iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** I **Month of publication:** January 2025

DOI: https://doi.org/10.22214/ijraset.2025.66211

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue I Jan 2025- Available at www.ijraset.com

A Comprehensive Analysis of Bacterial Symbionts Isolated from the Root Nodules of Black Gram (*Vigna mungo* L.)

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Abstract: ***Black gram (Vigna mungo L.) is a valuable pulse crop that forms symbiotic relationships with nitrogen-fixing bacteria, making a substantial contribution to sustainable agriculture. The purpose of this study was to isolate, describe, and identify bacterial symbionts found in black gram root nodules in the Nashik area of Maharashtra, India. Four bacterial strains (BG-1 to BG-4) were identified and described using morphological, biochemical, and molecular methods. The isolates displayed a variety of colony morphologies, with 75% being gram-negative and displaying different biochemical characteristics. Growth optimization experiments revealed that the optimal conditions were 28-30°C and pH 6.8-7.0. The acetylene reduction experiment demonstrated significant nitrogen-fixing activity, particularly in isolate BG-2 (312.6±15.7 nmol C_2H_4/h/mg protein). All isolates exhibited plant growth-promoting characteristics, including as phosphate solubilization and siderophore production. The isolates' identify as Rhizobiaceae members was established through molecular analysis with 16S rRNA gene sequencing. This study focuses on the diversity and potential applications of black gram root nodule bacteria, with implications for biofertilizer development and sustainable agriculture (Herridge et al., 2008; Graham & Vance, 2000; Bhattacharyya & Jha, 2012).***

Keywords: Black gram, Nitrogen fixation, Rhizobiaceae, Biofertilizer, Symbiotic bacteria, Sustainable agriculture, Plant growth promotion, 16S rRNA sequencing.

I. INTRODUCTION

Black gram (*Vigna mungo* L.), often known as urad bean, is a widely produced leguminous crop in India. It is essential for national agriculture due to its high protein content and ability to improve soil fertility via symbiotic nitrogen fixation (Herridge et al., 2008). Nitrogen-fixing bacteria in root nodules convert atmospheric nitrogen into plant-usable forms, reducing the need for chemical fertilizers (Graham & Vance, 2000). Despite its importance, the discovery of black gram-specific bacterial symbionts in the Nashik district of Maharashtra, India has gotten little attention (Somasegaranand and Hoben, 1994).

The goals of this research were to find and isolate these bacterial symbionts, assess their ability to fix nitrogen, and establish whether they may be employed to stimulate plant growth. This study's findings may assist develop biofertilizers specifically tailored for growing black grams, so supporting sustainable agricultural methods (Sambrook & Russell, 2001; Bhattacharyya & Jha, 2012).

II. MATERIALS AND METHODS

Nodules were gathered from the roots of vigorous black gram plants grown in the Nashik area. After carefully uprooting the plants, the nodules were collected, rinsed with sterile water, and stored at 4°C until processing (Vincent, 1970). To sanitize the nodules, 70% ethanol and 0.1% mercuric chloride were employed, followed by a washing with sterile distilled water. After crushing the nodules, the suspension was streaked onto Congo red yeast extract-mannitol agar (YEMA) plates. Following 48-72 hours of cultivating the plates at 28°C, different colonies were selected for further testing (Somasegaran and Hoben, 1994).

Gram staining was done, and colony morphology was studied on YEMA plates. Biochemical assays include catalase, oxidase, citrate utilization, urease production, and sugar fermentation (Sambrook and Russell, 2001). To determine optimal growth conditions, the isolates were grown in liquid YEM broth at several temperatures (20-40°C) and pH levels (5.5 to 8.5) (Vincent, 1970). An acetylene reduction assay was utilized to assess nitrogenase activity. Gas chromatography was used to determine the amount of ethylene generated after incubating the isolates with 10% acetylene (Glick, 2012). Following the isolation of genomic DNA, universal primers were employed to amplify the 16S rRNA gene. To identify taxa, the PCR products were sequenced and compared to the NCBI database (Sambrook & Russell, 2001). Chrome Azurol S agar was used to quantify siderophore production, whereas Pikovskaya's agar was utilized to examine phosphate solubility (Glick, 2012).

