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Statistical Optimization for Enhanced Sporulation of Probiotic Bacillus Coagulans BC LOG12 In a Shorter Fermentation Time

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***Abstract*— The cutting-edge research work focuses on growing a powerful fermentation medium that complements sporulation while shortening the fermentation period of Bacillus coagulans BC LOG12. Several optimizations of the media were executed to enhance both biomass and sporulation. The period of fermentation is also a critical thing. For the reason that the fermentation period for maximum probiotics in shake flasks levels from 80 to 105 hours, there's a need to create a medium that shortens this fermentation duration. The shake flask culture medium for Bacillus coagulans BC LOG12, which includes an optimized soluble gum fiber, glucose, and fructooligosaccharide (FOS) as carbon assets in conjunction with carefully adjusted requirements of nitrogen and minerals, changed into evolved. The findings imply that the medium containing soluble gum fiber ended in extensively better sporulation inside a reduced time-frame of 48 to 50 hours, whilst the mediums using glucose and FOS did no longer exhibit good enough sporulation, even after 105 hours of fermentation, that is the shortest fermentation duration referred to for the cultivation of Bacillus coagulans BC LOG12, accomplishing 75%-80% sporulation in shake flasks. The number one benefits of minimizing fermentation duration are the reduction of time required and an enhancement in the efficiency of probiotic preparations, additional experiments will be carried out in bioreactors to enhance the efficacy of the fermentation and sporulation techniques for Bacillus coagulans BC LOG12.**

***Keywords*— Bacillus coagulans; Probiotic; Fermentation; Sporulation.**

I. INTRODUCTION

The term probiotics means live microorganism which when consumed at a known dosage gives us health benefits. Probiotics has gained right reputation since ancient times, owing to their therapeutic potential and health benefits [1]. The use probiotics and their products are widely accepted across different sectors to formulate optimized veterinary and poultry feeds which can prevent infections and aid in growth and developments animal feeds, recent studies have shown that they are also well used in aqua feed as well [2,3]. As per the scientific studies, the probiotic and enzyme usage appear to be the most feasible and edible forms with minimal side effects which makes them as one of the healthiest food commodities with assured safety. Especially, the species like Lactobacillus and Bifidobacterium accounts to produce probiotic components across the globe with health benefits.

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Apart from these, there are other groups of microbial communities which encompasses Enterococcus, Streptococcus and spore probiotic Bacillus species [4]. Alongside few of Saccharomyces species. The Lactobacillus & Bifidobacterium species are sensitive and are usually stored at low temperature to maintain its viability, because of this they can't be utilized in warmness processing foods. Many spore producing bacterial population especially which falls under the group of Bacillus specie which includes Bacillus clausii, Bacillus subtilis, Bacillus coagulans etc.

These bacteria are isolated and screened for their potential to secrete probiotics-based metabolites. The screened efficacy and potential of probiotic bacteria makes them one of the interesting subjects across the microbial scientific community. Studies have also conferred that these bacterial spores are rigid and can withstand the process of large-scale cultivation and processing it into product with minimal cost and can be one of the ideal candidates for the developing health formulations. Most of the products available in the market are biocompatible and are easy and safe to use.

The inherent capacity of manufacturing of big quantity of diverse class of bioactive metabolites especially in the Bacillus species are among the ideal candidate for industrial application. One of the most probiotic spores researched is obtained from Bacillus coagulans, which is spore forming gram-positive bacteria which has accord attention from the food industries due to its probiotic functionalities and can withstand various parameters like high temperature, sever acid conditions, and also functions well in the gastrointestinal niches. The latest technologies provide excellent platforms to large scale cultivation of the desire probiotic bacteria with additional nutritive and health benefits which can be ideal fermented meal making them vital for business viability. In order to commercial these products, sequential research and development needs to be executed with optimized parameters and reproducibility under varied conditions which can give a roadmap for commercialization [5].

A large number of research have proven an increase in cell biomass and sporulation in most bacilli under the optimized culturing conditions which starts from the use of appropriate industrial media for maximum cell growth and desired yield of probiotic components. Studies have highlighted different components influencing the productivity for instance the presence of yeast extract, amino acids, protein concentrates, hydrolysates, vitamins and inorganic compounds inclusive of (NH4)2SO4 and (NH4)2HPO4. However, in comparison with other Bacillus species, Bacillus coagulans indicates problems in promoting sporulation [6-9]. The current available probiotic products are generally in the form of spores which have the capacity to survival at harsh conditions upon reaching the intestine and act efficiently and prevents the disorders and improves the health conditions [10,11].

