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Evaluation of Plant Growth-promoting Properties of Nitrogen-Fixing *Enterobacter mori* Strain P5 (MW242734.1) isolated from Spent Mushroom Substrates

Uchenna Oyeagu^{1,2}, Kingsley T. Ughamba^{1,3*}, Chukwuemeka C. Ezeh^{1,4}, Rufus O. Animashaun⁵, Nnamdi K. Ogbonna¹, Blessing N. Nebo¹, Somtochukwu C. Eze¹, Emmanuel U. Eke¹

¹Department of Microbiology, University of Nigeria, Nsukka, ²South East Zonal Biotechnology Research Centre, University of Nigeria, Nsukka, ³Department of Science Laboratory Technology, University of Nigeria, Nsukka, ⁴Central Science Laboratory, University of Nigeria, Nsukka, ⁵Department of Basic Sciences, Babcock University

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Uchenna Oyeagu^{1,2}, (Email: uchenna.oyeagu@unn.edu.ng, ORCID: <https://orcid.org/0000-0002-1282-5662>); Kingsley T. Ughamba^{1,3*}, (Email: kingsley.ughamba@unn.edu.ng, ORCID: <https://orcid.org/0000-0002-3082-1403> Tel: +2348066050782); Chukwuemeka C. Ezeh^{1,4}, (Email: eze.chukwuemeka@unn.edu.ng, ORCID: <https://orcid.org/0009-0001-0392-1185>); Rufus O. Animashaun⁵, Email: animashaunr@babcock.edu.ng, ORCID: <https://orcid.org/0009-0007-2729-1492>); Nnamdi K. Ogbonna¹, (Email: kingnamdi30@gmail.com, ORCID: <https://orcid.org/0009-0009-8012-8875>); Blessing N. Nebo¹, (Email: nebonmesomablessing@gmail.com, ORCID: <https://orcid.org/0009-0006-3323-8367>); Somtochukwu C. Eze¹, Email: chelseaeze124@gmail.com, ORCID: <https://orcid.org/0009-0006-1841-7877>); Emmanuel U. Eke¹, Email: ek668@gmail.com, ORCID: <https://orcid.org/0009-0006-1764-9492>)

*Corresponding Author: Kingsley T. Ughamba¹ Email: kingsley.ughamba@unn.edu.ng, Tel: +2348066050782

Abstract

Chemical fertilizers have significantly improved crop yield. Nevertheless, the health of our environment has been affected negatively owing to the toxic residues emanating from repeated chemical fertilizer application. The evaluation of spent mushroom substrate (SMS) as a source of plant growth promoting bacteria is considered a safe alternative to chemical fertilizers. Mannitol Ashby selective agar and semi solid bromthymol blue agar without nitrogen were used to isolate nitrogen-fixing bacteria and screen bacterial isolates for nitrogen fixation potentials respectively. Bergey's Systematic Bacteriology manual was used for initial identification. Secondary bacterial metabolites were quantified using standard microbiological methods. Seven isolates (C1 to C7) were confirmed to fix atmospheric nitrogen. Colony forms and edges of all isolates were all circular and entire respectively. Only C3 and C6 showed gray colony colours whereas other colonies were white. Isolates C2 and C3 gave high ammonia production and nitrogen fixing efficiency. Each isolate showed varying concentrations of all the secondary metabolites examined. Isolate C3 had significant peak indole acetic acid (IAA) production (300 ± 12.8 mg/L), gibberellins (410 ± 15.0 mg/L), 1-aminocyclopropane-1-carboxylate (ACC) deaminase secretion (3.07 ± 0.3 mg/mg protein) and hydrogen cyanide production (0.15 ± 0.007 mg/L). Isolate C3 was identified molecularly as *Enterobacter mori* strain P5 (MW242734.1) with 99.7% similarity. SMS can be considered as a better alternative to chemical fertilizer since they harbor diversity of bacteria species beneficial to plants such as *Enterobacter mori* strain P5 that is also environmentally friendly.

Keywords: SMS, phytopathogens, phytohormones, ACC deaminase, plant growth, hydrogen cyanide

Introduction

The use of chemical fertilizers to promote plant growth has over the years improved crop yield and productivity. Despite this, chemical fertilizers pose dangerous threat to the environment. The risks associated with chemical fertilizers originate from the point of manufacturing where toxic gases such as carbon dioxide, methane and ammonia are released to pollute the environment (Chandini et al., 2019). Even though high crop yield is recorded using chemical fertilizer, plants usually experience poor developments of effective roots and shoots (Chandini et al., 2019; Pahalvi et al., 2021). Another demerit is that chemical fertilizer application abuse can also lead to decreased soil organic carbon, acidification, increased attack by pests, crust in the soil and low crop yield (Pahalvi et al., 2021). To avert the dangers involved in the continuous use of chemical fertilizers, biological approaches using Spent Mushroom Substrates (SMS) from edible mushroom cultivation is encouraged. For instance, the work of Wiafe-Kwagyan and Osei (2025) recorded general significant growth and productivity of cowpea (*Vigna unguiculate L.*) and tomato (*Solanum lycopersicum L.*) when SMS were added to the soil. However, there has been increased demand for mushroom in recent times due to its beneficial values. Consequently, wastes are generated in significant tons as SMS and its production has been projected to record about 104 million tons by 2026 (Wiafe-Kwagyan and Osei, 2025). Management of millions of tons of released SMS constitutes big threat to health of the environment. Interestingly, reports have shown that SMS contain a wide distribution of microbes (especially bacteria) that possess varieties of benefits to plants (Sarkar et al., 2024, Mini, 2025). Additionally, Mini et al. (2025) recorded bio-control activities of bacteria species found in SMS when they were evaluated for biofertilizer potentials. The report of Neerja et al. (2025) revealed the presence of plant-growth beneficial bacteria in SMS. Out of a total of 25 bacteria isolated from SMS, 60 % were recorded to fix nitrogen, 40 % were screened to produce IAA whereas 36 % of them were found to produce HCN. Some bacteria are known to benefit host plants in many ways, especially in biologic fixation of nitrogen, production of a range of secondary metabolites such as phytohormones, hydrogen cyanide, ammonia, ACC deaminase and catalase (Fan et al., 2024, Singh et al., 2017). A good number of bacteria (*Azotobacter*, *Enterobacter*, *Arthrobacter*, *Bacillus*, *Klebsiella*, etc.) have been reportedly involved in plant growth-promoting activities (Abdullahi et al., 2022, Kumar et al., 2012). *Enterobacter* spp are mostly found in the soil around the roots of plants owing to their outstanding potential in fixing nitrogen for the plant, and for other plant-beneficial secondary metabolites excreted by them (Fan et al., 2024, Ortega-Ortega et al., 2024, Singh et al., 2021). Similarly, indole acetic acid (IAA) is a type of phytohormone that promotes shoots and roots progression whereas the facilitator of germinations in plant seeds is affirmed to be gibberellins (Goldberg-Moeller et al., 2013). Besides the microbial metabolites that are involved in direct plant growth, other microbial metabolites (hydrogen

