https://doi.org/10.48047/AFJBS.6.15.2024.252-263

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Isolation and Characterizations of Potential Plant Growth Properties of Some Bacterial Endophytes Isolated from Roots of *Ocimum sanctum* L.

Pham Hong Hien[1], Ngo Thị Lan[2], Dang Thuy Vy[3], Nguyen Phuc Anh[4], Giang Van Nguyen [2*]

[1]Vietnam Academy of Agricultural Sciences
[2] Faculty of Biotechnology, Vietnam National University of Agriculture
[3]Hanoi - Amsterdam High School for the Gifted
[4]Kenedy Catholic High School

[*]*Corresponding author: nvgiang@vnua.edu.vn*

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 04 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.252-263

Abstract

Bacterial endophytes can offer several benefits to the host plant, particularly growth promotion as phytohormone biosynthesis, phosphate solubilization, siderophore production and stimulation of nutrient adsorption, and increase of pathogen resistance of host plants. In this study, there were 04 bacterial endophytic strains, namely RH2.1, RH2.2, RH5.1, and RH5.2, isolated from the roots of *Ocimum sanctum* L., and their plant growth-promoting properties were evaluated. All of these strains exhibited phosphate solubilizing ability, siderophore production, and IAA (indole-3-acetic acid) synthesis. Among the strains, RH2.2 showed the highest IAA-producing activity after 72 hours of incubation at pH 7.0 and a temperature of 30-35^{o}C in a culture medium containing glucose, lactose, and peptone as carbon and nitrogen sources. These selected endophytic bacteria may be used as the potential materials for microbial inoculants to develop sustainable agriculture production.

Keywords: Endophytic bacteria, phosphate solubilizing, siderophore, IAA production, *Ocimum sanctum* L.

Introduction

Ocimum sanctum L, commonly known as holy basil or tulsi, is a small perennial or biennial herb. Its stems and branches are covered in purple hairs and bear purple flowers in clusters of 6-8 blossoms per bundle. Holy basil is widely cultivated and thrives in various regions from lowlands to high mountains in Vietnam. Traditional medicine attributes warming and spicy properties to purple basil. The different parts of the plant, including leaves, stems, flowers, roots, seeds, and the entire plant, are utilized for treating various ailments such as colds, sore throats, bronchitis, malaria, diarrhea, dysentery, skin diseases, arthritis, eye conditions, insect bites, and for their antibacterial, cardioprotective, analgesic, and antispasmodic effects (Loi, 2001). The essential oil extracted from purple basil comprises approximately 0.2 - 0.3% of the plant's fresh weight, containing constituents like eugenol (45 - 70%), approximately 20% methyl ether of eugenol, 3% carvacrol, o-cymene, p-cymene, camphene, limonene, α, and β pinene. Eugenol is an essential component to use in dentistry and vanillin synthesis (Loi, 2001).

Endophytic bacteria are microorganisms that live within plants without harming the host. Instead, they provide several benefits for plant growth and development (Diep et al., 2021; Adeleke et al., 2022). One significant role of endophytic bacteria in plant growth is their ability to produce phytohormones like Indole 3 acetic acid (IAA), which promotes plant growth (Adeleke et al., 2022; Mohite, 2013). Additionally, endophytes can increase mineral content, improve disease resistance, withstand stressful conditions, and enhance plant immunity. Currently, numerous worldwide studies have explored the use of these microorganisms in the production of agricultural fertilizers, pesticides, and biological products, as well as in the pharmaceutical and cosmetic industries. This study aims to isolate a variety of endophytic bacterial strains from the purple basil plant that has the potential to enhance plant growth. The practical goal is to use these strains in basil cultivation, with a focus on safety, quality assurance, and the production of pharmaceutical-grade yields.

Materials and Methods

Sample collection

Healthy purple basil root samples, devoid of any disease symptoms, were gathered from the Institute of Medicinal Materials Duong Ngoc Hoi, located in Ngu Hiep, Thanh Tri, Hanoi. Subsequently, these samples were transported to the laboratory of the Department of Microbiology, Faculty of Biotechnology, Vietnam National Academy of Agriculture, for research purposes. The samples were then stored at 4°C until the isolation was further made.

Methods

Isolation of Endophytic Bacteria

Following the collection, the purple basil root samples underwent multiple rinses with clean water to eliminate soil adhering to the roots. The roots were then cut into small pieces measuring 1-2 cm in length. These root segments were immersed in 70% ethanol for 3 minutes, followed by another rinse using sterile distilled water. Further disinfection was carried out by treating the segments with NaOCl for 3 minutes, followed by a 70% ethanol treatment lasting 30 seconds. A final rinse with distilled water was performed to eliminate any residual chemicals on the root surface. To verify the sterility of the root surface post-sterilization, 0.1 mL of the final rinse was inoculated onto a petri dish containing Nutrient Agar (NA). These plates were then placed in an incubator set at 30°C for 48 hours, and observations were made for bacterial growth (Giang et al., 2022). In the absence of microbial growth, it was established that the sample

sterilization process had been effective. After successful sterilization, the basil root samples were cut into 0.5 cm segments and placed onto NA agar. Incubation at 30°C ensued, followed by monitoring for colony formation for 2 to 4 days. Colonies that displayed purity were subcultured on LB medium and preserved in inclined agar tubes at 4ºC, earmarked for subsequent experiments (Giang et al., 2020; Hien et al., 2021).

