



Characterization of Rhizobacteria for Sustainable Agriculture and Biofertilizer Development

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DOI: <https://doi.org/10.53762/grjnst.03.01.34>

Abstract

Okra (*Abelmoschus esculentus* L.) is recognized as one of the most nutritionally and economically significant vegetable crops cultivated extensively across tropical and subtropical regions. Its pods are enriched with vitamins, minerals, dietary fiber, and mucilage, making it a vital component of food security strategies in many developing countries where malnutrition and dietary deficiencies remain pressing challenges. Despite its importance, okra productivity is frequently constrained by multiple factors, including nutrient



deficiencies, pest infestations, and abiotic stresses such as drought, salinity, and erratic rainfall patterns. These limitations have prompted the search for sustainable solutions that can enhance crop yield while maintaining soil health. Plant growth-promoting rhizobacteria (PGPR), which inhabit the rhizosphere, have emerged as promising eco-friendly alternatives to chemical fertilizers and pesticides. PGPR are known to fix atmospheric nitrogen, solubilize phosphorus and zinc, produce phytohormones such as indole-3-acetic acid, and secrete metabolites that suppress phytopathogens. Their multifunctional roles not only improve nutrient availability but also strengthen plant tolerance to environmental stressors. In the present study, five bacterial isolates (MH01, MH03, MH04, MH05, MH06) were characterized for their biochemical traits to evaluate their potential as PGPR candidates for okra cultivation. A series of enzymatic assays revealed strong amylase and cellulase activity in strain MH04, highlighting its capacity to degrade complex carbohydrates and contribute to nutrient cycling. Protease production was most pronounced in MH03, suggesting its role in protein turnover and nitrogen mineralization. Phosphate solubilization was highest in MH05, while zinc solubilization was most effective in MH01, indicating their importance in mobilizing essential macronutrients and micronutrients. Catalase activity was consistently positive across all isolates, reflecting their resilience under oxidative stress conditions. In addition, exopolysaccharide production and ammonia release were observed in several strains, further emphasizing their functional diversity and potential contributions to soil aggregation, nutrient enrichment, and stress tolerance. Collectively, these findings demonstrate that the isolates possess complementary biochemical traits that can be harnessed in combination as biofertilizers and biocontrol agents. Their application in okra cultivation could reduce reliance on synthetic inputs, improve soil fertility, and enhance crop yield under diverse environmental conditions. This study provides valuable insights into the functional diversity of okra-associated rhizobacteria and highlights their potential role in advancing sustainable agriculture.

Keywords: Rhizobacteria, Sustainable Agriculture, Biofertilizer Development, vegetable crops, Okra, Characterization

Introduction

The rhizosphere is widely recognized as one of the most biologically active interfaces in agricultural soils, where plant roots interact with diverse microbial communities that regulate nutrient cycling, stress tolerance, and overall plant health (Shameer & Prasad, 2018; Vejan et al., 2016). Within this zone, plant growth-promoting rhizobacteria (PGPR) have emerged as crucial allies in sustainable agriculture. PGPR influence plant growth through direct mechanisms such as nitrogen fixation, phosphate and zinc solubilization, and phytohormone production, as well as indirect mechanisms including pathogen suppression, induction of systemic resistance, and mitigation of abiotic stresses (Backer et al., 2018; Ahemad & Kibret, 2014). Their multifunctional roles make them indispensable for improving crop productivity while reducing reliance on synthetic inputs.

Okra (*Abelmoschus esculentus L.*) is a vegetable of global nutritional and economic importance. Its pods are rich in vitamins (C, K, B6, folates), minerals (Ca, Fe, P), dietary fiber, and mucilage, which contribute to digestive health, antioxidant activity, and immune function (Gemedede et al., 2015;

Elkhalifa et al., 2021). In many developing countries, okra serves as a food security crop, providing essential nutrients to populations vulnerable to malnutrition. However, okra productivity is often constrained by nutrient deficiencies, pest infestations, and climate variability, leading to yield losses of up to 50% in some regions (Saima et al., 2022; Massrie, 2025). Conventional reliance on chemical fertilizers and pesticides has improved yields but has also contributed to soil degradation, water pollution, and ecological imbalance.

PGPR offer an eco-friendly alternative that can simultaneously enhance crop yield and soil health. Recent studies have demonstrated that PGPR inoculation in okra increases photosynthetic efficiency, nitrogen content, and root biomass, while reducing dependence on synthetic inputs (Mustapha et al., 2025). PGPR strains have also been shown to produce indole-3-acetic acid (IAA), reduce ethylene accumulation under stress, and enhance antioxidant activity, thereby improving crop resilience under salinity and drought conditions (Ng et al., 2024; Zou et al., 2024; Zhang et al., 2025). Despite these advances, limited information is available on the biochemical diversity of rhizobacteria associated specifically with okra roots, particularly in terms of their enzymatic activities and nutrient-solubilizing potential. This study was designed to: Characterize bacterial isolates obtained from the okra rhizosphere based on their biochemical traits. Evaluate enzymatic activities including amylase, protease, pectinase, cellulase, and catalase production. Assess functional traits such as phosphate and zinc solubilization, exopolysaccharide formation, and ammonia production. Identify promising PGPR strains with potential application as biofertilizers and biocontrol agents for sustainable okra cultivation.