cyanide and ammonia) can indirectly enhance plant development by inhibiting the growth of phytopathogens. Hydrogen cyanide is a microbial secondary metabolite produced by a variety of bacteria. They are volatile and inhibit the growth of phytopathogens (Abd El-Rahman et al., 2019; Reetha et al., 2014). For instance, *Enterobacter* sp. and *Citrobacter freundii* also showed significant antagonistic actions against known plant pathogen owing to the secretion of high concentration of hydrogen cyanide (Olayemi and Odedara, 2017). In addition, ammonia is also another toxic compound produced by some microbes in the soil to inhibit phytopathogens. Bacteria-secreted ammonia contributes largely to the health status of plants (Abdullahi et al., 2022). Moreover, some enzymes such as ACC deaminase and catalase have been instrumental in modern agriculture and plant growth (Das and Roychoudhury, 2014; Saini and Khanna, 2013). A stress hormone called ethylene is known to be localized in plant roots area to hinder nitrogen fixation and nodulation in plants (Saini and Khanna, 2013). However, the microbial metabolite ACC deaminase reduces this ethylene concentration in the plant roots to improve crop productivity when secreted by microbes (Saini and Khanna, 2013). In addition, reactive oxygen species (ROS) is another plant growth hindrance that exerts oxidative stress on plants (Das and Roychoudhury, 2014). This ROS constructively destroys cells by obstructing some critical metabolic pathways especially the DNA and proteins in plants (Mansoor et al., 2022). Nevertheless, some microbes help plants build defensive measures against oxidative stress in plants by secreting catalase enzymes. Catalases breakdown toxic H₂O₂ (critical to the destructive activities of ROS) into non-toxic compounds (Mansoor et al., 2022). Generally the role of plant growth-promoting bacteria cannot be overemphasized in modern agriculture. A report by Ortega-Ortega et al.(2024) showed that the use of *Enterobacter* inoculants as biofertilizers resulted in 20% and 40% increased tomato (*Solanum lycopersicum L.*) height and root biomass respectively. Notably, the role and contributions of plant growth-promoting bacteria to agriculture and human existence is enormous. In a nutshell, SMS harbor varieties of bacteria capable of improving plant growth and development in addition to continuous dependent on the use of chemical fertilizer. Microbial involvement in plant-growth promotion solemnly acts as biofertilizers and does not lead to the formation of any poisonous compound as residue in the environment (Rathour et al., 2017). The search for microbial species capable of offering a holistic advantage in terms of improving plant health is still an on-going process. Therefore, this paper addressed the evaluation of bacteria isolated from SMS on nitrogen fixation capacities, phytohormone production, ammonia production and bio secretion of other plant growth-promoting secondary metabolites (catalase, ACC deaminase, hydrogen cyanide). The aim of this research was to evaluate plant growth-promoting potentials of *Enterobacter mori* Strain P5 (MW242734.1) isolated from SMS.

Materials and Methods

SMS Source

Five SMS were obtained from Mushroom research unit at the South East Zonal Biotechnology Research Centre, University of Nigeria, Nsukka. Samples were transported to laboratory for microbiological study.

Isolation of Bacteria

A 10 g quantity of SMS was added to 90 ml of diluent (sterile distilled water) in a 250 mL Erlenmeyer flask. The mixture was agitated for 30 minutes at 120 rpm using IKA Incubator shaker (KS 3000 I control). A serial dilution of the mixture was prepared up to 10^{-7} before inoculating into Petri dishes containing mannitol Ashby selective agar (20 g mannitol, 5 g CaCO_3 , 0.2 g K_2HPO_4 , 0.2 g NaCl, 0.1 g K_2SO_4 , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 15 g agar, and 1 L of distilled water) using 10^{-5} and 10^{-7} dilutions. The cultures were incubated for seven days at room temperature ($28 \pm 2^\circ\text{C}$). Thereafter, distinct colonies were sub-cultured and stored in Luria-Bertani (LB) (Catalog Number. J75852.30) slants awaiting use. All chemicals used were of analytical grade from HIMEDIA (Maharashtra, India)

Biochemical Identification of Nitrogen-Fixing Bacteria

The methods for bacterial identification as contained in the Bergey's Manual of Systematic Bacteriology Identification of bacterial isolates were adopted in the determination of bacteria colonial morphology and the determination of urease, citrate, oxidase and indole reactions (Krieg and Holt, 1994; Singh et al., 2022).

Nitrogen fixation Screening Experiments

Bacteria were cultivated on a semi-solid nitrogen-fixing isolation media according to Widawati (2019). Test tube cultures were incubated for seven days at room temperature. Nitrogen-fixing bacterial colonies were thereafter identified with their characteristic fog that is circular and beneath the surface of the test tube culture in the form of a ring. All reagents used were of analytical quality.

