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RESEARCH

Evaluation of Bengal gram (*Cicer arietinum*) as a cost-effective nitrogen substitute for the optimized growth of probiotic *Lactobacillus* spp.

M. Kaviyanjali[1], A. Premlatha[2] and S. Kaneez Fathima[1]*

[1]P.G. & Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai, India
[2]Department of Zoology, Meenakshi College for Women Autonomous, Chennai, India

***Correspondence:**
S. Kaneez Fathima,
kaneezfathima.s@jbascollege.edu.in

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Probiotic *Lactobacillus* species are nutritionally fastidious microorganisms that require protein-rich nitrogen sources for optimal growth, making conventional culture media such as Luria-Bertani (LB) and Nutrient Agar economically inappropriate for large-scale and industrial applications. The present study analyzes the potential of Bengal gram (*Cicer arietinum*) powder as a low-cost, plant-based nitrogen source for cultivating *Lactobacillus spp.,* In order to determine the protein concentration per gram of substrate, Bengal gram powder was quantitatively estimated using Lowry's method. Based on this estimation, Bengal gram powder was added into the culture medium in the place of peptone and tryptone in the culture medium formulation, thereby serving as the primary nitrogen source. The basal medium was consists of beef extract, sodium chloride, agar and distilled water, while standard Luria medium and Nutrient Agar served as control media. The growth performance of selected probiotic *Lactobacillus* strains was analyzed by biomass yield and observing growth during incubation. The findings highlights that the Bengal gram–based medium effectively promoted the bacterial growth, yielding growth curves and biomass production compared to those of traditional control media. *Lactobacillus* species nutritional requirements were sufficiently satisfied by Bengal gram's natural micronutrient profile and high protein content. This study reveals that Bengal gram powder can effectively replace peptone as a nitrogen source, cost-effective, sustainable, and scalable alternative culture medium for the fermentation and industrial productions.

Keywords: *Lactobacillus*, probiotics, Bengal gram (*Cicer arietinum*), alternative media, fermentation, sustainable media

Introduction

Probiotic microorganisms, particularly Lactic acid bacteria (LAB) are essential to the food, pharmaceutical and aquaculture industries because they improve host nutrition, strengthen immune responses, and preserve gut health. These bacteria are naturally fastidious require complex organic nitrogen source, amino acids and vitamins for optimal growth (1). Traditional, culture medium such as Luria-Bertani (LB) broth and Nutrient agar incorporated with tryptone and peptone have been extensively used for large scale probiotic production. Moreover, the expensive nature and animal derived origin of peptone limit their utilization in industrial scale production (2).

The increasing demand for cost-and sustainable plant-based alternative has driven researchers to investigate agricultural substrate as a potential nutrient source for microbial growth. According Campos-Vega et al. (3), pulses are specifically promising because of their high protein content, essential amino-acids, minerals and bioactive compounds that supports bacterial metabolism. Among these, Bengal gram (*Cicer arietinum*) is notable for being widely available, affordable and nutrient-rich containing about 18–22% protein, carbohydrates, vitamins and essential micronutrients (4).

Several analyses have shows the effective use of plant-derived substrates such as soybean, chickpea and grain flours can be used effectively as substitute nitrogen sources for LAB that exhibit growth equivalent to that of traditional media (5, 6). Nevertheless, few studies have specifically assessed Bengal gram powder as a direct replacement of peptone in standard bacteriological medium formulation.

Therefore, the present study aims to evaluate the Bengal gram powder potential as a low cost plant based nitrogen source for the cultivation of probiotic *Lactobacillus* species. The protein content of Bengal gram powder was quantified using Lowry's method, followed by its incorporated in to Nutrient agar as a substitute for peptone. The growth of *Lactobacillus* Strains was observed and compared with traditional LB and Nutrient agar media to determine its large scale and sustainable probiotic production.

Methodology

Preparation of Bengal gram powder

The Bengal gram seeds (*Cicer arietinum*) were washed thoroughly, shade-dried and ground into a fine powder using a sterile mechanical grinder. The powder sample was then stored in airtight container until required.

