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Bacillus aerius: A Promising Probiotic for Poultry Feed Use

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A B S T R A C T

This article describes the beneficial effect of *Bacillus aerius* probiotic formulation on the growth of poultry chicks. *B.aerius* was cultivated by submerged fermentation. Biomass was harvested and formulated using white dextrin. Four kg of cream free flowing powder formulation was obtained from 50L broth after spray drying. Poultry field trials on a group of day old chicks were carried out. Live body weight being significantly higher ($P<0.05$) and FCR being significantly lower ($P<0.05$) as compared with the control group, with no mortality was reported. The study indicates that *B.aerius* proves to be a growth promoter and thus is a promising candidate for commercial probiotic for poultry.

Introduction

Enteric diseases are an important concern to the poultry industry because of loss in productivity, increased mortality, and the associated contamination of poultry products for human consumption. With increasing threats about antibiotic resistance, and the ban on sub therapeutic antibiotic usage in many countries, there is a growing interest in finding alternatives to antibiotics for poultry production (Rahimi, 2009). Colonization of the digestive tract with commensals in the form of probiotics, induces mucosal barrier fortification, innate immune responses and promotion of nutrient metabolism (Timmerman *et al.*, 2006).

Probiotic strains have been shown to inhibit pathogens both *in vitro* and *in vivo* through different mechanisms. Probiotics in poultry maintain normal intestinal microflora by competitive exclusion and antagonism, alter metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (Al-Barwary *et al.*, 2012). A well-accepted method to quickly introduce a commensal microflora in hen-deprived chicks is through the administration of probiotics (Timmerman *et al.*, 2006). *B. subtilis* and *B. licheniformis* are being used in products, Biosporin and BioPlus 2B. BioPlus 2B is used in animal feed and Biosporin is

licensed as a medicine (Xiaolu Liu *et al.*, 2012). Most research findings on probiotics have demonstrated the use of mono strain or multi strain probiotic microbes belonging to the same species or genus (Shim *et al.*, 2010).

Most commercially used probiotic products when used in the range of 10^7-10^9cfu/day are able to show their probiotic property and thus exert their health benefit. A large number of the applied microorganisms are deactivated during the passage through GIT due to high acidity and bile salt effect. Thus, large numbers of cells are required for each dose. (Elmarzugi *et al.*, 2010). For commercial usage of spores at a less expensive rate and in higher concentrations, high cell density and good sporulation efficiency is must.

The spore forming ability of genus *Bacillus* provides a high level of resistance to extreme environment conditions which makes these bacteria good candidates for developing stable and efficient commercial products. Formulation can be prepared using different drying methods, including freeze drying, spray drying and fluidized bed drying (Yánez- Mendizabal *et al.*, 2011). Althoughfreeze drying is the widely used method, spray drying provides an economical technology due to higher productivity per unit time and practicability ease in large scale production. Spray drying functions as a microencapsulation technique when an active material is dissolved or suspended in a solution and becomes trapped in the dried material (Vidhyalakshmi, 2009). A number of studies have reported survival rate and performance of a variety of probiotic cultures after spray drying (Yadav *et al.*, 2009).

Animals reared under commercial field conditions are subjected to immunological stress, especially in the early stages of life. This depends on the pathogen load in their environment. When the microorganism load in the gut is unbalanced, beneficial results can be achieved through the use of dietary probiotics (Midilli *et al.*, 2008). *Bacillus* species are found to predominate the poultry intestinal tract. Certain strains of *Bacillus* have also been used for probiotic purpose (Duc *et al.*, 2004).

The present study is aimed at large scale biomass production and formulation of a novel probiotic strain of *B.aerius* (Lavanya & Aparna, 2015 & 2016).Supplementation to the diet of broiler chickens with the prepared formulation through water on their growth is also investigated to know if it can be considered to be as a potential candidate for commercial probiotic production.

Materials and Methods

Biomass production: The inoculum was prepared in 1L medium (2.5% Soya Peptone, 0.5% NaCl) and incubated at 37^0C/24 hr at 100rpm. 0.5% of this inoculums was transferred to a 100L fermenter containing batch volume of 50L medium (6% Soya flour, 0.5% NaCl, 0.1% alkaline protease, 0.1% Polypropylene Glycol (PPG), 0.5% Amylase. Sterilization: 123 ± 1^0C, 30min, 1.25 Kg/cm^2)and incubated at $38^0C\pm1^0$C with an agitation of 275rpm and aeration of 75lpm (Air supplied at the rate of 1vvm with the help of a pipe sparger) till complete sporulation of the vegetative cells was observed microscopically by Gram staining.