Evaluation of Phosphate solubilizing ability of Bacterial Strains

After purification, the bacterial strains were cultivated in test tubes containing 5.0 mL of liquid NBRIP medium with the following composition per liter: glucose 10 g, $Ca_3(PO_4)_2$ 5.0 g, $MgCl_2.6H_2O$ 5.0 g, $MgSO_4.7H_2O$ 0.25 g, KCl 0.2 g, $(NH_4)_2SO_4$ 0.1 g. The cultures were incubated at 30°C for 4 days with continuous shaking at 200 rpm. Following incubation, the culture broth was then centrifugated at 10000 rpm for 10 minutes at 4°C. The $PO_4$3- content was collected and determined utilizing the Green Molybdate method as described by Hien et al. (2021). In brief, 50 µL of supernatant was transferred into test tubes, followed by the addition of 3.45 mL of distilled water and 1.5 mL of supernatant reagent solution (ratio of 7:3). The mixture was incubated at 37°C for 1 hour. Subsequently, the absorbance at 820 nm was measured, and the obtained optical density (OD) value was substituted into the calibration curve equation $y = 0.2878x + 0.0429$ ($R^2 = 0.9919$) to calculate the concentration of phosphate released into the medium.

Evaluation of Siderophore Producing Ability

The endogenous bacterial strains were inoculated onto a concentrated CAS medium and incubated at 30°C for 48 hours. The utilization of iron by bacteria in the medium resulted in the secretion of siderophores, causing the formation of an orange area around the bacterial colony. The CAS medium was prepared using the following components: chrome azurol S (CAS) 60.5 mg, hexadecyltrimethylammonium bromide (HDTMA) 72.9 mg, Piperazine-1,4-bis (2-ethanesulfonic acid) (PIPETS) 30.24 g, 1 mM $FeCl_3$.$6H_2O$ in 10 mM HCl 10 mL, and agar (0.9% w/v) (Schwyn and Neilands, 1987).

Determination of Indole-3-Acetic Acid (IAA) Synthesizing Ability

Newly isolated bacterial strains were cultivated in Nutrient Broth (NB) medium supplemented with 1 g/L of tryptophan and grown at 30°C with continuous shaking at 200 rpm. The quantification of IAA in the bacterial culture was carried out using Salkowski's reagent according to the method outlined by Glickmann and Dessaux (1995). After 72 hours of cultivation, the bacterial solution was centrifuged at 6000 rpm for 10 minutes, and the clear supernatant was collected. One milliliter of the clear solution was then transferred to tubes and mixed with 2 mL of Salkowski's reagent (comprising 300 mL of 98% H2SO4 and 15 mL of 0.5 M $FeCl_3$). The mixture was shaken well and left in the dark at room temperature for 20 minutes for the reaction to occur and coloration to develop. The absorbance was measured at $\lambda = 530$ nm. The OD measurements of the isolates were substituted into the equation of the standard curve $y = 0.0054x + 0.0096$ ($R^2 = 0.994$) to calculate the concentration of IAA synthesized by the experimental bacterial strains.

Effects of culture conditions on IAA concentration

Effect of pH, medium temperature and incubation time: The experiment was arranged in a single-factor design. Bacterial strains were cultured on NB medium supplemented with L-tryptophan (1g/L) with initial media pH values of 5.0, 6.0, 7.0, 8.0, and 9.0, and IAA content in the culture solution was determined after 72 h of culture. After determining the appropriate environmental pH, the ability of bacterial strains to synthesize IAA was determined after 1, 2, 3, 4, and 5 days of culture, respectively.

Effects of carbon and nitrogen sources: Selected bacterial strains were grown in an experimental medium consisting of basic mineral salts with 1% addition of carbon sources (lactose, sucrose, starch, D-sorbitol, mannitol) to determine the effect of the carbon source. To find a suitable nitrogen source, bacterial strains were grown in a suitable carbon source medium supplemented with 0.1% nitrogen source (peptone, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, KNO_3) for 72 h at room temperature of 30°C, then determine the IAA content as previously described.

Culture media

Basic mineral environment to investigate the influence of carbon source (g/L): $(NH_4)_2SO_4$ 2; $MgSO_4.7H_2O$ 0.2; $NaH_2PO_4.H_2O$ 0.5; $CaCl_2.2H_2O$ 0.1; KH_2PO_4 0.5; H_2O 1 liter. Basic mineral environment to investigate the influence of nitrogen source (g/L): KH_2PO_4 1.36; $CaCl_2$ 0.03; NaH_2PO_4 2.13; $MgSO_4.7H_2O$ 0.2; $FeSO_4.7H_2O$ 0.01; source C (suitable for each microorganism strain): 10; H_2O 1 liter. Nutrient agar (NA) (g/L): Pepton 5; NaCl 5; high meat 1; yeast extract 2; agar 18. nutrient broth (NB) (g/L): Pepton 5; NaCl 5; high meat 2; yeast extract 3, distilled water 1000 mL. This study was conducted from October 2022 to May 2023 at the Laboratory of Microbiology Technology Department, Faculty of Biotechnology, and Laboratory of Plant Protection Research Institute, Vietnam Academy of Agricultural Science (VAAS).