Bovine serum albumin (BSA) standard preparation

A stock solution of bovine serum albumin (BSA) was prepared at 1 mg/mL concentration in distilled water. Working standards were then prepared by pipetting 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the BSA solution into separate test tubes, representing 200–1000 μg of protein and adjusting the final volume with 0.1 N NaOH. A blank was prepared without BSA. To each tubes 4.5 mL of alkaline was added and incubated at room temperature for 15 minutes. Then 0.5 mL of Folin-Ciocalteu reagent was introduced and the mixture is allowed to stand for 30 minutes to develop color. The absorbance was measured at 580 nm using a spectrophotometer and a standard curve was plotted to estimate the protein content of the sample.

Estimation of protein content in Bengal gram powder

The protein content of Bengal gram (*Cicer arietinum*) powder was estimated by the Lowry's method (7). The powdered sample was processed to extract protein, which was then analyzed using a colorimetric assay. A blue color complex is formed after reacting with alkaline copper reagent and Folin-Ciocalteu reagent and its intensity was measured at 580 nm with a spectrophotometer. The protein concentration of the sample was quantified using a standard calibration curve and reported as milligrams of protein per gram of Bengal gram powder.

Preparation of Bengal gram–based culture medium

Based on the protein estimation, Bengal gram powder was incorporated into the culture medium as a replacement for peptone. The modified medium consists of beef extract, sodium chloride, agar, Bengal gram powder (as the nitrogen source), and distilled water. The overall composition of the Bengal gram–based medium was maintained similar to that of conventional Nutrient Agar and LB medium, except that peptone and tryptone was replaced by Bengal gram powder as the nitrogen source. The medium pH was adjusted to neutral and sterilized by autoclaving at 121 °C for 15 minutes.

Control media

Standard LB medium and conventional Nutrient Agar were procured in dehydrated form from a commercial supplier and prepared according to the manufacturer's instructions by dissolving the appropriate quantity of powder in distilled water followed by autoclaving at 121 °C for 15 minutes.

Bacterial strains and growth assessment

Selected probiotic *Lactobacillus* strains were aseptically inoculated into the Bengal gram–based medium and control media. The cultures were incubated at suitable growth temperature under standard conditions. Bacterial growth was observed by measuring optical density at regular intervals to analyze growth kinetics and biomass yield was recorded for comparative analysis.

Results

Protein estimation of Bengal gram powder

The protein content of Bengal gram (*Cicer arietinum*) powder was examined using Lowry's method with BSA serving as the standard. A linear standard calibration curve was obtained, and the absorbance values of the Bengal gram extract were used to calculate protein concentration. The standard curve is shown in **Figure 1**, and the calculated protein concentration of Bengal gram powder is presented in **Table 1**.