Formulation: After complete sporulation, 50L of broth was spray dried with 5% white dextrin under optimized conditions (air inlet temperature 105^0C -110^0C and air outlet temperature 65^0C -70^0C) (Yadav *et al.*, 2009). The viability of the spores and acid

and bile salt tolerance of the final product obtained was subsequently checked as per the method mentioned in Lavanya & Aparna 2016.

Field trials: A total of 24 one-day old cob-400 broiler chicks (Source: Venkateshwara Hatcheries, Pune), with an average weight of 50g, were subjected to a 42-day experimental period. The chicks were randomly divided into two experimental groups of 12 chicks in each group - Group 1 as the Control group and Group 2 as the Test group. Both the groups were fed broiler starter from day 1to 21 and broiler finisher from day 22 to 42 (Shim *et al.,* 2010). Group 1 (Control) were provided only water and group 2 (Test) with water with $4.1x10^8$cfu/ml of *B.aerius* spore powder formulation added as per standard protocol.(Xiaolu Liu *et al.,* 2012 and Rahimi, 2009). Chicks in both groups were vaccinated against Newcastle disease via eye drops and subcutaneously on days 7 and 21 of age; while on day 14 of age, against IBD (Inflammatory Bowel Disease).

Body weight and feed intake of birds was checked every alternate day Feed Conversion Ratio (FCR) was calculated as the ratio between feed intake and body weight gain (FCR= feed intake/average body weight) at the end of each week (Rahimi, 2009).

Statistical Analysis: Data was analyzed with the help of Univariate Analysis of Variance using the General Linear Models (GLM) procedure of SPSS 17.0. When significant differences were noted, means were compared using independent t-test.

Results and Discussion

Fermentation: Submerged liquid fermentation involves growth of microbes in an aqueous medium and currently this method is most commonly used for probiotic production (Shim *et al.,* 2010). To increase the spore production, high cell density needs to be achieved followed by sporulation to occur (Monteiro *et al.,* 2005).Every 5hours, samples were checked microscopically for sporulation (Fig 1).

During the logarithmic growth phase of the culture i.e. from 0-40 hours of the bioreactor run, <5% sporulation was observed indicating that the cells are in their vegetative state. At the start of stationary phase (observed around 40 hr), a steep increase in sporulation was observed with >20% sporulation 60hr to Complete sporulation (approximate 100%) within 108hours of incubation. A spore count of $9x10^9$cfu/ml was recorded after harvesting.

Formulation: Spray drying is a mild technique due to its very short drying time to which the product is exposed. However, compared to freeze or vacuum drying, spray drying process is more prone to damaging heat sensitive components such as enzymes and probiotic bacteria. (Schutyser *et al.,* 2012). A variety of protectants like milk powder, whey protein, sucrose, lactose, glucose, etc are been added to the drying medium during spray drying to protect their viability (Yadav *et al.,* 2009).

Dextrin (carrier) mixed with the substance to be encapsulated (Spores of *Bacillus* in the fermenter broth) were homogenized and spray dried. Four kg of cream free flowing powder formulation was obtained from 50L broth after spray drying. Addition of carbohydrates contributes to the formation of glassy, amorphous powder and during storage; sugars are reported to be effective protectants against oxidation damage (Schutyser *et al.,* 2012).

After complete sporulation, the broth was harvested. A spore count of $9x10^9$cfu/ml

was obtained and was further used for spray drying. A yield of $4.1x10^7$cfu/gm formulation was obtained with dextrin. Acid and bile salt tolerance of the formulated spores was observed to be at par with the earlier results in i.e. for growth in gastric juice the optimum pH is 2.5 and the Bile salt tolerance was observed to be up to 2% and growth ability up to 1% (Lavanya & Aparna 2016).

Field trials: The health promoting effect of probiotic in the gastrointestinal tract has been mainly associated with their capacity to stimulate the immune response and to inhibit the growth of pathogenic bacteria (Rahimi, 2009).