Statistical analysis

All raw data were collected and mean comparisons were then statistically analyzed using Excel version 2016.

Results and Discussion

Isolation and Selection of Phosphate solubilizing Endophytic Bacterial Strains

In this study, from the basil samples collected at the Institute of Medicinal Materials in Ngoc Hoi, Thanh Tri, Hanoi, a total of 18 distinct endogenous bacterial strains were isolated on LB agar. These isolated bacterial strains were subsequently subcultured into NBRIP medium to assess their proficiency in degrading insoluble phosphate. Following a 48-hour incubation period at 30°C and a shaking speed of 200 rpm, the culture solutions were centrifuged, and the supernatant was collected. The outcomes were determined through colorimetric analysis using the technique detailed by Hien et al. (2021). The findings demonstrated that 10 out of the 18 strains displayed the capacity to degrade insoluble phosphate, namely H1, H9, H3, H5, RH2.1, RH2.2, RH5.1, RH5.2, RH6.1, and RH6.2 (as shown in Figure 1). The concentration of PO_4^{3-} released by these endogenous bacterial strains ranged from 0.15 to 6.92 mg/L. Remarkably, the bacterial strain RH2.2 exhibited the most robust phosphate-solubilizing activity among the collected strains.

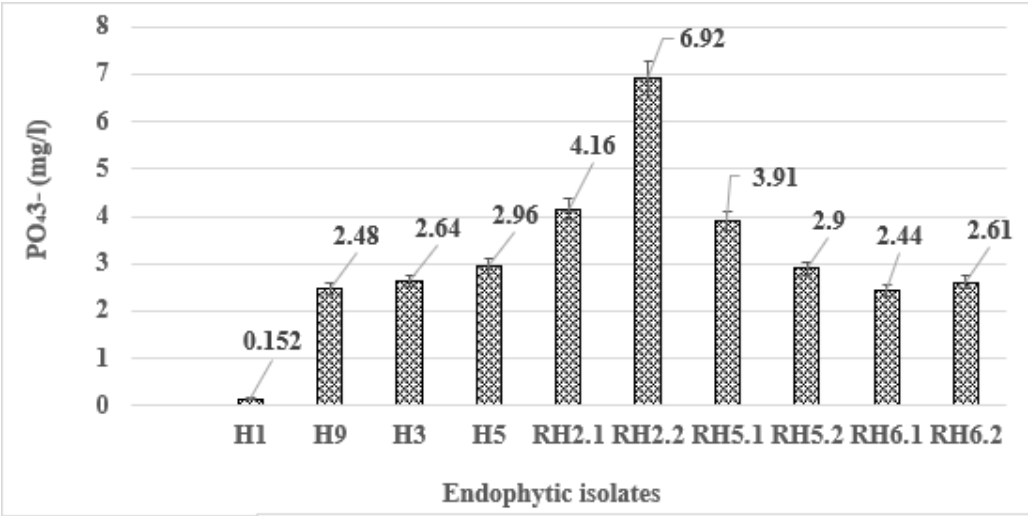

Figure 1. Phosphate solubilizing activity of endophytic isolates

Phosphorus stands as a pivotal nutrient element, actively participating in various vital processes within plants, encompassing metabolism, respiration, photosynthesis, nucleic acid structure, and the formation of high-energy molecules like ATP, ADP, and phospholipids. Phosphorus-containing fertilizers play a significant role in promoting root growth, reinforcing plants, enhancing flower and seed formation, and elevating overall product quality. A phosphorus deficiency can significantly impede plant growth and development. To counter such deficiencies, producers often resort to the application of chemical phosphate fertilizers. However, a portion of these phosphate fertilizers introduced into the soil tends to be immobilized by metal cations such as Ca^{2+}, Fe^{3+}, or Al^{3+}, leading to the formation of insoluble phosphate compounds, which subsequently undermines the efficiency of phosphate fertilizer utilization.

Microorganisms employ various strategies to enhance phosphate solubilization. They secrete organic acids and synthesize siderophores, among other methods. Organic acids, armed with carboxyl and hydroxyl groups, exhibit the ability to chelate metal cations that attach to PO_4^{3-} or lower the ambient pH, thereby facilitating the release of PO_4^{3-}. Additionally, organophosphorus compounds are subject to mineralization by enzymes secreted by microorganisms (Oteino et al., 2015). In this experiment, since inorganic phosphorus compounds were employed, it can be deduced that the newly isolated endogenous microorganisms likely secreted siderophore compounds or synthesized organic acids to heighten phosphate solubilization in the culture medium. Moreover, endogenous bacterial strains demonstrating the capacity to degrade insoluble phosphate were evaluated for their ability to synthesize siderophores.