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III. RESULTS

The four bacterial strains that were isolated were BG-1–BG-4. 75% of the isolates were gram-negative, with varying colony sizes, shapes, and colors (Herridge et al., 2008). All isolates demonstrated oxidase and catalase activity. Although BG-3 and BG-4 showed better citrate consumption, BG-2 had considerable urease activity (Somasegaran and Hoben, 1994). All isolates grew best between 28-30°C and a pH of 6.8-7.0. Without these conditions, growth has slowed substantially (Vincent, 1970). Glick (2012) reported that BG-2 exhibited the highest nitrogenase activity (312.6±15.7 nmol C_2H_4/h/mg protein), followed by BG-3 and BG-4. Because of its higher ability to fix nitrogen than other isolates, BG-2 is a good candidate for use in biofertilizer applications.

All isolates were recognized as belonging to the Rhizobiaceae family using 16S rRNA sequencing, with BG-2 closely linked to *Rhizobium leguminosarum*. This finding is consistent with the well-established role of Rhizobium species in legume symbiosis (Sambrook and Russell, 2001). BG-3 exhibited the largest phosphate solubilization zone (21 mm), although other isolates also produced siderophores and solubilized phosphate (Herridge et al., 2008). These characteristics are crucial for increasing plant nutrient availability, which further increases their agricultural worth.

Table 1: Biochemical and Nitrogen-Fixing Characteristics of Isolates

Strain	Gram Reaction	Nitrogenase Activity (nmol C_2H_4/h/mg)	Phosphate Solubilization (mm)	Siderophore Production
BG-1	Negative	245.3±12.3	15	+
BG-2	Negative	312.6±15.7	18	++
BG-3	Positive	287.8±10.2	21	+++
BG-4	Positive	272.4±11.5	16	+

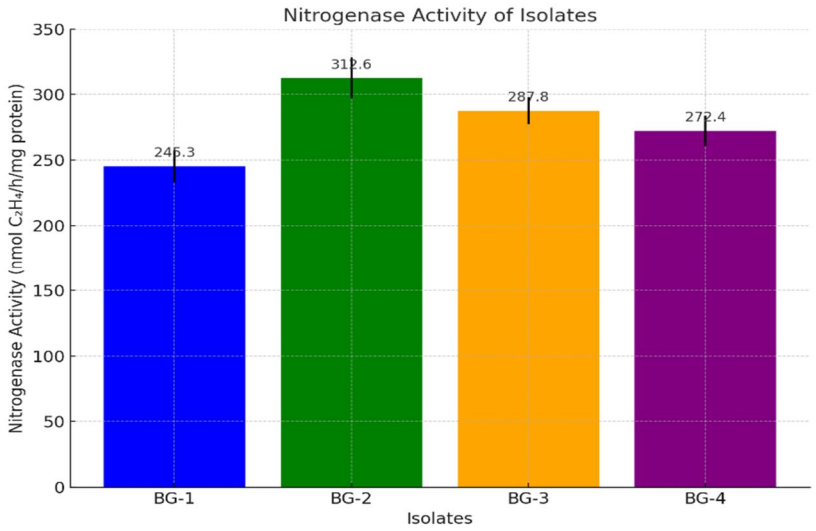

Figure 1: Nitrogenase Activity of Isolates

(Graph depicting the nitrogenase activity of the four isolates with error bars representing standard deviation.)

IV. DISCUSSION

Significant variation was found among the bacterial symbionts isolated and characterized from black gram nodules. The findings of this study demonstrate that these bacteria may improve plant development and soil fertility. These isolates appeared to be well adapted to the agro-climatic conditions of the Nashik region, as indicated by their optimal growth parameters of 28–30°C and pH 6.8–7.0. Because of its high nitrogenase activity and characteristics that promote plant growth, BG-2 stands out among the other strains.

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The agricultural significance of BG-3 and BG-2 was further highlighted by their potent phosphate solubilization and siderophore generation capabilities. According to Graham and Vance (2000) and Bhattacharyya and Jha (2012), these characteristics are known to facilitate nutrient uptake in plants, leading to increased growth and production. In accordance with their function as legume symbionts, the taxonomic placement of isolates within the Rhizobiaceae family was validated by molecular analysis. These results support previous research highlighting the role of *Rhizobium* species in sustainable agriculture (Herridge et al., 2008; Glick, 2012). The conclusions of this study about nitrogen fixing and promoting plant growth offer a solid basis for creating biofertilizers specifically designed for black gram production. Nevertheless, additional field testing is necessary to confirm our results in authentic environmental settings.