Table.1 Effect of probiotic supplementation on Live body Weight (Mean±SEM*) and Feed Conversion Ratio in Broiler chickens

Age (day)	Parameter	Control (Group 1)	Test (Group 2)
7	Live body Weight(g)	162.41± 0.37[a]	170.08± 0.28[b]
	FCR	1.02	0.95
14	Live body Weight(g)	450.58± 0.35[a]	461.25± 0.25[b]
	FCR	1.29	1.11
21	Live body Weight(g)	637.50± 0.75[a]	650.66± 0.35[b]
	FCR	1.65	1.53
28	Live body Weight(g)	1289.75± 0.35[a]	1307.16± 0.66[b]
	FCR	1.75	1.63
35	Live body Weight(g)	1835.66± 1.51[a]	1876.50± 2.60[b]
	FCR	1.96	1.80
42	Live body Weight(g)	2296.66± 3.76[a]	2463.08± 3.11[b]
	FCR	2.18	1.94

*SEM= Standard error of means
In each row, means with different superscript letters are significantly different (P<0.05).

Fig.1 Age vs Sporulation &Age vsO.D.

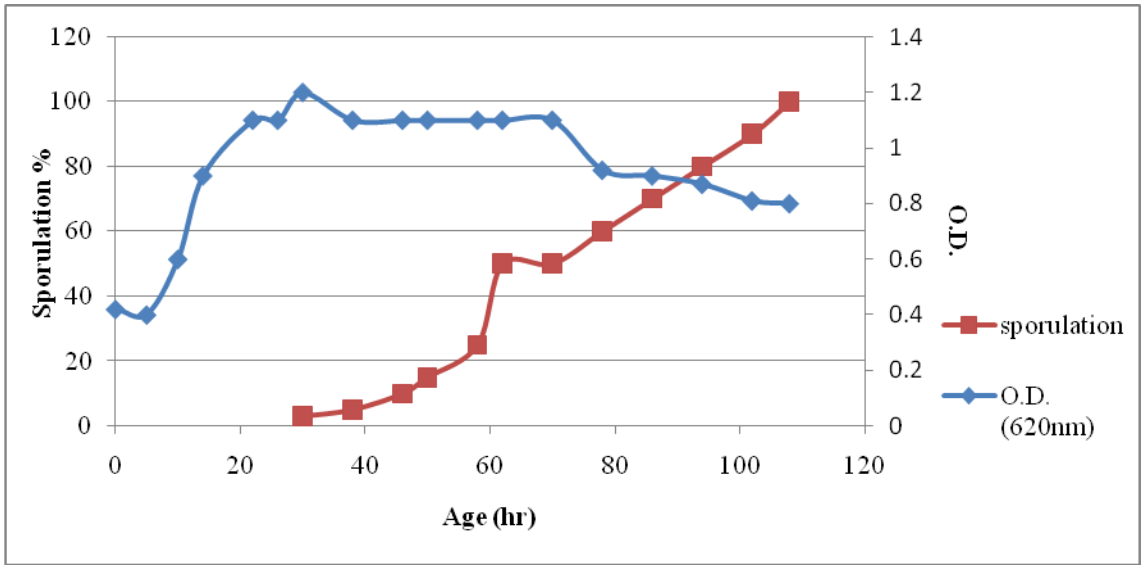

Table 1- The average live weight of broiler chickens was found to be significantly higher ($P<0.05$) in chicks which had received the probiotic than the control group. Also, feed conversion ratio (FCR) was significantly lower ($P<0.05$) in chicks which received *B.aerius* supplements from that of group 1 during overall experiment period. No mortality was recorded in probiotic supplemented group throughout the experiment. These results are in agreement with the findings of Anjum *et al.*, 2005 who reported that the use of probiotic in broiler chicken's diet significantly improved the daily body weight gain. Feed conversion ratio is a measure of how well a flock converts feed intake into live weight and as feed costs represent 60-70% of thetotal cost of broiler production, the efficient conversion of feed into live weight is essential for profitability(Arbor Acres Service Bulletin, 2011).The effect of probiotic on body weight was significantly higher ($P<0.05$)in test group as compared to control group from 28-42 days and FCR was found to decrease in accordance with increase in body weight, with highest difference of FCR between control and test from 35-42 days suggesting an improved intestinal balance of microbial population in probiotic treatments, which is in accordance to the work performed by Xiaolu Liu *et al.*, 2012.

In conclusion, probiotic strain of *B.aerius* was scaled up to 50L broth in fermenter; with a harvest of approximately 4kg of biomass with dextrin and $4.7x10^7$cfu/gm of spore count. The formulation, when fed to broiler chicks, the body weight of chicks was found to be significantly higher & FCR was significantly lower for chicks supplemented with *B.aerius* as compared with that of the control group, with no mortality. Thus, *B. aerius* proves as a promising candidate for commercial probiotic production.

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