Evaluation of the ability to synthesize siderophore

Siderophores represent compounds secreted by bacteria as a means to gather iron ions (Fe^{2+}) from their environment, particularly in instances of iron-deficient conditions. This mechanism aids plants in surmounting challenges arising from iron deficiency. While pathogens also necessitate iron for their growth, the affinity of both plants and beneficial microorganisms for iron surpasses that of pathogenic microorganisms. Consequently, this disparity diminishes the ability of pathogens to thrive within the medium (Chung et al., 2005), thereby fostering the growth and progression of host plants (Louden et al., 2011).

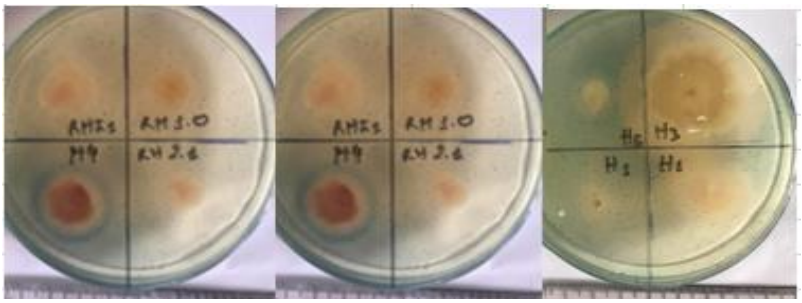

Figure 2. The siderophore-producing ability of endophytic isolates on CAS medium

The attained results as presented in Figure 2, unveiled the emergence of an orange-yellow hue encircling the colonies of specific newly isolated endogenous bacterial strains, namely RH2.1 and RH5.1, both endowed with the capability to degrade insoluble phosphate. This observable color alteration serves as evidence of their capacity for siderophore production. This assertion aligns with the classification of siderophore-producing strains established by Louden et al. (2011), where microorganisms with the ability to generate siderophores typically manifest a yellow-orange appearance when cultivated on CAS medium.

IAA biosynthesis ability of selected bacterial strains

Bacterial strains that exhibited proficiency in degrading insoluble phosphates were cultivated in NB medium supplemented with 1 g/L of tryptophan, maintained at 30°C, and agitated at 200 rpm. Following a 72-hour incubation period, the bacterial cultures were subjected to centrifugation, enabling the collection of the supernatant for the determination of IAA concentration within the solution. The findings showcased that certain strains, namely RH2.1, RH2.2, RH5.1, and RH5.2, displayed the potential for IAA synthesis, with IAA concentrations reaching 36.0, 73.9, 49.95, and 8.16 µg/mL, respectively. The dual ability of these bacterial strains to degrade insoluble phosphate and synthesize IAA establishes their suitability for subsequent experimental utilization.

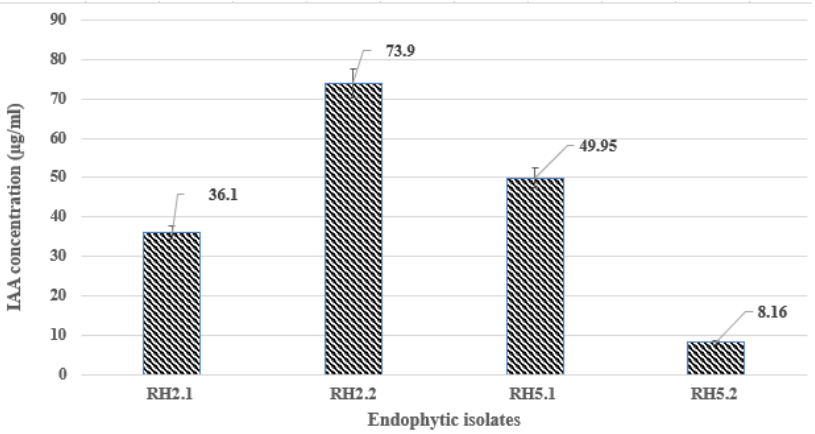

Figure 3. The IAA concentration produced by endophytic isolates

IAA is an essential plant growth hormone that is synthesized by both plants and microorganisms. Microorganisms employ diverse IAA biosynthetic pathways, with certain bacterial strains even utilizing more than one pathway for IAA synthesis. The concentration of IAA produced hinges on factors such as the chosen biosynthetic pathway, the genomic location of pertinent genes within the IAA biosynthetic process (whether on chromosomes or plasmid DNA), and regulatory mechanisms governing IAA biosynthesis. Enzymes may also participate in the

conversion of active IAA to an inert or inactive state (Patten and Glick, 1996). Consequently, endogenous microbial strains originating from various plants yield distinct quantities of IAA.

For instance, Trang et al. (2018) isolated four endogenous bacterial strains from pepper plant roots, with IAA production ranging from 24 to 68 µg/mL. In the case of endogenous bacteria isolated from *aloe vera* plant roots, IAA synthesis yielded concentrations spanning from 17.18 to 23.23 µg/mL (Giang et al., 2016). In another study of Hien et al. (2021), endogenous bacterial strains sourced from black tiger roots showcased IAA content ranging between 4.34 and 47.02 µg/mL. These variations underscore the influence of both microbial origin and specific biosynthetic pathways on IAA production levels.

