of theireconomic

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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 aquafeedsproduced are positive indicators for industrial level applications[12]. Future research efforts shouldfocus on better understanding of the modes of action, which must include a better understanding of thecomposition and activity of indigenous microbiomes, aswell as the effects on the hostitself, so that optimization of probiotic/prebiotic selection, dosage and application strategiescan occur[13].

## Material and Methods:-

All chemicals were procured from HiMedia Laboratories, Mumbai. Microbial pack was procured from MTCC, 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 LB agar at $37^0$C for 24 h. A total of 45 sterile falcon tubes were taken and grouped into three categories, every five tubes were added having density of $2.25*10^7$ cells/ml, inoculated a flask containing 250ml of sterile culture medium and labelled inoculated aseptically, incubated for overnight at $37^0$C for 120rpm to obtain a concentration of 1.5x 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) 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 organism 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**

Viable cell counts of cultures were determined by plating 100µl of $10^6$ dilution of the appropriate culture grown in LB broth on LB agar plates and counting the colonies after aerobic incubation at 37 °C for overnight period. In order toverify the influence of bacterial cell count, values were taken after every 5 minutes interval. Maximum yield is observed with fresh culture compared to the cultures stored for several months.

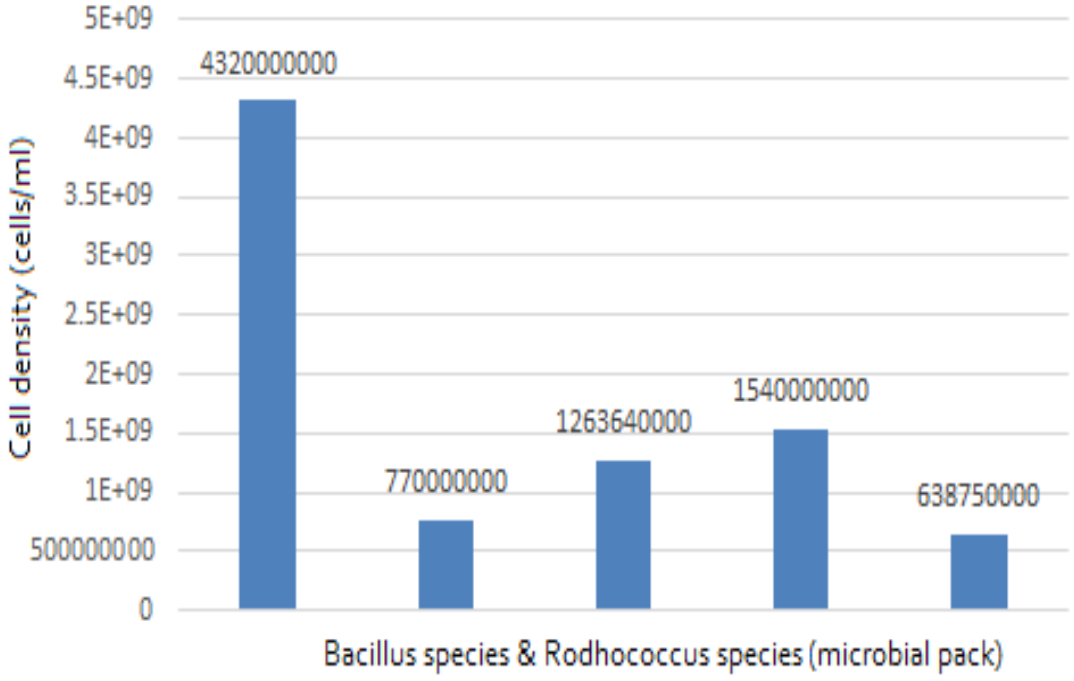


**Figure 1:-** Viable cell count of microbial pack.

**Plasmid stability studies**

Plasmid stability has been problematic in bacterial studies, and historically antibiotics have been used to ensure plasmid stability for the growth of bacteria. In the present study, we used different combinations of starter culture to maintain stability of microbial growth that obviate antibiotic usage. The samples were then run on 1% agarose gel together with 1kb ladder DNA for reference and checked for the purity.

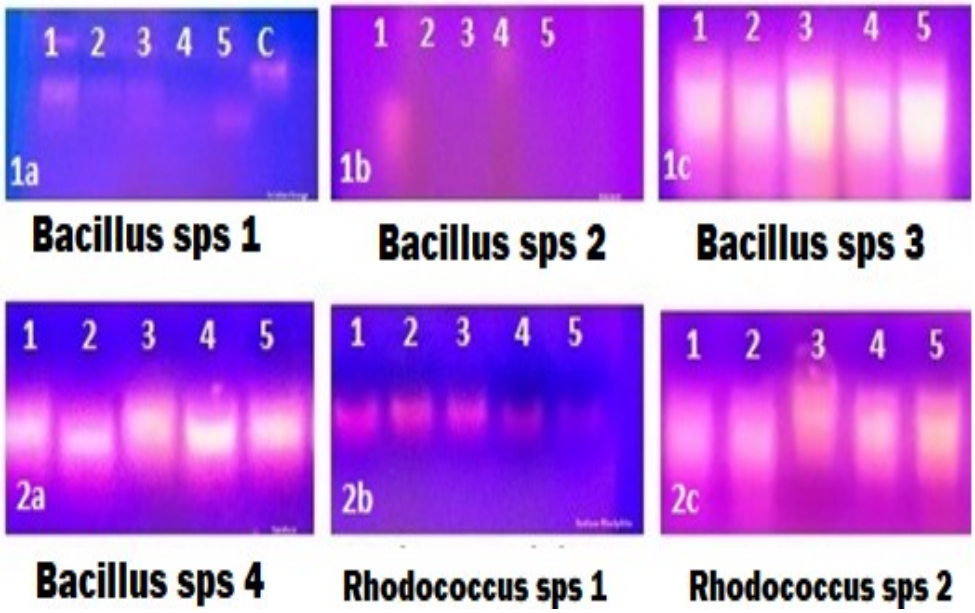


**Figure 2:-** High-copy number plasmid DNA was isolated from overnight Bacillus and Rhodococcusculture and purified plasmid DNA was analyzed by agarose (1%) electrophoresis.

## Conclusion:-

Comparative studies between microbial pack have further elucidated for the viability and stability of strains and its activity. However, further studies like 16s rRNA sequencing, knock out technology has to be verify the stability of strains. Our study, proved that microbial pack system as fresh starter culture has maximum yield of plasmids than old culture system which can retain stability over a multitude of generations both in vitro and in vivo without antibiotic selection. Thus, our approach can be used as a powerful meal or feed supplement for obtaining maximum yield in aquaculture.

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