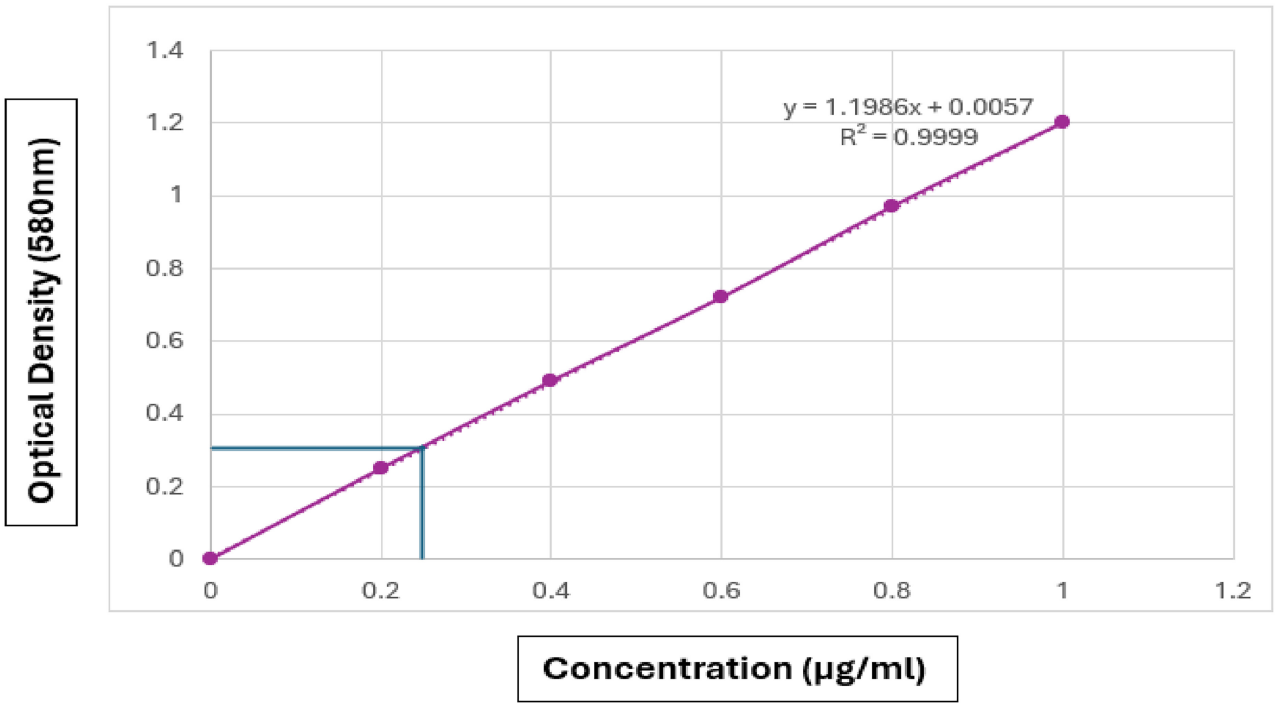

FIGURE 1 | Standard curve for protein estimation of Bengal gram powder. Protein concentration of Bengal gram powder: 0.25 mg/g

TABLE 1 | Protein estimation of Bengal gram powder using Lowry's method.

S. no.	Particulars	Standard (BSA)						Test sample (Bengal gram)
		Blank	S1	S2	S3	S4	S5	
1.	Volume of standard solution	-	0.2	0.4	0.6	0.8	1.0	Protein precipitate 0.5 mL
2.	Volume of 0.1 N NaOH	1.0	0.8	0.6	0.4	0.2	-	0.5 mL
3.	Volume of Alkaline copper reagent	4.5	4.5	4.5	4.5	4.5	4.5	4.5 mL of alkaline reagent
Allow it to stand for 15 minutes								
4.	Volume of Folin's reagent	0.5	0.5	0.5	0.5	0.5	0.5	0.5 mL
Allow it to stand for 30 minutes								
5.	Optical density of 580 nm	-	0.25	0.49	0.72	0.97	1.2	0.30 mL

Comparison of culture media composition

The composition of the Bengal gram–based medium was compared with standard LB medium and conventional Nutrient Agar. All media contained similar basal components, with variation only in the nitrogen source, where tryptone and peptone in control media were replaced by Bengal gram powder in the experimental medium. The comparative composition of the media is presented in **Table 2**.

Growth of *Lactobacillus spp*. at 24 and 48 hour

The growth of *Lactobacillus spp.*, on agar plates prepared using LB medium, Nutrient Agar and Bengal gram based medium was examined after 24 and 48 hours of incubation. Diverse bacterial colony formation was detected on all media at both time intervals. Representative agar plate images showing bacterial growth in control and experimental media are presented in **Figure 2**.

TABLE 2 | Comparison of culture media composition.

S. no.	Components	Luria–Bertani (LB) medium	Nutrient agar medium	Bengal gram medium
1.	Nitrogen source	Tryptone	Peptone	Bengal gram powder
2.	Beef extract	Yeast extract	Present	Present
3.	Sodium chloride	Present	Present	Present
4.	Agar	Present	Present	Present
5.	Distilled water	Present	Present	Present
6.	PH	Neutral	Neutral	Neutral