Phytohormone Production Screening

Gibberellin Production in Nitrogen-Fixing Bacterial Isolates

Seven nitrogen-fixing bacterial isolates were inoculated differently into 100 ml nutrient broth media in a 250 mL Erlenmeyer flask and incubated for 2 days. The 48 h-old cultures were centrifuged (Model no: GT116C, Hunan Kecheng Instrument Equipment Co., Ltd) at 10,000 rpm for 20 min. Culture supernatants were recovered and adjusted to a pH of 2.5 using 3.75 N HCl. Ethyl acetate / NaHCO_3 (liquid-liquid) extraction technique was adopted in extracting culture supernatants obtained (Pandya and Desai, 2014). Gibberellic acid contents were obtained from the ethyl acetate phase at 254

nm using Beckman Coulter DV 800 visible/UV spectrophotometer and the corresponding blank

Indole Acetic Acid (IAA) Production in Nitrogen-Fixing Bacterial Isolates

IAA production was determined by inoculating bacterial culture in broth media containing tryptophan (0.2 g) and 50 mL of King Broth (0.15% K_2HPO_4 , 2% peptone, and 0.15% MgSO_4). The set up in 250 mL Erlenmeyer flasks were incubated for seven days at room temperature. Thereafter, 2 ml of culture media were sampled and centrifuged at 10,000 rpm (Model no: GT116C, Hunan Kecheng Instrument Equipment Co.,Ltd) for 5 minutes. The supernatant collected was added to 4 mL of Salkowski reagent (15 mL of 0.5M FeCl_3 , 300 mL of conc. H_2SO_4 and 500 mL of distilled water) according to Ahmed et al. (2010). IAA quantity produced was established using a Beckman Coulter DV 800 visible/UV spectrophotometer at a wavelength of 540 nm.

Screening for catalase production in nitrogen-fixing bacterial isolates

A nutrient broth containing yeast extract (3 g/L), peptone (10 g/L), and NaCl (1 g/L) with pH (Model no: PHS – 25, Techmel USA) adjusted to 7.2 was used in the cultivation of rhizospheric nitrogen-fixing bacterial isolates (Beers and Sizer, 1952). After 24 h of cultivation the cultures of all isolates were individually standardized to optical density of 0.6 using Beckman Coulter DV 800 visible/UV spectrophotometer at 600 nm. The culture was re-inoculated into a fresh broth media and incubated for 24 h using a rotary shaker at 30°C . The cultures were centrifuged (Model no: GT116C, Hunan Kecheng Instrument Equipment Co.,Ltd) for 10 min at 4500 rpm thereafter to recover the crude extract. A 1.5 mL (30mM H_2O_2) was dispensed into the crude extract (0.1 mL) containing 50 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ (2.7 mL). Optical density was obtained at 240 nm using a Beckman Coulter DV 800 visible/UV spectrophotometer

Screening for ACC deaminase activity from nitrogen-fixing bacterial isolates

Each isolate was cultivated on Tryptic soy broth medium (5 mL) at 38°C to get the isolates to their stationary phase. The cells were harvested by centrifuging and washing with 0.1 M Tris-HCl (pH 7.5). They were suspended in modified DF minimal medium (2 mL) containing 3 mM final concentration of ACC without polyethylene glycol. The media was incubated for 72 h at 28°C , with constant agitation using IKA Incubator shaker (KS 3000 I control). ACC deaminase was quantified by formation of α -ketobutyrate using 540 nm (Sadasivam and Manickam, 1992; Cappuccino and Sherman, 1992). In addition, ammonia was also released through cleavage of ACC owing to the ACC deaminase formed.

Qualitative determination of Ammonia production by nitrogen fixing rhizospheric bacterial isolates

10 mL of nutrient broth was used to cultivate 24 h old bacterial isolates for 48 h at 30 °C using a rotary shaker (Platform type: Model No. HV-RS-154). Nessler's reagent (0.5 mL) was gently dispensed to each tube. Positive ammonia production was indicated by a colour change from yellow to brown (Cappuccino and Sherman, 1992)

Determination of Hydrogen Cyanide production by nitrogen-fixing bacterial isolates

The isolates were inoculated to kings B broth media in Erlenmeyer flask according to Sadasivam and Manickam (1992). The kings B broth media containing 4.4 g/L of glycine was prepared to also have hanging strips of 10 x 0.5 cm² dimensioned filter paper soaked in alkaline picrate solution. The incubation took 48 h at room temperature. A colour change of the filter paper to red confirms evolution of HCN at the end of incubation. The filter paper was thereafter inserted into 10 mL of distilled water in a test tube to elute before taking the optical density using Beckman Coulter DV 800 visible/UV spectrophotometer at 625nm.

Molecular identification of the best plant growth promoting isolate (Isolate C3)

Extraction of DNA was carried out using the ZR fungal/bacterial DNA miniprep according to the manufacturer's instruction. Subsequently a pair of primers (27F: AGAGTTTGATCMTGGCTCAG) and (1525R: AAGGAGGTGWTCARCCGCA) was involved in the reaction to target the 16S rRNA on the DNA of bacteria. Agarose powder (0.9 %) was prepared and later mixed with DNS stain (10 µL EZ vision) to enable easy DNA visualization using ultraviolet light. Part of the fragments that was amplified was thereafter sequenced with a sequencer (Genetic Analyzer 3130xl) originating from Applied Biosystems using Big Dye terminator v3.1 cycle sequencing kit. Genetic analysis using the result obtained was handled with MEGA X version 10.0.5.

Statistical Analyses

Experiments were performed in triplicates whereas data analysis was conducted using a One-way ANOVA and the means separated using LSD ($p < 0.05$).