Effect of culture conditions on the IAA biosynthesis of endophytic isolates

Effect of medium pH and temperature

The selected endophytic bacterial strains, namely RH2.1, RH2.2, RH5.1, and RH5.2, were cultivated in NB medium supplemented with 1 g/L of tryptophan, maintained at a temperature of 30°C, and subjected to agitation at a rate of 200 rpm. The cultures were initiated at distinct initial media pH values, specifically 5, 6, 7, 8, and 9, respectively. Following a cultivation period of 72 hours, the culture solutions underwent centrifugation, and the quantification of IAA was conducted utilizing the method outlined in section 2.2.4.

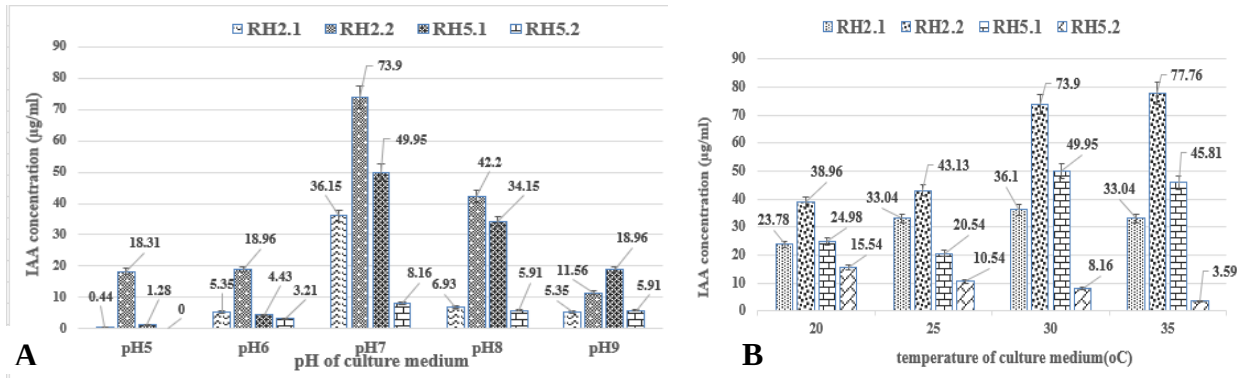

Figure 4. Effects of pH (A) and temperature of culture medium (B) on IAA production of endophytic isolates

When cultivated within a medium of pH 7.0, the experimental bacterial strains exhibited the highest synthesis of IAA, with IAA content ranging from 8.16 to 73.9 µg/mL (Figure 4A). Strikingly, when the pH of the culture medium deviated from 7.0, particularly in acidic conditions, the amount of IAA synthesized by these bacterial strains experienced a significant decline (Figure 4A). Investigation into the effects of culture environment temperature (ranging from 20 to 35°C) on the concentration of IAA synthesized by strains RH2.1, RH2.2, RH5.1, and RH5.2 was further made. The optimal temperature range for IAA synthesis by the experimental strains was determined between 30 and 35°C (Figure 4B). Subsequently, temperatures that were lower than 30-35°C, specifically within the range of 20-25°C, resulted in a reduction of IAA content synthesized by the experimental strains.

Low pH levels pose limitations on plant growth due to the potential for soil acidity. Such conditions lead to the accumulation of certain metal ions, reaching toxic concentrations detrimental to both soil and plant health (Mohite, 2013; Chandra et al., 2018). Mohite (2013) concluded that bacterial strains demonstrating high IAA synthesis were the most prominent within an environmental pH range of 7.0 to 9.0, respectively. Similar observations were described by Hien

et al. (2022), who found that the highest IAA synthesis by bacterial strains HY9 and TT3 occurred at a culture medium pH of 7.0. Endogenous bacterial strains derived from black tiger roots displayed maximum IAA synthesis within the pH range of 7.0 to 8.0 (Giang et al., 2021). Moreover, Chandra et al. (2018) reported that microbial strains could synthesize IAA effectively within the pH range of 5.0 to 9.0, respectively. Additionally, Adeleke et al. (2022) found that the *Stenotrophomonas indicatrix* BOVIS40 strain exhibited peak IAA synthesis at pH 7.0. These findings collectively underscore the influence of soil pH on various biological processes occurring within the rhizomes of plants. Microbial strains engage in IAA synthesis pathways regulated by gene activity and catalyzed by distinct enzymes (Patten and Glick, 1996), making them sensitive to temperature variations that impact the genes and enzyme activity (Duca et al., 2014). Also, Adeleke et al. (2022) reported that endogenous bacterial strains exhibited peak IAA synthesis at pH 7.0 and temperatures within the range of 22 to 37°C, respectively. Mohite (2013) identified the temperature of 30°C as the optimal temperature for microbial strains in IAA synthesis. Different bacterial strains, such as *Rhizobium* spp. and *Bacillus* spp., demonstrated the highest IAA synthesis at 37°C (Sudha et al., 2012). Chandra et al. (2018) concluded that the CA 1001 strain's optimal temperature for IAA synthesis was 37°C. Other studies reported by Giang et al. (2021) and Hien et al. (2022), who highlighted optimal IAA synthesis at pH 7.0 and temperatures of 30-37°C. Furthermore, Giang et al. (2016) reported that the endogenous bacterial strain derived from *Aloe vera* plant roots achieved peak IAA synthesis at 35°C.