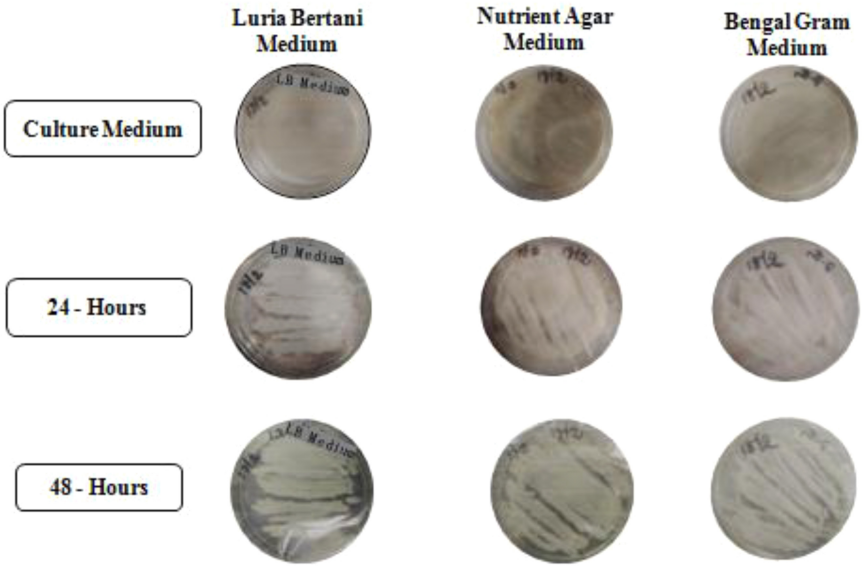

FIGURE 2 | Growth of *Lactobacillus* spp. at 24 and 48 hour.

Discussion

In the present study Bengal gram (*Cicer arietinum*) powder was evaluated as an alternative plant-based nitrogen source for microbial culture media and its protein content was determined to be 0.25 mg/g using Lowry's method. Bengal gram has a relatively low protein concentration when compared to conventional laboratory nitrogen sources such as peptone and tryptone, which usually contain higher soluble protein content 10–20 mg/g (2, 7). However, present studies have highlight that total protein concentration alone does not determine the microbial growth efficiency, especially for LAB, which can metabolize complex plant-derived substrates effectively.

Studies report that legumes such as Bengal gram contain storage proteins, peptides and non-protein nitrogen compounds that are gradually released and utilized during microbial metabolism (4). Campos-Vega et al. (3) demonstrated that chickpea-based substrates support microbial growth due to their balanced composition of proteins, carbohydrates and micronutrients even when measurable soluble protein content is comparatively lower. This confirms the present study that *Lactobacillus spp.*, grow on Bengal gram-based medium at a protein concentration of 0.25 mg/g as indicated by visible colony formation similar to that observed on standard Nutrient Agar and LB agar.

Additionally, plant based medium enriched with cereals or legumes, where nitrogen is progressively released during fermentation can support the growth of LAB (5). These results are consistent with the present study, which showed gradual nutrient utilization from Bengal gram powder and increased colony density after 24 and 48 hours. Despite the lower initial protein concentration, the persistent growth pattern implies that Bengal gram proteins and related nutrient were metabolically available.

Several analysis emphasize the need of developing low-cost, non-animal-derived culture media for industrial microbiology and probiotic application (2, 6). Consistent with these findings, the present study shows that Bengal gram powder, can effectively substitute peptone and tryptone without compromising bacteria even at a protein concentration of 0.25 mg/g. This comparative analysis confirmed the potential of Bengal gram as a sustainable and economical alternative nitrogen source, especially suitable for industrial application and large-scale probiotic cultivation.

Conclusion

This study shows that Bengal gram (*Cicer arietinum*) powder can be effectively used as a plant-based alternative nitrogen source in microbial culture media. Despite exhibiting a relatively low protein concentration of 0.25 mg/g, as determined by Lowry's method, Bengal gram–based medium successfully supported the growth of probiotic *Lactobacillus* strains, with visible robust colony development comparable to conventional Nutrient Agar and LB media. The increase in growth observed after 24 and 48 hours interval indicates gradual nutrient availability and efficient utilization of pulses-derived proteins by the microorganisms. These results highlight the potential of Bengal gram powder as a cost-effective, non-animal-derived substitute for peptone and tryptone in microbiological media preparation, especially for fermentation, probiotic research and sustainable industrial applications.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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