Results and Discussion

Nitrogen fixation screening

A total of seven bacteria colonies (C1, C2, C3, C4, C5, C6 and C7) were isolated from SMS using a nitrogen-fixing selective medium (mannitol Ashby). All selected isolates were able to fix nitrogen with additional beneficial roles to plants. Isolate C3, (*Enterobacter mori* strain P5) significantly ($P \leq 0.05$) showed outstanding roles in nitrogen fixation against five

isolates (C1, C4, C5, C6, C7) investigated in this work, evidenced by the selective media (mannitol Ashby) in which it was cultivated (**Figure 1**). Other evidences can be drawn from the work of Ji et al. (2020) who classified *Enterobacter cloacae* strain HG-1 isolated from wheat soil rhizosphere to be highly efficient in nitrogen fixation. Ogbo and Okonkwo (2012) equally reported successful isolation of nitrogen-fixing bacteria from maize soil rhizosphere. To the best of our knowledge, this study is the first to report the involvement of *Enterobacter mori* strain P5 on nitrogen fixation. It can notably be suggested that rhizosphere soil of crops harbor nitrogen-fixing bacteria and remains an important microbial habitat for plants.

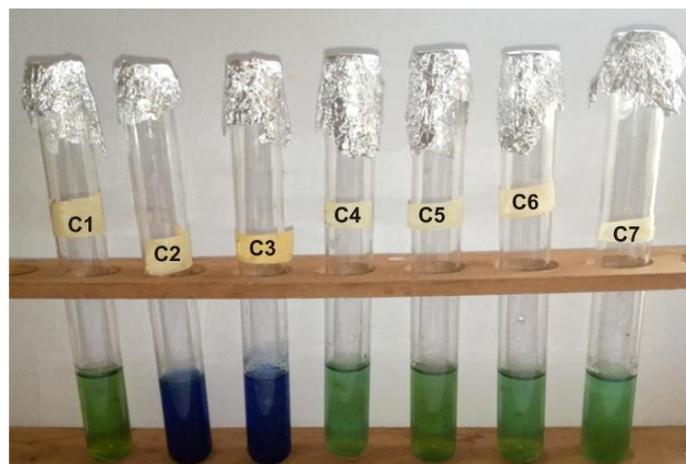


Figure 1: Screening of isolates for nitrogen fixation efficiency indicating outstanding nitrogen fixations by isolate C2 and C3 (colour change to blue)

Preliminary identification

Colony morphological characteristics affirmed all to be circular in form with entire edges (**Table 1**). C1 and C7 were observed to have raised colonies while the rest were flat. Two isolates (C3 and C6) had gray colony colours whereas others were all whitish. When the cell characteristics were examined under the microscope, C1 and C4 were Gram positive while others were Gram negatives. In addition, Isolate C1 and C7 were cocci shaped cells while others were Gram negative rods when examined under the microscope (**Table 1**). Thus, there were varying cell arrangements on individual isolates. Biochemical examinations also revealed that Isolate C1 and C7 were negative to both citrate and urease enzymes even when all isolates investigated were confirmed positive to catalase and indole enzymes. The Gram reaction, citrate and catalase characteristics of isolate C3 (*Enterobacter mori* strain P5) in this study were in agreement with the findings of Fadiji et al (2023). Although colony colour of *Enterobacter mori* strain AYS9 as recorded by Fadiji et al (2023) was creamy as against the gray colony colour obtained in this study. This disparity can be attributed to the existence of varying strains of *Enterobacter mori* from different ecological niche.

Table 1: Colony morphology and biochemical characteristics of nitrogen-fixing bacteria

Characteristics	Isolate C1	Isolate C2	Isolate C3	Isolate C4	Isolate C5	Isolate C6	Isolate C7
Colour	white	White	Gray	White	White	Gray	White
Form	Circular						
Edge	Entire						
Elevation	raised	flat	flat	Flat	flat	Flats	Raised
Gram reaction	+	-	-	+	-	-	-
Shape	cocci	Rods	Rods	Rods	Rods	Rods	Cocci
Cell Arrangement	chains	Singles	In pairs	Chains	In pairs	in pairs	Chains
Nitrogen fixation	+	+++	+++	+	+	+	+
Oxidase	+	-	-	+	-	-	+
Citrate	-	+	+	+	+	+	-
Indole	+	+	+	+	+	+	+
Urease	-	+	+	+	+	+	-
Ammonia production	+	+++	+++	++	++	++	+

Phytohormone production test

Out of seven bacterial isolates studied, all were capable of producing phytohormones. Isolate C3 (*Enterobacter mori* strain P5) had peak significant phytohormone production with 300 ± 2.8 mg/L and 410 ± 5.0 mg/L for indole acetic acids and gibberellins respectively. In contrast, bacterial isolates with the least indole acetic acid were C1 (60 ± 14.1 mg/L) and C7 (80 ± 15.0 mg/L) as shown in **Figure 2** whereas C1 (162 ± 22.0 mg/L) and C7 (170 ± 13.9 mg/L) were least in gibberellins production (**Figure 3**). As a matter of fact, indole acetic acids and gibberellins are important plant growth promoting hormones with growth-enhancing characteristics on plant. Notably, the root and shoot developments in plants are mostly initiated by indole acetic acid (Sosnowski, 2023) while gibberellins are known to facilitate germination in seeds, including other major roles in the commencement of flowering processes in plants (Goldberg-Moeller et al., 2013). Soil rhizosphere is considered a habitat for plant growth-promoting bacteria. With this in mind, a guinea corn rhizospheric *Lactobacillus bulgaricus* was reported to improve guinea corn growth with $418.29 \mu\text{g/ml}$ of indole acetic acid (Abdullahi et al., 2022). More so, Luziatelli et al. (2017) optimized the production of indole acetic acid to 17.2 mg/L/h using *Enterobacter* sp. strain P-36. Summarily, *Enterobacter mori* strain P5 was reported to secrete indole acetic acids and gibberellins and can play major plant growth promotion roles when used as biofertilizer inoculants. However, plant growth hormone productions by microbes are economically strategic in agricultural biotechnology especially when their stimulations and production were orchestrated by rhizospheric bacteria.