Presently maximum of the industrial producers use complex carbon and nitrogen resources for growth of Bacillus coagulans and takes longer fermentation time [12-19]. Supplementing the tradition medium with soluble fibers alongside commercially available growth supplements results in reduced fermentation time with good sporulation, as when compared to glucose & FOS as carbon sources. The aims of the present investigation is to evaluate the growth conditions of Bacillus coagulans BC LOG12 the using three extraordinary carbon sources i.e. glucose, soluble gum fiber and a prebiotic FOS were hired to expand a bioprocess for decreasing fermentation time maintaining in control growth and sporulation efficiencies and reduced media cost for fermentation. Exceptional techniques like altered carbon/nitrogen ratio, mode of fermentation [20-25], The efficacy of superior sporulation and different concentration of sugar were studied as parameters which studied sequentially to reach maximum bioprocess. Also, in the present investigation, soluble gum fiber was used as sole source of carbon for attaining quick fermentation time for the probiotic culture Bacillus coagulans BC LOG12 was evolved.

II. Materials and Methods

The spore probiotic strain Bacillus coagulans BC LOG12 was cultured as per good manufacturing practices (GMP) guidelines. Bacillus coagulans BC LOG12 was grown aerobically at 37°C, 150 rpm in Lactobacilli MRS broth (Himedia) as primary growth media. Bacillus coagulans BC LOG12 is a thermostable lactic acid producing strain used globally in different food and nutraceutical applications due to its ability to withstand harsh manufacturing conditions and GI stability.

A. Preparation of inoculum, media composition and growth conditions

The viable cells of the Bacillus coagulans BC LOG12 was aseptically transferred from 1 mL of MRS media and incubated at 37oC until visible growth was observed which is usually 24 hours. The pH was monitored and shaking conditions were maintained with 150 rpm, once the maximum growth was obtained, the culture broth was used for future studies [13,14].

TABLE I. COMPOSITIONS OF THE SECONDARY AND PRODUCTION MEDIA USED FOR THE GROWTH OF BACILLUS COAGULANS BC LOG12

Components	Media Concentration (g/Lit)		
	M1	M2	M3
Glucose	-	20.0	-
Soluble gum fiber	10.0	-	-
Fructooligosaccharide	-	-	10.0
Peptone	10.0	10.0	10.0
Yeast extract	7.5	7.5	7.5
Urea	10.0	10.0	10.0
Magnesium sulphate.$7H_2O$	0.05	0.05	0.05
Manganous sulphate	2.5	2.5	2.5
Potassium dihydrogen phosphate	0.5	0.5	0.5
Calcium carbonate	3.0	3.0	3.0
Zinc Sulphate	1.0	1.0	1.0

Note: M1: medium 1, M2: medium 2, M3: medium 3

B. Production inoculum preparation

The inoculum was prepared with 50 mL x 3 actively growing 20-24 hours pure culture suspension which was aseptically transferred into 2000 mL flask containing 450 mL of production media with different carbon sources (M1- soluble gum fiber, M2- glucose & M3- FOS) in duplicates Table 1. The bacterium was grown under the influence of 150 rpm and at 37o C until adequate spore suspension was achieved. Flasks were incubated on orbital shaker at 37°C with 150 rpm until good sporulation is obtained. Samples were drawn at different time intervals and monitored for pH, cell density, sugar utilization and sporulation. The complete parameters implemented to carry out the experiments is cited in Table 2. All the experiments were performed in triplicates and to check the efficacy, three different carbon sources were screened for experiments.

C. Fermentation conditions

TABLE II. SHAKE FLASK PARAMETERS FOR THE GROWTH OF BACILLUS COAGULANS BC LOG12

Parameters	Details
Total volume	450 mL media x 2 (2000 mL Erlenmeyer flasks)
Incubation conditions	37 °C, 150 rpm
pH at transfer	6.5 ± 0.3
Inoculum (%)	10.0

D. Process parameters

The culture conditions of the bacterium was monitored with respect to parameters like temperature, sugar (total reducing sugar), rpm for agitation and optical density was measured at A630 nm, the pH was monitored followed by the sterility check which as carried out using plating techniques.