Materials and Methods

Collection of Plant Samples and Isolation of Rhizobacteria

Healthy okra (*Abelmoschus esculentus L.*) plants were selected from experimental fields, and rhizospheric soil samples adhering to roots were collected under aseptic conditions. The soil was serially diluted and plated on nutrient agar medium to obtain distinct bacterial colonies, following standard microbiological protocols (Cappuccino & Sherman, 2014). Colonies with unique morphology were purified through repeated streaking and maintained on nutrient agar slants at 4 °C for subsequent biochemical characterization.

Enzymatic Assays

To evaluate the functional diversity of isolates, a series of enzyme production assays were conducted:

Amylase activity was assessed on starch agar plates. After incubation at 30 °C for 24 h, plates were flooded with iodine solution, and clear zones around colonies indicated starch hydrolysis (Sen et al., 2014; Pranay et al., 2019).

Protease activity was determined using skim milk agar. Transparent halos around colonies after incubation confirmed protease secretion (Vijayaraghavan & Lalithakumari, 2013).

Pectinase activity was tested on citrus pectin agar. Plates were treated with acidic ethanol after incubation, and halo formation indicated pectin degradation (Oumer & Abate, 2018).

Cellulase activity was measured on carboxymethyl cellulose agar. Plates were stained with Congo red and destained with NaCl to visualize hydrolysis zones (Shaikh et al., 2025).

Catalase activity was detected by adding hydrogen peroxide (5%) to colonies on glass slides. Immediate bubble formation indicated catalase production (AlAli et al., 2021).

Nutrient Solubilization Assays

Functional traits related to nutrient mobilization were examined:

Phosphate solubilization was tested on Pikovskaya's agar containing tricalcium phosphate. Clear zones around colonies after 72 h incubation indicated solubilization (Pikovskaya, 1948; Zaidi et al., 2009).

Zinc solubilization was evaluated using insoluble zinc compounds in agar medium, following the protocol of Fasim et al. (2002). Transparent zones confirmed solubilization.

Exopolysaccharide and Ammonia Production

Exopolysaccharide (EPS) production was assessed on RCV-glucose medium. Viscous colony morphology after 7 days incubation indicated EPS formation (Ashraf et al., 2004).

Ammonia production was measured in peptone water broth. After incubation, cultures were treated with Nessler's reagent, and the development of yellow to brown coloration indicated ammonia release (Vyas et al., 2018).

Data Recording and Analysis

For enzyme assays, solubilization intensity (SI) and solubilization efficiency (SE) were calculated using halo and colony diameters, following the formulae described by AlAli et al. (2021). Each experiment was conducted in triplicate, and results were expressed as mean values to ensure reproducibility. Statistical consistency was maintained by comparing replicate values, and isolates were ranked based on their relative efficiency in each biochemical trait.

Results

In this experiment the comprehensive biochemical screening of rhizobacterial isolates were checked, a diverse set of observations highlight the functional variability among strains. The outcomes are presented systematically, beginning with enzymatic activities and extending to nutrient solubilization, exopolysaccharide formation, and ammonia production. These results provide a comparative overview of the isolates' capabilities, allowing identification of strains with the most promising traits for plant growth promotion and sustainable agricultural applications.

Amylase Activity

All isolates, except strain MH05, demonstrated measurable amylase activity. The highest solubilization intensity (SI = 2.7) and efficiency (SE = 170%) were recorded for strain MH04, indicating strong starch-degrading potential. Moderate activity was observed in MH03 (SI = 2.0; SE = 30%) and MH01 (SI = 1.5; SE = 54%), while MH06 exhibited comparatively lower activity (SI = 1.2; SE = 20%). These results highlight MH04 as the most effective amylolytic strain, capable of mobilizing carbohydrates in the rhizosphere (Table 1).

Protease Activity

Protease production varied considerably among isolates. MH03 showed the highest activity (SI = 4.1; SE = 210%), followed by MH05 (SI = 2.6; SE = 160%) and MH04 (SI = 3.1; SE = 106%). MH01 and MH06 displayed only modest activity (SI = 1.5; SE = 50%). The strong proteolytic capacity of MH03 suggests its potential role in protein turnover and nitrogen mineralization, which are critical for sustaining plant growth (Table 2).