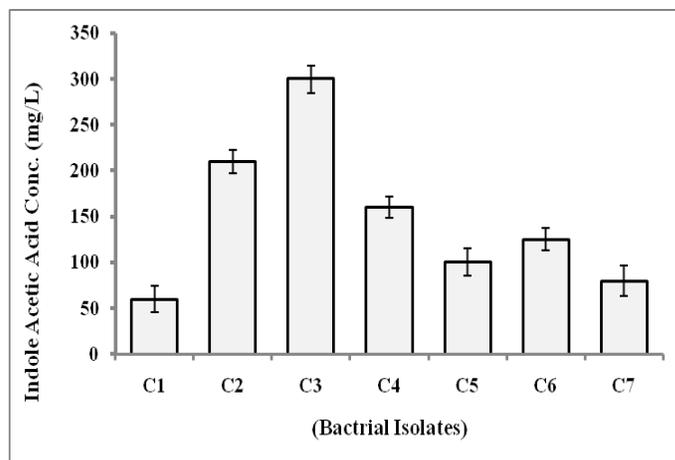


Figure 2: Production of Indole acetic acids in nitrogen-fixing bacteria

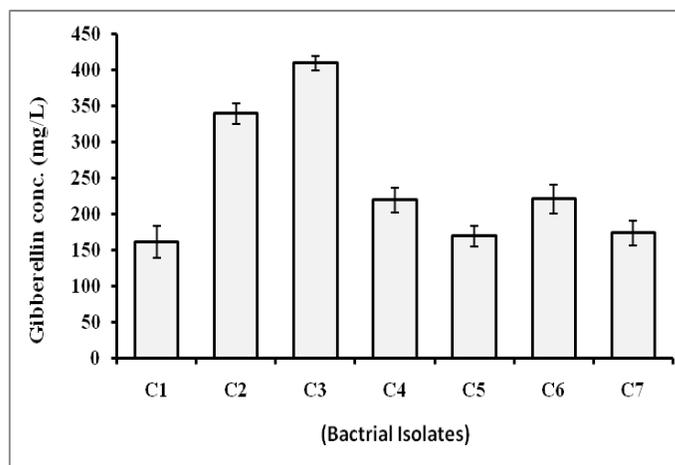


Figure 3: Gibberellins production in nitrogen-fixing bacteria

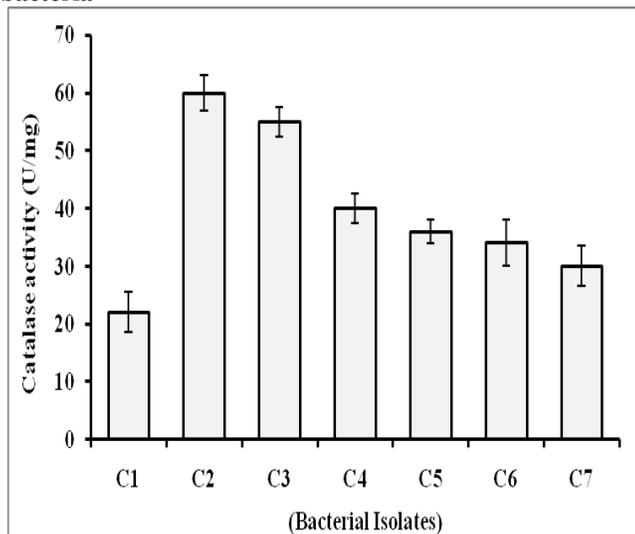


Figure 4: Catalase production in nitrogen-fixing bacteria

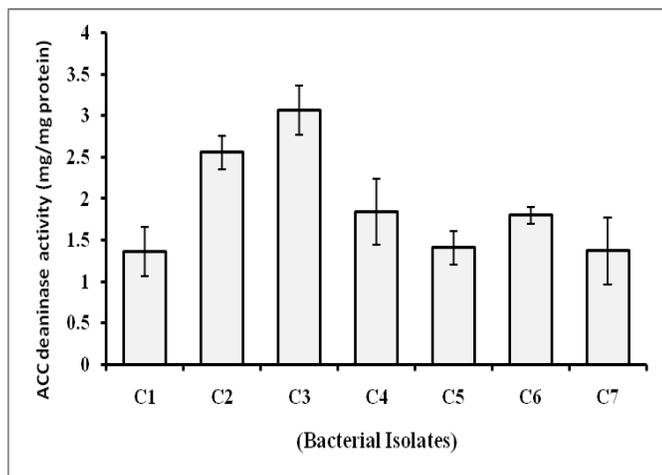


Figure 5: Production of ACC deaminase in nitrogen-fixing bacteria

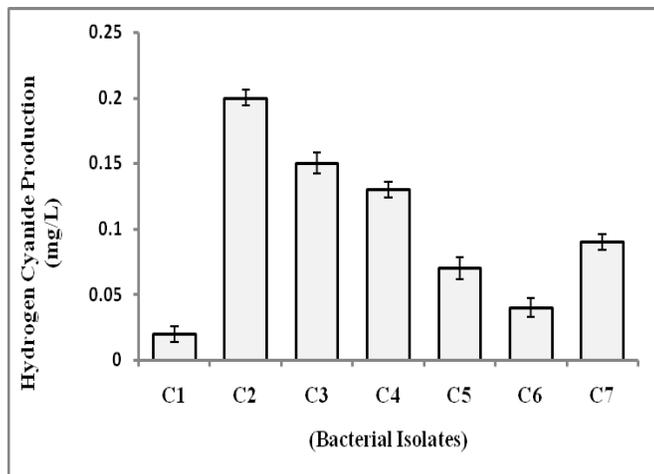


Figure 6: Hydrogen cyanide production in nitrogen-fixing bacteria

Catalase enzyme screening

Values obtained from catalase production experiments ranged from 22 ± 3.5 U/mg (isolate C1) to 60 ± 3.1 U/mg (isolate C2). Despite, the second-best position of isolate *Enterobacter mori* strain P5 (C3) in catalase production (55 ± 2.6 U/mg), it was also leading significantly ($P \leq 0.05$) on the IAA and gibberellins production as discussed earlier. *Enterobacter mori* strain P5 had outstanding evidence of catalase production notwithstanding its second position (Figure 4). As an antioxidant, catalase produced by rhizospheric bacteria helps annul the effect of oxidative stress impacted on plants tissues by reactive oxygen species (Das and Roychoudhury, 2014). In other words, catalases stimulate the biological breakdown of toxic H_2O_2 to non-toxic oxygen and water in protection of the host plant cells. High accumulation of H_2O_2 encourages the destructive activities of reactive oxygen species (ROS). However, stabilized reactive oxygen species (ROS) is required for some cellular activities to be activated in plants, especially in conditions necessary for seed germination (Das and Roychoudhury, 2014). Most plant growth- promoting bacteria secrete catalase to encourage the germination processes in seeds. It was widely documented that rhizospheric bacteria possess catalase production potentials (Kumar et al., 2012, Rathour et al., 2017).

1-aminocyclopropane-1-carboxylate (ACC) deaminase screening

Isolate C3 (*Enterobacter mori* strain P5) was on a significant lead with 3.07 ± 0.3 mg/mg protein for ACC deaminase enzyme production as shown in Figure 5. All isolates examined produced ACC deaminase with isolate C3 (*Enterobacter mori* strain P5) having a significant lead (3.07 ± 0.3 mg/mg protein). This denotes that *Enterobacter mori* strain P5 (C3) has the potential to reduce the concentration of ethylene localized in the roots of plants to annul crop developmental hindrances and promote its growth. Availability of ACC deaminase at the roots of plants indirectly enhances plant growth by the progressive reduction of ethylene. In contrast, ethylene (stress hormone) accumulation in the root area of plant has been attributed to reduced plant growth owing to declined nodulation and nitrogen fixation in plants (Saini and Khanna, 2013). Saini and Khanna (2013) affirmed this claim when they reported improved growth rates of lentil plants as a result of ACC deaminase production (1.120 optical density at 600 nm) by rhizospheric bacteria.

Ammonia and hydrogen cyanide screening

