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RESEARCH ARTICLE

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IMPROVING GUT IMMUNITY FOR HOMEOSTASIS USING MICROBIAL PACK OF PROBIOTIC SUPPLEMENTS

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Manuscript Info

Key words:-

Probiotics, Microbial pack, Immunity, Homeostasis, Shrimps, Microorganisms

Abstract

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* and *Rhodococcus 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.

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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 low cultivability, 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 diverse communities 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 two decades to fortify these communities and maintain microbial balance[4]. Among such efforts the applications of probiotics and prebiotics have been at the forefront[5-7]. The scientific evidence which underpins the efficacy, and to some extent elucidates their modes of action, has been comprehensive, although not always reproducible[8]. This body of

evidence has helped to create a market and drive demand for commercial 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 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 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].

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°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°C for 120rpm to obtain a concentration of 1.5×10^8 cells/ml [14].

Bacterial Colony forming units

The main culture was inoculated with preculture, approximately generating a start OD₆₀₀ of 0.1. After growing at 37°C for several hours to a final OD₆₀₀ 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 100 µl 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 to verify 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.

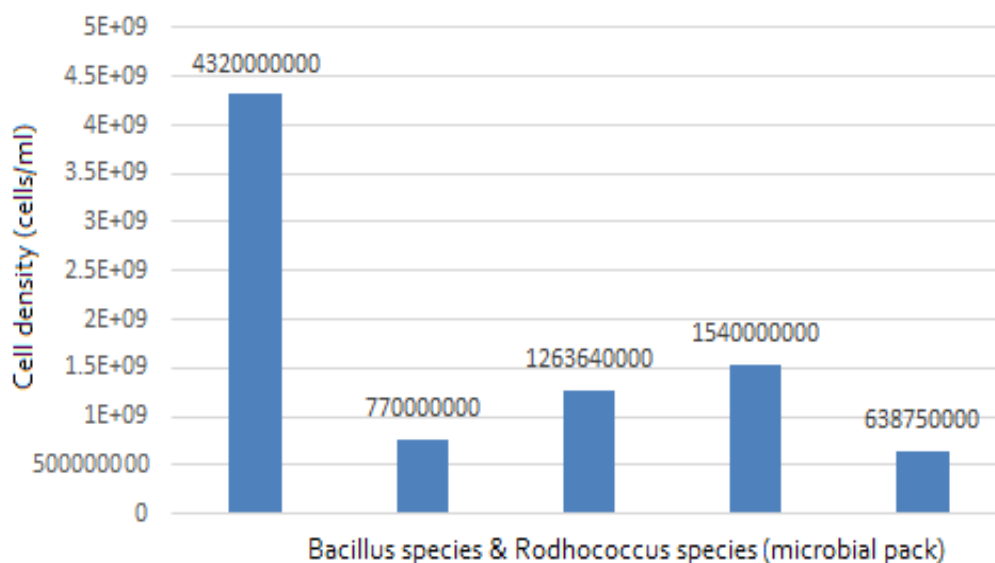


Figure 1:- Viable cell count of microbial pack.

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.

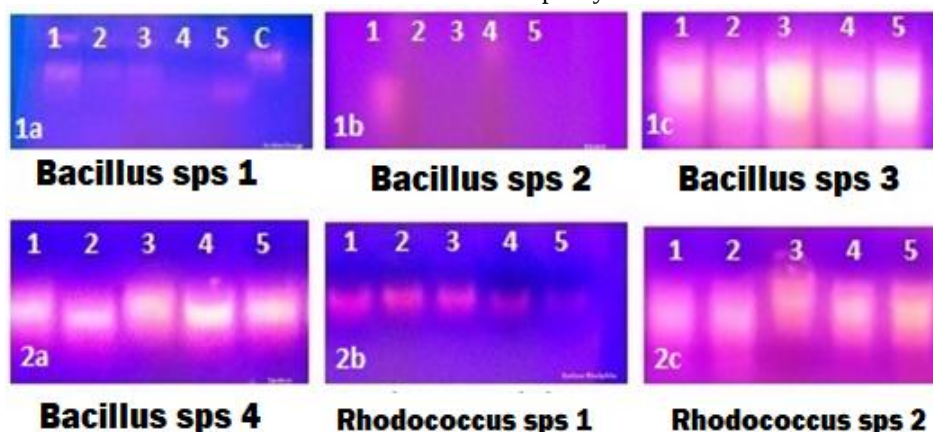


Figure 2:- High-copy number plasmid DNA was isolated from overnight *Bacillus* and *Rhodococcus* culture and purified plasmid DNA was analyzed by agarose (1%) electrophoresis.

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 etc 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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