Effects of carbon and nitrogen sources in the culture medium

The experimental bacterial strains (RH2.1, RH2.2, RH5.1, and RH5.2) were cultured in media enriched with various carbon sources, encompassing lactose, glucose, sorbitol, and sucrose. The extent of IAA synthesis by these strains exhibited variability contingent upon the particular carbon source within the medium (Figure 5A). For the strain RH2.2, glucose and lactose emerged as the most suitable carbon sources, resulting in the synthesis of 82.57 and 65.63 µg/mL of IAA, respectively. This synthesis plummeted to 7.02 and 9.24 µg/mL when the culture medium was furnished with sorbitol and sucrose. In contrast, for the strain RH2.1, sorbitol and sucrose proved to be the optimal carbon sources, yielding an impressive IAA content of 76.83 and 76.46 µg/mL, respectively.

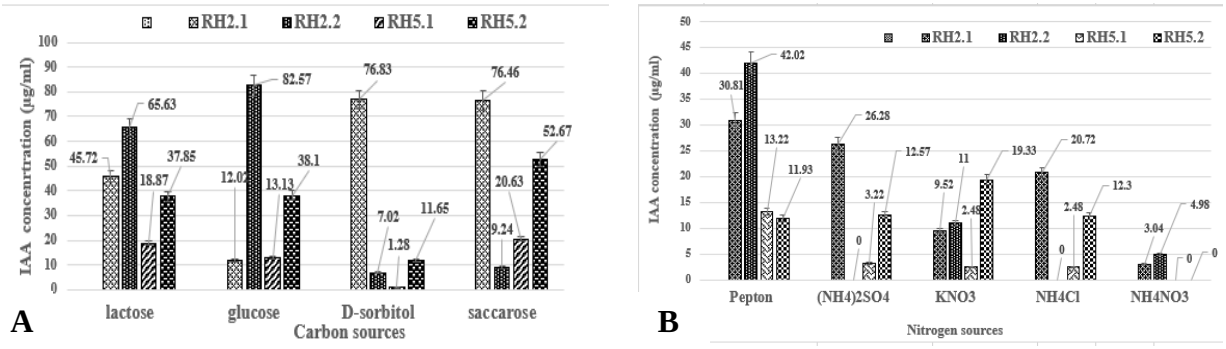

Figure 5. Effects of carbon (A) and nitrogen sources (B) on IAA concentration produced by endophytic isolates

Upon cultivation in a medium enriched with diverse nitrogen sources, including peptone, NH_4Cl, $(NH_4)_2SO_4$, KNO_3, and NH_4NO_3, the selected endophytic bacterial strains exhibited varying levels of IAA synthesis. The most substantial IAA synthesis was observed in the presence of peptone within the culture medium. In contrast, when the culture medium featured inorganic

nitrogen sources, the concentration of IAA synthesized by the endophytic bacterial strains experienced a marked reduction. For strain RH2.1, $(NH_4)_2SO_4$ and NH_4Cl proved suitable, resulting in the highest IAA synthesis concentrations (26.28 and 20.72 µg/mL, respectively). Strain RH5.2 showcased a preference for KNO_3, generating an IAA concentration of 19.23 µg/mL, surpassing that synthesized by other strains. The least amount of IAA synthesis was recorded in the medium containing NH_4NO_3 (Figure 5B). Microorganisms utilize carbon sources to synthesize vital cellular components and secondary metabolites, thereby influencing cellular biosynthetic processes. The chosen carbon source significantly impacts the efficiency of IAA synthesis by microbial strains due to its ability to modulate biochemical reaction rates within the cell. This subsequently affects the microorganism's growth rate and metabolite synthesis. Recent reports of Hien et al. (2022) and Panigrahi et al. (2020) demonstrated that the addition of sucrose to the culture medium led to increased IAA synthesis by endogenous bacterial strains. Similarly, Emami et al. (2019) found that the highest IAA synthesis occurred in the presence of glucose, followed by sucrose. Adeleke et al. (2022) documented that *Stenotrophomonas maltophilia* JVB5 and *Bacillus cereus* T4S exhibited the highest IAA concentrations when cultured with sucrose and glucose sources. Notably, lactose and glucose are considered optimal carbon sources for IAA synthesis by *Enterobacter* sp. and *Rhizobium* spp. (Basu and Ghosh, 2001). This underscores the role of carbon sources as energy providers for cellular processes and enhancers of secondary metabolite synthesis in microorganisms (Chandra et al., 2018).