Ammonia production experiments conducted in this study gave evidence that although all isolates produced ammonia, isolate C2 and C3 (*Enterobacter mori* strain P5) performed best in ammonia production using the ammonia screening technique. Isolates C1 and C7 had minimal ammonia production as shown

in Table 1. Similarly, C2 had peak hydrogen cyanide concentration (0.20 ± 0.006 mg/L) followed by *Enterobacter mori* strain P5 (C3) with hydrogen cyanide concentration of 0.20 ± 0.006 mg/L (Figure 6), whereas C1 showed the least hydrogen cyanide (0.02 ± 0.006 mg/L). The formation of toxic ammonia and hydrogen cyanide conferred *Enterobacter mori* strain P5 an additional protective role to the plant. Toxicity of ammonia and hydrogen cyanide were believed to have the potentials of inhibiting the proliferation of phytopathogens on plants. Perhaps production of hydrogen cyanide in soil bacteria is an added advantage to improving the resistance patterns of host plants towards phytopathogens (Whipps, 2001). Similarly, Reetha et al. (2014) reported the antagonistic actions of *Pseudomonas fluorescence* with hydrogen cyanide production against a plant pathogen (*Macrophomina phaseolina*). Thus, ammonia secretions by bacteria have also been studied to possess antagonistic actions against some specific phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum* etc (Yildiz, 2012). Abdullahi et al. (2022) also confirmed ammonia production ($2452.65 \mu\text{g/ml}$) and its plant growth-promoting potentials of *Bacillus licheniformis* isolated from the rhizosphere of guinea corn. However, these additional roles of nitrogen-fixing bacteria are interesting turning points in bio-control and indirect plant growth-enhancing technology

Molecular Identification

After DNA isolation, the 16s rRNA gene was successfully extracted (Figure 7). The Maximum Composite Likelihood technique was adopted to determine the distance in evolution at substitutions base per location. Clustering patterns base according to ancestral relatedness were observed after phylogenetic tree construction (Figure 8). Isolate C3 using a set of primers on target (16S rRNA) revealed *Enterobacter mori* strain P5 (MW242734.1.) with 99.7 % similarity. Based on the phylogenetic analysis, other bacterial strains closest neighbours such as *Enterobacter ludwigii* strain KA24, *Enterobacter kobei* MAT- 3a and *Pantoea agglomerans* E77 recorded 99.6 %, 99.6 % and 99.5 % respectively. As a matter of fact, all these bacterial strains with nearest similarities were all Gram negatives. The similarity percentage of the best isolate in this work to *Enterobacter mori* strain P5 was 99.7%. Other authors have also affirmed growth-promoting potentials of *Enterobacter* sp. For instance, Fadiji et al. (2023) isolated *Enterobacter mori* AYS9 that induced drought resistance to their host plants thereby promoting their growth. Additionally, a rhizospheric *Enterobacter* sp was recorded to facilitate growth developmental process in maize plants (Ogbo and Okonkwo, 2012). Consequently, Ji et al. (2020) complimented this by reporting improved nitrogen fixation by *Enterobacter cloacae* strain HG-1 of wheat soil rhizosphere.