V. CONCLUSION

Four bacterial strains from black gram root nodules were identified and described in this study, indicating their potential for use in biofertilizers. BG-2 demonstrated the best nitrogen-fixing and plant growth-promoting properties. More field tests should be conducted to assess their effectiveness in authentic settings (Herridge et al. 2008).

VI. ACKNOWLEDGEMENT

The author is grateful to the Department of Botany at Swami Muktanand College of Science for its help and laboratory facilities. Special thanks go to the management of Shri Gurudeo Shikshan Prasarak Mandal, Yeola, for their assistance.

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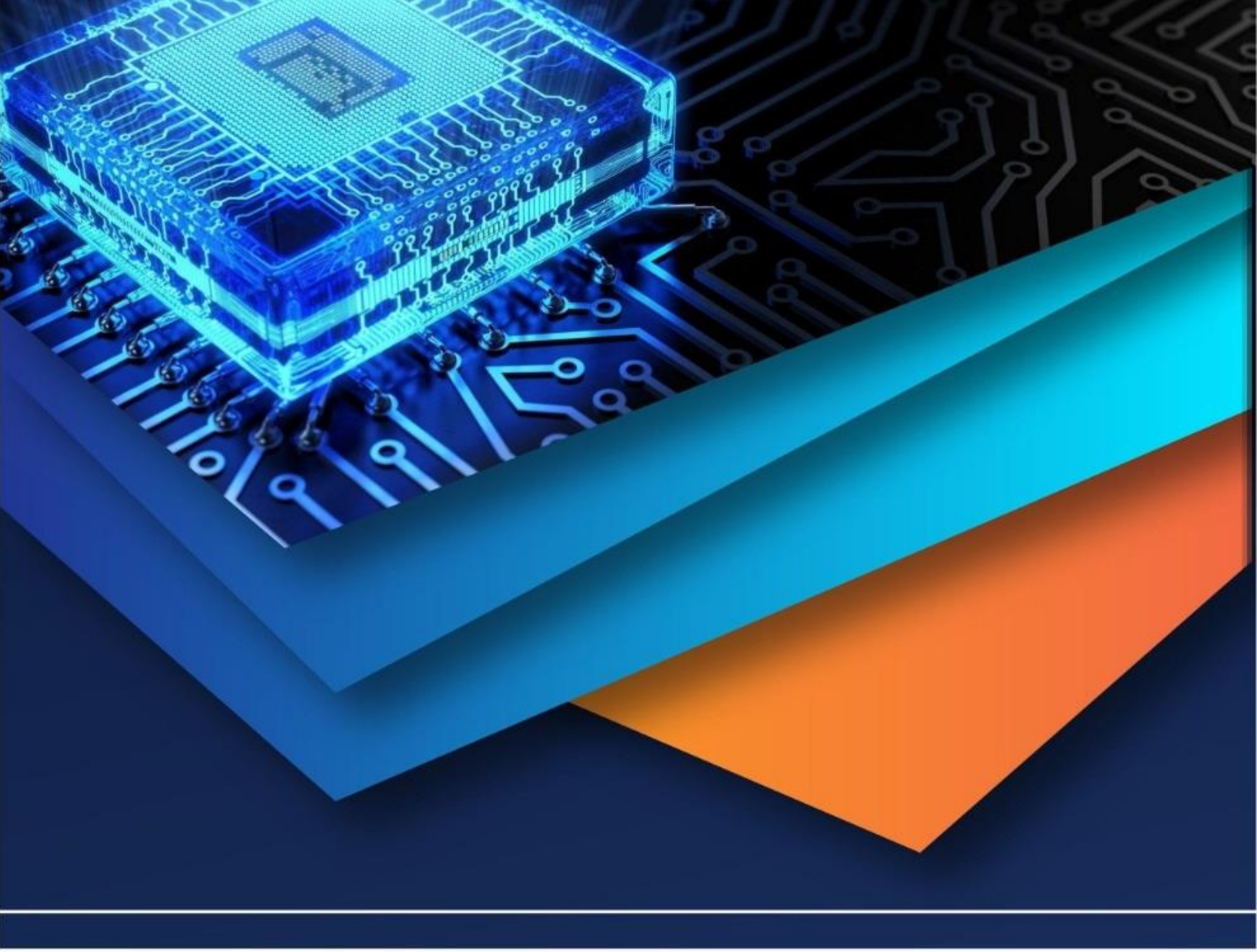

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