E. Batch harvest

The down streaming to the fermentation process was initiated by monitoring the changes in the parameters such as elevated pH and sporulation efficiency which was attenuated more than 75% and there was reduction in the reducing sugar concentration. The biomass density was also monitored. The M1, M2 and M3 medium flasks were harvested at different time intervals depending on the sporulation obtained. The harvest broth was centrifuged at 4000 rpm and cell pellet was suspended in sterile water and dried in oven at 100°C to obtain a free-flowing powder. This powder was taken up for total viable spore count analysis.

F. Measurement of dry cell mass and Total reducing sugar estimation

The cell mass was measured by drawing the samples at regular intervals followed by washing with sterile distilled water and the optical density was measured at 600 nm, the cells were further dried at 80oC for 8 hours and the dry weight was measured and calibration curve was plotted with cell density versus dry cell weight (g/L). The total sugar reduction was measured as per the protocol of anthrone method[Fog.4-6].

Total Viable Spore Count

The total viable cells of Bacillus coagulans was determined with sequential dilution followed by incubation at 70oC for 30 mins and reducing the temperature. The plating techniques was carried out by culturing the dilutions from 104 to 108 and the incubated for 48 to 72 hours and the colonies emerging on the petriplate were counted as colony forming unit [Table 3-5].

III. Results

A. M1 Shake flask fermentation of Bacillus coagulans yield

TABLE III. RESULTS OF M1 ON GROWTH OF BACILLUS COAGULANS BC LOG12

Cultural media M1	Details
Sporulation	**75% - 80%**
Fermentation time	48h - 50h
Biomass	3.4 g/L
Total viable spore count	**1.2 x 10^9 Cfu/g**

B. M2 Shake flask fermentation of Bacillus coagulans BC LOG12 yield

TABLE IV. RESULTS OF M2 ON GROWTH OF BACILLUS COAGULANS BC LOG12

Cultural media M2	Details
Sporulation	50% - 55%
Fermentation time	100h - 101h
Biomass	2.1 g/L
Total viable spore count	**0.5 x 10^9 Cfu/g**

C. M3 Shake flask fermentation of Bacillus coagulans BC LOG12 yield

TABLE V. RESULTS OF M3 ON GROWTH OF BACILLUS COAGULANS BC LOG12

Cultural media M3	Details
Sporulation	5% - 10%
Fermentation time	100h - 101h
Biomass	3.3 g/L
Total viable spore count	**0.1 x 10^9 Cfu/g**

IV. Discussion

The use of optimized concentration of soluble gum fiber along with optimized concentration of nitrogen & trace minerals resulted in reduction of fermentation time to 48 - 50 hours with 75%-80% sporulation in shake flask comparing to more than 100 hours of fermentation time using glucose and FOS as carbon sources. The pH trend using gum soluble fiber was maintained at 6.0 ± 0.5 throughout the fermentation favoring the growth of Bacillus coagulans BC LOG12 compared to glucose and FOS where pH drop was observed [Fig.1-3]. Currently available commercial media with complex carbon and nitrogen sources fermentation takes more than 100 hours without much sporulation in shake flasks. The current data we observed the lowest fermentation time reported values for cultivation of Bacillus coagulans BC LOG12 with a 75% - 80% sporulation and better biomass in shake flask when compared to glucose and FOS [Fig.10 -13].