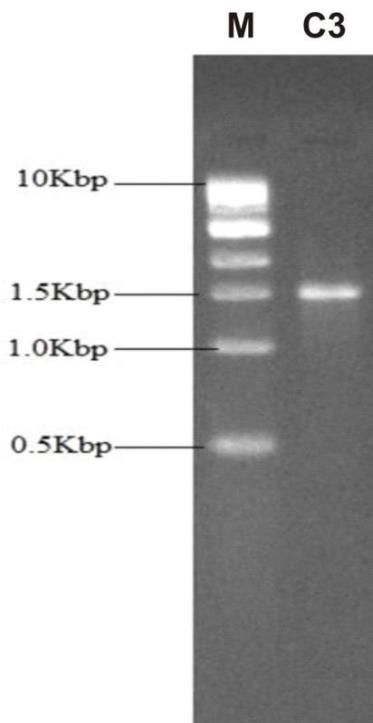


Figure 7: Amplified gel image of the house keeping gene (16SrRNA) of C3 isolate at 1500bp

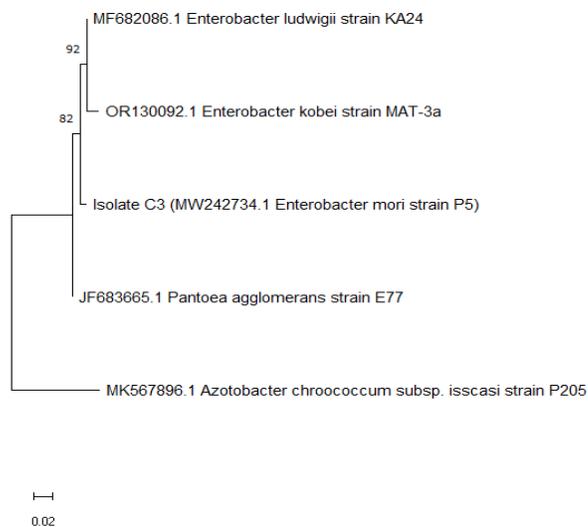


Figure 8: Phylogenetic identification of isolate C3

Conclusion

This research provides evidence to the fact that SMS harbor diversity of bacteria possessing plant growth-promoting roles. This claim can be explained by the production of growth-promoting secondary metabolites by all isolates investigated. Specifically, *Enterobacter mori* strain P5 was outstanding in almost all growth-enhancement parameters including nitrogen fixation examined in this research. Growth-promoting compounds such as phytohormones (indole acetic acid and gibberellins), ACC deaminase and catalase investigated in this paper were all reportedly produced by *Enterobacter mori* strain P5 isolated from SMS. In addition, this research also showed that *Enterobacter mori* strain P5 has also been associated with production of bio-control-related compounds such as ammonia and hydrogen cyanide. Owing to this, *Enterobacter mori* strain P5 possessed antagonistic potentials against phytopathogens. Interestingly, bacterial inoculants of SMS have the capacities to promote plant growth and encourage better root development without harming the environment. Therefore, *Enterobacter mori* strain P5 has shown potentials to be used as biofertilizer against currently used chemical fertilizers whose by products pollute the environment.

Recommendation

Even though HCN and ammonia productions were documented to be produced by *Enterobacter mori* strain P5 in this study, further research is also important to conduct cultural antagonistic experiment and also profile other possible antimicrobial bioactive compounds that may be produced by *Enterobacter mori* strain P5. It is also important to demonstrate and understand the holistic growth promoting impact of *Enterobacter mori* strain P5 inoculant on crop growth and yield.

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References

- Abd El-Rahman, A.F., Shaheen, H.A., Abd El-Aziz, R.M., and Ibrahim, D.S.S. (2019). Influence of hydrogen cyanide-producing rhizobacteria in controlling the crown gall and root-knot nematode, *Meloidogyne incognita*. *Egyptian Journal of Biological Pest Control*. 29(41):1-11
- Abdullahi, M., Oyeyiola, G.P. and Sule, I.O. (2022). Assessment of phosphate solubilization, indole acetic acid and ammonia production by bacteria and fungi isolated from the rhizosphere of guinea corn (*Sorghum bicolor*). *UMYU Journal of Microbiology Research*. 7 (1): 47-55
- Ahmed, M., Stal, L.J., and Hasnain, S. (2010). Production of indole-3-acetic acid by the cyanobacterium *Arthrospira platensis* strain MMG-9. *Journal of Microbiology and Biotechnology*. 20(9):1259-65.
- Beers, R.F. and Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*. 195(1):133-40.
- Cappuccino, J.G. and Sherman N. (1992). Biochemical activities of microorganisms: Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co. 1245 p.
- Das, K., and Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, 2:(53):1-13
- Fadiji, A.E., Ayangbenro, A.S. and Babalola, O.O. (2023) Genomic Assessment of *Enterobacter mori* AYS9: A Potential Plant Growth-Promoting Drought-Resistant Rhizobacteria. *Spanish Journal of Soil Science*. 13, 11302. doi: 10.3389/sjss.2023.11302
- Fan, Y., Wang, H., Zhang, Z., Li, Y.X., Zhao, Z. and Ni, X. (2024). Mechanisms involved in plant growth promotion by *Enterobacter cloacae* DJ under salinity and alkalinity stress. *Chemical and Biological Technologies in Agriculture*, 11:12
- Goldberg-Moeller, R., Shalom, L., Shlizerman, L., Samuels, S., Zur, N., Ophir, R., Blumwald, E. and Sadka, A. (2013). Effects of gibberellin treatment during flowering induction period on global gene expression and the transcription of flowering-control genes in Citrus buds. *Plant Science*. 198:46-57.
- Ji, C., Liu, Z., Hao, L., Song, X., Wang, C., Liu, Y., Li, H., Li, C, Gao, Q. and Liu, X. (2020). Effects of *Enterobacter cloacae* HG-1 on the nitrogen-fixing community structure of wheat rhizosphere soil and on salt tolerance. *Frontiers in Plant Science*. 11:1094.
- Krieg, N.R. and Holt, J.G. (1994). *Bergey's Manual of determinative bacteriology*. (9th ed.) Baltimore, London: Williams and Wilkins; 1994.
- Kumar, A., Kumar, A, Devi, S., Patil, S., Payal, C. and Negi, S. (2012). Isolation, screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent Research in Science and Technology*, 4(1): 01-05
- Kumar R, Kumar R, Prakash O. (2019). Chapter-5 the impact of chemical fertilizers on our environment and ecosystem. Ed. 35(69):1173-89.
- Luziatelli, F., Melini, F., Bonini, P., Melini, V., Cirino, V. and Ruzzi, M. (2021). Production of indole auxins by *Enterobacter* sp. strain P-36 under submerged conditions. *Fermentation*. 7(3):1-12.