Pectinase Activity

Clear halo formation confirmed pectinase secretion in most isolates, except MH05. MH03 (SI = 2.2; SE = 118%) and MH06 (SI = 2.2; SE = 121%) were the most efficient strains, while MH01 (SI = 2.0; SE = 100%) and MH04 (SI = 1.5; SE = 80%) showed moderate activity. The ability of these isolates to degrade pectin indicates their contribution to root penetration and nutrient mobilization (Table 3).

Catalase Activity

All five isolates tested positive for catalase production, as evidenced by rapid bubble formation upon exposure to hydrogen peroxide. This uniform response indicates that the strains possess oxidative stress tolerance, a trait beneficial for survival in diverse soil environments and for protecting plants under stress conditions (Table 4).

Cellulase Activity

Cellulase activity was detected in four isolates, with MH04 exhibiting the highest values (SI = 7.0; SE = 500%). MH03 (SI = 6.6; SE = 460%) and MH01 (SI = 5.3; SE = 433%) also showed strong activity, whereas MH06 displayed moderate levels (SI = 4.0; SE = 300%). MH05 did not produce detectable cellulase. These results suggest that MH04 is particularly effective in cellulose degradation, which may enhance organic matter turnover in the rhizosphere (Table 5).

Phosphate Solubilization

Phosphate solubilization was most pronounced in MH05 (SI = 3.0; SE = 200%), followed by MH06 (SI = 2.3; SE = 128%). MH01 and MH03 showed weak activity (SI = 1.6; SE = 56% and 20%, respectively), while MH04 did not exhibit solubilization. The superior performance of MH05 highlights its potential for mobilizing phosphorus in soils where availability is limited (Table 6).

Zinc Solubilization

Zinc solubilization was observed in MH01 (SI = 4.5; SE = 350%), MH04 (SI = 3.0; SE = 100%), and MH03 (SI = 2.6; SE = 67%). Strains MH06 and MH05 did not show activity. The superior performance of MH01 highlights its potential for micronutrient mobilization, which is critical for improving crop nutritional quality (Table 7).

Exopolysaccharide Production

EPS production was confirmed in MH01, MH03, and MH06, all of which produced viscous colonies (+++). MH04 and MH05 did not exhibit EPS formation. EPS secretion is known to improve soil aggregation, enhance water retention, and protect roots under stress conditions, suggesting that these strains may contribute to soil structure and resilience (Table 8).

Ammonia Production

Ammonia release varied among isolates. MH01 and MH03 produced strong positive reactions (+++), MH05 showed moderate activity (++) , MH04 weak activity (+), while MH06 was negative. The strong ammonia production in MH01 and MH03 indicates their potential role in nitrogen enrichment of the rhizosphere (Table 9).

Taken together, the biochemical assays revealed distinct functional profiles among the five isolates. MH04 excelled in amylase and cellulase production, MH03 was dominant in protease activity, MH05 was most efficient in phosphate solubilization, and MH01 showed superior zinc solubilization. The consistent catalase activity across all strains underscores their resilience under oxidative stress. These results suggest that different isolates may

contribute complementary functions in the rhizosphere, supporting okra growth through nutrient mobilization, enzyme secretion, and stress tolerance.

Discussion

The present study provides new insights into the biochemical diversity of rhizobacteria isolated from the okra (*Abelmoschus esculentus*) rhizosphere, highlighting their potential role in plant growth promotion and sustainable crop management. The enzymatic assays revealed that several isolates possessed strong hydrolytic capacities, particularly MH04, which exhibited superior amylase and cellulase activity. These enzymes are critical for the degradation of complex carbohydrates into simpler sugars, thereby enhancing nutrient cycling in the rhizosphere. Similar findings have been reported in PGPR associated with cereals, where amylase and cellulase activity contributed to improved root colonization and nutrient mobilization (Ma et al., 2019; Sen et al., 2014).

Protease activity was most pronounced in MH03, suggesting its contribution to protein turnover and nitrogen mineralization. Protease secretion by PGPR has been linked to both nutrient release and pathogen suppression, as proteolytic enzymes can degrade structural proteins of soil-borne pathogens (Vijayaraghavan & Lalithakumari, 2013; Backer et al., 2018). The strong proteolytic capacity of MH03 therefore indicates its dual role in nutrient cycling and biocontrol. Pectinase activity, observed in MH03 and MH06, further supports the role of these isolates in degrading plant polymers, which may facilitate root penetration and improve nutrient availability (Oumer & Abate, 2018).

Catalase activity was consistently positive across all isolates, indicating their resilience under oxidative stress. This trait is particularly relevant in soils exposed to abiotic stresses such as salinity and drought, where reactive oxygen species accumulate in root tissues. PGPR capable of detoxifying hydrogen peroxide enhance plant tolerance and support root health under adverse conditions (Ha-Tran et al., 2021). The uniform catalase activity observed in this study suggests that these isolates are well adapted to stress-prone environments, making them suitable candidates for biofertilizer development.