V. pH Kinetics of Bacillus coagulans BC LOG12

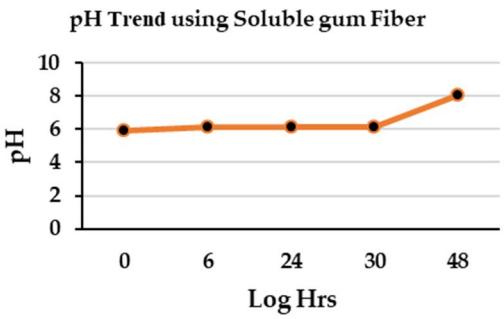

Fig.1. pH trend of Bacillus coagulans BC LOG12 using Soluble gum fiber

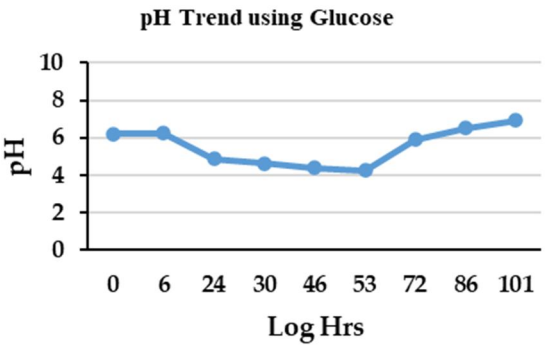

Fig.2. pH trend of Bacillus coagulans BC LOG12 using glucose

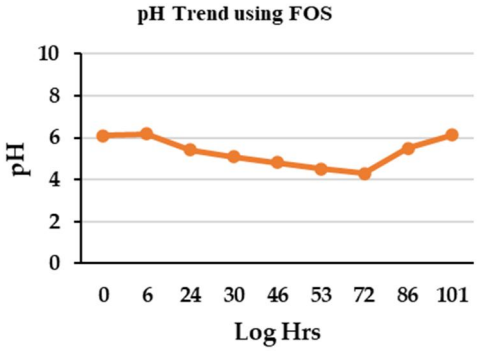

Fig.3. pH trend of Bacillus coagulans BC LOG12 using FOS

VI. Total Reducing Sugar Estimation

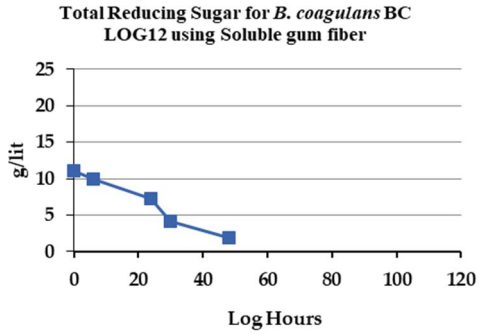

Fig.4. Total reducing sugar of Bacillus coagulans BC LOG12 using soluble gum fiber