The nitrogen source within the culture medium stands as a pivotal factor impacting both microbial growth and metabolite synthesis. Distinct nitrogen sources exert varying influences on IAA synthesis, and microorganisms adeptly exploit suitable nitrogen sources to optimize this process. NH_4NO_3 and NH_4Cl have been identified as fitting nitrogen sources for endogenous microorganisms isolated from black tiger roots (Giang et al., 2021). Similarly, two strains of bacteria, namely HY9 and TT3, isolated from honeysuckle roots, exhibited maximum IAA synthesis when cultured in a medium containing NH4Cl (Hien et al., 2022). In the study conducted by Chandra et al. (2018), CA 2004 strain achieved the highest IAA concentration within an environment featuring NH_4NO_3, and high beef concentrations. Notably, the concentration of IAA synthesized by bacterial strains CA1001 and CA2003 declined when exposed to an inorganic nitrogen source within the medium. In the fact that the aforementioned two strains failed to grow when cultivated within an environment featuring inorganic nitrogen sources. Conversely, strain CA2004 displayed the peak IAA concentration when cultured in a medium enriched with KNO_3 and NH_4NO_3. Sridevi et al. (2008) revealed that the inclusion of inorganic nitrogen sources within the culture medium elevated the concentration of IAA synthesized by *Rhizobium* spp., while the presence of organic nitrogen sources led to a reduction in IAA concentration synthesized by the same strain.

Morphological and biological characteristics of the selected bacterial strains

Five distinct strains of bacteria were judiciously chosen, each characterized by a unique morphology and color, as illustrated in Figure 6. Notably, all colonies showcased smooth and rounded surfaces, exhibiting shades of white, off-white, or pale yellow. Two of these strains, specifically RH2.2 and RH5.2, featured rod-shaped cells that stained purple in the Gram staining process. Conversely, strains RH2.1 and RH5.1 displayed rod-shaped cells that manifested a pink hue during Gram staining. In terms of biochemical reactions, both strains RH2.1 and RH2.2 exhibited positivity across four reactions, namely catalase, Methyl Red (MR), citrate, and Voges-Proskauer (VP). Strain RH5.1 showcased a positive response for catalase, citrate, and VP reactions, yet demonstrated no reactivity towards Methyl Red. On the other hand, strain RH5.2

tested positive for catalase and citrate reactions, while remaining unresponsive to Methyl Red and VP testing.

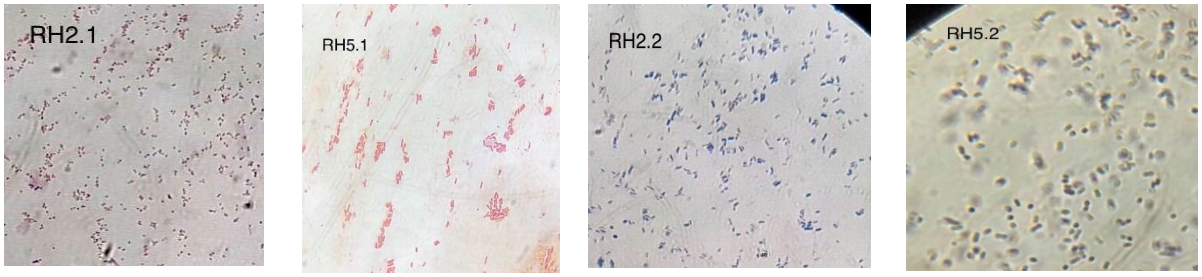

Figure 6. Gram reaction of endophytic isolates of the RH2.1, RH.5.1, RH2.2 and RH5.2.

Conclusion

In conclusion, our findings provide valuable information and insights into the endophytic bacterial strains isolated from the roots of purple basil, shedding light on their *in vitro* culture conditions and their impact on IAA synthesis. Specifically, strains RH2.1, RH2.2, RH5.1, and RH5.2 were isolated and selected from purple basil roots. All strains demonstrated remarkable capability in degrading insoluble phosphate and synthesizing IAA at substantial concentrations. Under *in vitro* culture conditions, these strains exhibited the capacity to harness distinct carbon and nitrogen sources to facilitate IAA synthesis. For strain RH2.1, sorbitol and sucrose emerged as the most favorable carbon sources, while sucrose proved suitable for strain RH5.2. Additionally, lactose and glucose spurred increased IAA synthesis for strain RH2.2. Notably, these selected endogenous microorganisms show the highest IAA synthesis levels within a peptone-enriched medium. Further exploration should be done to assess the efficacy of these strains *in vivo* to validate the actual potential stimulating plant growth for sustainable agriculture production.

References

Adeleke B.S, Ayangbenro, A.S. and Babalola, O.O. (2022) *In vitro* screening of sunflower associated endophytic bacteria with plant growth-promoting traits. *Frontiers in Sustainable Food Systems*, 6: 903114.

Diep, V.T.N., Huyen, P.K., Giang, N.V., Trung, K.H., Khanh, T.D. (2021). Roles of bacteria in ACC deaminase biosynthesis in minimizing abiotic stress in plants. *Vietnam Journal of Science and Technology*, 9:56-59.