Nutrient solubilization assays revealed functional specialization among isolates. MH05 demonstrated the highest phosphate solubilization efficiency, while MH01 excelled in zinc mobilization. Phosphate-solubilizing bacteria are known to excrete organic acids and phosphatases that release bound phosphorus, increasing its availability to plants. Field trials have shown that phosphate solubilization by PGPR can reduce fertilizer dependency by up to 25% (Zaidi et al., 2009; Pan & Cai, 2023). Zinc solubilization is equally important, as micronutrient deficiencies are widespread in tropical soils and directly affect crop yield and nutritional quality (Alori et al., 2017). The superior performance of MH01 in zinc mobilization highlights its potential for improving the nutritional value of okra pods.

Exopolysaccharide (EPS) production was confirmed in MH01, MH03, and MH06. EPS secretion is known to improve soil aggregation, enhance water retention, and protect roots under stress conditions (Ashraf et al., 2004; Bulgari et al., 2019). The ability of these isolates

to produce EPS suggests that they may contribute to soil structure and resilience, particularly under drought conditions. Ammonia release, strongest in MH01 and MH03, further supports their role in nitrogen enrichment of the rhizosphere. Ammonia production by PGPR, combined with root exudates, has been reported to enhance nutrient uptake and stimulate root growth (Aasfar et al., 2021).

Taken together, the biochemical traits observed in these isolates demonstrate complementary functions that can collectively enhance okra growth. MH04 contributes to organic matter degradation, MH03 provides proteolytic activity, MH05 mobilizes phosphorus, and MH01 ensures zinc availability. The uniform catalase activity across all strains adds resilience to oxidative stress, a critical factor in climate-affected soils. These findings reinforce the concept that PGPR consortia, rather than single strains, may offer the most effective biofertilizer solutions (Liu et al., 2018; Abdelaziz et al., 2023).

Implications for Sustainable Agriculture

The integration of PGPR into okra cultivation systems offers multiple benefits: improved nutrient cycling, reduced reliance on chemical fertilizers, enhanced stress tolerance, and biocontrol against pathogens. Given the increasing demand for eco-friendly agricultural practices, these isolates represent promising candidates for bioinoculant development. Their application could contribute to higher yields, better soil health, and improved nutritional quality of okra, particularly in regions facing resource constraints and climate variability.

Table 1. Amylase production by rhizobacterial isolates from okra rhizosphere, showing solubilization intensity (SI) and solubilization efficiency (SE).

Strains ID	MH01	MH03	MH04	MH05	MH06
SI	1.5	2.0	2.7	-	1.2
SE	54	30	170	-	20

Table 2. Protease activity of okra-associated rhizobacteria, expressed as solubilization intensity (SI) and efficiency (SE).

Strains ID	MH01	MH03	MH04	MH05	MH06
SI	1.5	4.1	3.1	2.6	1.5
SE	50	210	106	160	50

Table 3. Pectinase production by bacterial isolates, with comparative solubilization intensity (SI) and efficiency (SE).

Strains ID	MH01	MH03	MH04	MH05	MH06
SI	2	2.2	1.5	-	2.2
SE	100	118	80	-	121

Table 4. Catalase activity of rhizobacterial strains, indicating positive or negative enzyme production.

Strains ID	MH01	MH03	MH04	MH05	MH06
Result	Positive	Positive	Positive	Positive	Positive

Table 5. Cellulase activity of isolates, measured as solubilization intensity (SI) and efficiency (SE).

Stains ID	MH01	MH03	MH04	MH05	MH06
SI	5.3	6.6	7.0	-	4.0
SE	433	460	500	-	300

Table 6. Phosphate solubilization potential of rhizobacteria, expressed in terms of solubilization intensity (SI) and efficiency (SE).

Strains ID	MH01	MH03	MH04	MH05	MH06
SI	1.6	1.6	-	3.0	2.3

SE	56	20	-	200	128
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Table 7. Zinc solubilization activity of isolates, showing solubilization intensity (SI) and efficiency (SE).

Strains ID	MH01	MH03	MH04	MH05	MH06
SI	4.5	2.6	3.0	-	-
SE	350	67	100	-	-

Table 8. Exopolysaccharide (EPS) production by rhizobacterial isolates, represented by qualitative scoring (+++ or -).

Strain ID	MH01	MH03	MH04	MH05	MH06
Result	+++	+++	-	-	+++

Table 9. Ammonia production by bacterial strains, expressed as qualitative intensity (+++, ++, +, -).

Strain ID	MH01	MH03	MH04	MH05	MH06
Result	+++	+++	+	++	-

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