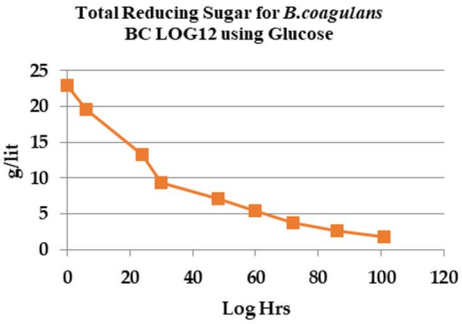

Fig.5. Total reducing sugar of Bacillus coagulans BC LOG12 using glucose

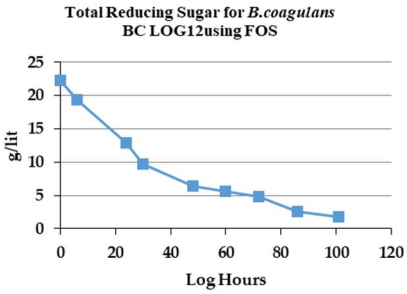

Fig.6. Total reducing sugar of Bacillus coagulans BC LOG12 using FOS

VII. Dry Cell Weight Estimation

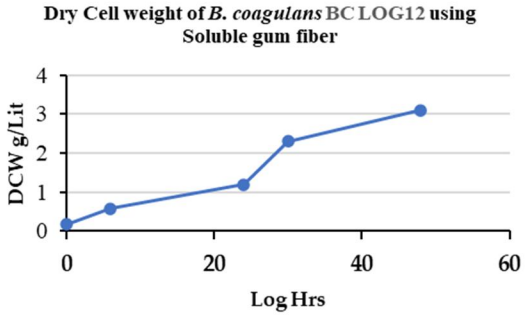

Fig.7. Dry cell weight of Bacillus coagulans BC LOG12 using soluble gum fiber

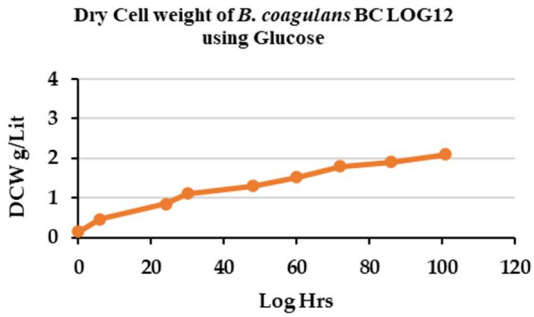

Fig.8. Dry cell weight of Bacillus coagulans BC LOG12 using glucose

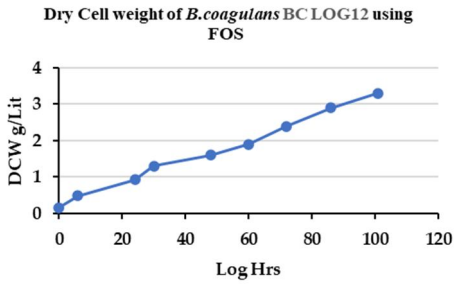

Fig.9. Dry cell weight of Bacillus coagulans BC LOG12 using FOS

VIII. Effect of Sporulation using Different Carbon Source

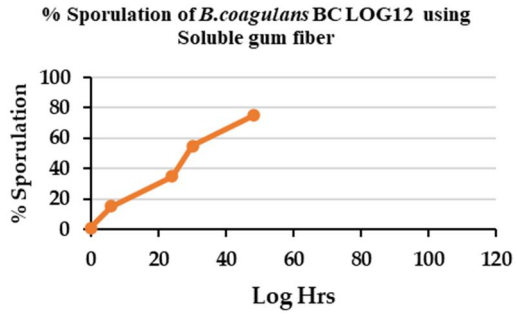

Fig.10. Effect of sporulation of Bacillus coagulans BC LOG12 using soluble gum fiber

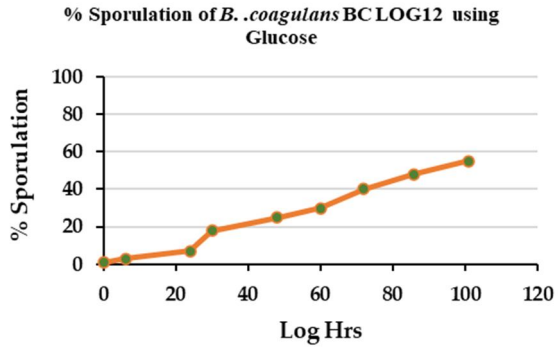

Fig.11. Effect of sporulation of Bacillus coagulans BC LOG12 using glucose

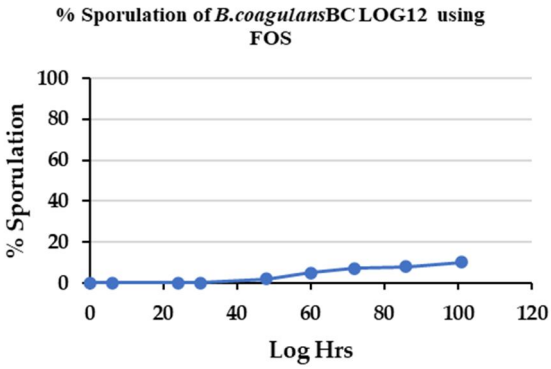

Fig.12. Effect of sporulation of Bacillus coagulans BC LOG12 using FOS

IX. TOTAL VIABLE SPORE COUNT OF BACILLUS COAGULANS BC LOG12 DRIED POWDER USING DIFFERENT CARBON SOURCES

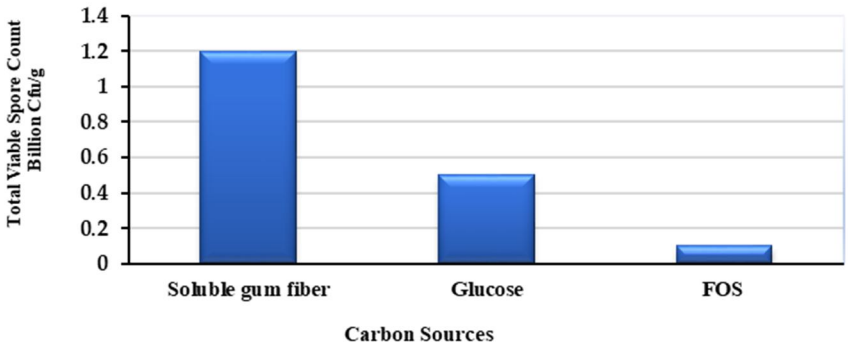

Fig.13. Total viable Spore count of Bacillus coagulans BC LOG12 using different carbon sources

X. CONCLUSION

The study overall displayed significant results pertaining to develop culture media for optimal growth parameters. The results showed that soluble gum fiber medium promoted significant sporulation in comparison with the other carbon sources which indicates the cost effectiveness of the fermentation media at large scale. The study forms the preliminary investigation which envisions the different parameters like elevated pH influencing the sporulation of Bacillus coagulans BC LOG12 which can be exploited for large scale production of probiotic functionalities. To best of our knowledge scanty reports are available on the use of soluble gum fibers which act as one of the best suited and minimal media for fermentation usage.

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