- Mansoor, S., Ali-Wani, O., Lone, J.K., Manhas, S., Kour, N., Alam, P., Ahmad, A. and Ahmad, P. (2022). Reactive oxygen Species in Plants: From Source to Sink. *Antioxidants*. 11 (225):1-14
- Mini, P.U., Onwukwe, C.D., Ezeonuegbu, B.A, Daodu, B.T., Stanley, H.O. and Ariole, C.N. (2025). Sustainable Production of Liquid Biofertilizer: Utilizing Spent Mushroom Substrate and Watermelon Peels. *Biotechnology Journal International*. 17;29(1):72-93.
- Neerja, R., Arti, G. and Shivani C. (2025). Characterization of Bacteria that Promote Growth from Spent Mushroom Compost and their Effectiveness on the Yield and Quality of White Button Mushrooms. *Asian Journal of Soil Science and Plant Nutrition*. 11(1):359-80
- Ogbo, F. and Okonkwo J. (2012). Some characteristics of a plant growth promoting *Enterobacter* sp. isolated from the roots of maize. *Advancements in Microbiology*. 2(03):368-374.
- Olayemi, O.P. and Odedara, O.O. (2017). Screening of endophytic plant growth-promoting bacteria isolated from two Nigerian rice varieties. *Nigerian Journal of Biotechnology*, 33:1-10.
- Ortega-Ortega, Y., Sarmiento-L'opez, L.G., Baylon-Palomino, A., Vazquez-Lee, J., Maldonado-Bonilla, L.D., Flores-Olivas, A, and Valenzuela-Soto, J.H. (2024). *Enterobacter* sp. DBA51 produces ACC deaminase and promotes the growth of tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.) plants under greenhouse condition. *Current Research in Microbial Science*. 6:1-11
- Pahalvi, H.N., Rafiya, L., Rashid, S., Nisar, B., and Kamili, A.N. (2021). Chemical fertilizers and their impact on soil health in microbiota and biofertilizers. In: Dar GH, Bhat RA, Mehmood MA, Hakeem KR (Eds.). Switzerland. Springer: Cham, 2. 1–20 p.
- Pandya, N.D. and Desai, P.V. (2014). Screening and characterization of GA3 producing *Pseudomonas monteilii* and its impact on plant growth promotion. *International Journal of Current Microbiology and Applied Science*. 3(5):110-115.
- Rathour, R.K., Ahuja, V. and Sharma N, Negi S (2017). Isolation and screening of plant growth promoting *Pseudomonas* sp. from phyllosphere and rhizosphere. *International Journal of Creative Research Thoughts*. 5(4): 182 – 194
- Reetha, A.K., Pavani, S.L. and Mohan, S. (2014). Hydrogen cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on *Macrophomina phaseolina* (Tassi. *International Journal of Current Microbiology and Applied Science* 3(5): 172-178
- Sadasivam S, Manickam A. (1992). Biochemical Methods for Agricultural Sciences. New Delhi: Wiley Eastern Ltd. 246p.
- Saini P, Khanna V. (2013). Preliminary screening for acc-deaminase production by plant growth promoting rhizobacteria. *Journal of Pure and Applied Microbiology*. 7(1): 573-576
- Sarkar, S., Bayen, S., Samanta, S., and Pal D (2024). Spent Mushroom Substrate- Prospects and Challenges of Agrowaste management into sustainable solutions: A Review. *International Journal of Agriculture, Environment and Biotechnology*, 17(04): 731-741.
- Singh P, Singh, R.K., Li, H.B., Guo, D.J., Sharma, A., Lakshmanan, P., Malviya, M.K., Song, X.P., Solanki, M.K., Verma, K.K., Yang, L.T. and Li, Y.R.. (2021). Diazotrophic bacteria *Pantoea dispersa* and *Enterobacter asburiae* promote sugarcane growth by inducing nitrogen uptake and defense-related gene expression. *Frontiers in Microbiology*. 11:1-20
- Singh, R.P., Jha, P. and Jha, P.N. (2017). Bio-inoculation of plant growth-promoting rhizobacterium *Enterobacter mori* strain P5ZNP-3 increased resistance against salt and temperature stresses in wheat plant (*Triticum aestivum* L.). *Journal of Plant Growth Regulation*. 36:783–798.
- Singh, S., Choure, K., Rai, P.K., Gour, S.S., and Agnihotri, V.K. (2022). Evaluation of plant growth-promoting activities of endophytic bacteria of *Musa acuminata* and their characterization. *Journal of Applied Biology*. 10(5): 94-101
- Sosnowski, J., Truba, M., and Vasileva, V. (2021). The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture*, 13(3):724-33.
- Whipps, J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*. 52(1):487-511.
- Wiafee-Kwagyan, M., Osei-Tutu, C. and Kotey M. (2025). Potential of spent mushroom compost from *Pleurotus ostreatus* strain EM-1 as organic soil amendment for growing cowpea and tomato under screen house conditions. *Advances in Agricultural Technology and Plant Sciences*, 8(1):180173
- Widawati, S (2019). Role of indigenous nitrogen-fixing bacteria in promoting plant growth on post-tin mining soil. *Makara Journal of Science*. 23(1):28-38
- Yildiz, H., Altinok, H.A. and Dikilitas, M. (2012) Screening of rhizobacteria against *Fusarium oxysporum* f. sp *melongenae*, the causal agent of wilt disease of egg plant. *African Journal of Microbiology Research*. 6(15):3700-370

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