Giang, N.V., Quan, N.T., Nhung, N.T., Linh, T.T.D., Minh, A.H.T., Trung, D.T., Thuy, T.T.D., Tran, N.P.B., Trung, K.H., Khanh, T.D. (2022). Evaluation of the IAA-producing ability of some endophytic bacteria isolated from *Panax pseudogingseng* roots. *Vietnam Journal of Agriculture and Rural Development*, 3(2):2-10.

Giang, N.V., Hoai, T.T., Trang, V.M., Trung, K.H., Xuan, T.D., Duong, V.X., Huyen, P.K., Diep, V.T.N., Trung, N.T., Khanh, T.D., Thu, P.T.L. (2020). Isolation and Characterization of Potassium Solubilizing Bacteria in some Vietnamese Soil Samples. *International Journal on Emerging Technologies*, 11(3):639-643.

Hien, P.H., Tuoi, V.T., Linh, V.T., Giang, N.V. (2021). Isolation and evaluation of biological characteristics of endophytic bacteria from roots of river mangrove. *Journal of Vietnam Agricultural Science and Technology*, 7(128):71-75

Loi, D.T. (2001). Vietnamese medicinal plants and herbs. Medical Publishing House, Hanoi (*in Vietnamese*).

Giang, N.V., Dao, T.T., An, T.T.T. (2016). Isolation and Characteristics of Some Bacterial Endophytes from Root of *Aloe vera*. *Vietnam Journal of Agricultural Science*, 14(5): 772-778.

Giang, N.V., Tuoi, V.T., Linh, V.T., Hien, P.H. (2021). Effects of culture media and condition on IAA producing ability of endophytic bacteria isolated from roots of river mangrove (*Aegiceras corniculatum*). *Journal of Vietnam Agricultural Science and Technology*, 06(127): 49-55.

Trang, N.T., Ha, T.T.T., Truong, N.X., Giang, N.V. (2018). Isolation and characterization of endophytic bacteria from roots of black pepper. *Journal of Vietnam Agricultural Science and Technology*, 10(95): 85-90 (*in Vietnamese*).

Hien, P.H., Dao, T.T., Trang, T.H., Lieu, H.T., Giang, N.V. (2022). Effects of Culture Conditons on IAA Synthetic Ability of Endophytic Bacterial Strains HY9 and TT3. *Vietnam Journal of Agricultural Science*, 20(12): 1640-1648.

Basu, P. and Ghosh, A. (2001). Production of indole acetic acid in cultureby a Rhizobium species from the root nodules of a monocotyledonoustree, *Roystonea regia*. *Acta Biotechnolica*. 21, 65–72. doi: 10.1002/1521-3846(200102)21:1

Chandra, S., Askari, K., Kumari, M. (2018). Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. *Journal of Genetic Engineering and Biotechnology*;16(2):581-586. doi: 10.1016/j.jgeb.2018.09.001.

Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H., Sa, T. (2005). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biology & Biochemistry*, 37(10): 1970-1974.

Duca, D., Lorv, J., Patten, C.L., Rose, D., Glick, B.R. (2014). Indole-3-acetic acid in plant-microbe interactions. *Antonie van Leeuwenhoek*; 106(1):85-125. doi: 10.1007/s10482-013-0095-y.

Emami, E., Alikhani, H.A., Pourbabaei, A.A., Etesami, H., Sarmadian, F., Motessharezadeh, B. (2019) Assessment of the Potential of Indole-3-Acetic Acid Producing Bacteria to manage Chemical Fertilizers Application. *International Journal of Environmental Research*. 13: 603-611.

Glickmann, E. and Dessaux, Y. (1995). A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied. Environmental. Microbiology*. 61(2): 793-796. doi: 10.1128/aem.61.2.793-796.1995

Louden, B.C., Haarmann, D., Lynne, A.M. (2011). Use of Blue Agar CAS Assay for Siderophore Detection. *Journal of Microbiology & Biology Education,* 2011 May 19;12(1):51-3. doi: 10.1128/jmbe.v12i1.249. PMID: 23653742; PMCID: PMC3577196.

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant Nutrition*, 13(3): 638-649.

Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J. and Dowling, D.N. (2015). Plant growth promotion induced by phosphate solubilizingendophytic *Pseudomonas* isolates. *Frontiers in Microbiology*, 6:745. doi: 10.3389/fmicb.2015.00745

Panigrahi, S., Mohanty, S., Chandi, C.R. (2020). Characterization of endophytic bacteria Enterobacter cloacae MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. *South African Journal of Botany*. 134: 17-26.

Patten, C. L. and Glick, B.R. (1996) Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*. 42: 207-220, https://doi.org/10.1139/m96-03.

Schwyn, B. and Neilands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160: 47-56

Sridevi, M., Yadav, N.C.S., Mallaiah, K.V. (2008) Production of indole acetic acid by Rhizobium isolates from Crolataria species. *Research Journal of Microbiology*. 3(4):276–81.

Sudha, M., Shyamala, G.R., Prbhavati, P., Astapritya, P., Yamuna Devi, Y., Saranya, A. (2012). Production and optimization of Indole acetic acid by indigenous microflora using agro waste as substrate. *Pakistan Journal of Biological Sciences*. 15, 39-43.