EXPLORATION OF PLANT GROWTH-PROMOTING ENDOPHYTIC BACTERIA FROM THE ROOTS OF NATIVE PLANT *Uncaria borneensis* IN SARAWAK, BORNEO

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Abstract

Endophytes refers to as the microorganisms that reside in a host plant and are in a relationship with the abiotic and biotic factors. These factors are responsible to influence the function and structure of the communities. There are few studies done about bacteria of endophytic from the tress of forest, though there have various studies been conducted. The recent study has the aim to isolate some endophytic bacteria from Borneo base forest of Universiti Putra Malaysia Kampus Bintulu (UPMKB). The test has been conducted for the growth of plant. About 61 bacteria have been isolated and tested to produce IAA or, indole-3-acetic acid, siderophore production, and solubilize phos-phate. About a number of 35 isolates have been discovered to solubilize the phosphate and 70 percent of the phosphate belong to bacteria have produced. There have found 220 to 99 g/ml of concentration for IAA. In order to produce siderophore, there found 8 isolates. Enterobacter sp. and Pantoea has been found as the general that is most abundant and recovered from the DNA se-quencing of 16s ribosome. Different bacteria had been tested in vitro to promote the ability for the growth of plant. There have found 5 strains capable for the growth of the tomato plant. This study may be the first ever report for performing the isolation of the plant growth that promotes the Uncaria borneensis by promoting endophytic bacteria. It should be concluded that, the successful interaction between the strains of endophytic such as the Uncaria tree, J-112, J-111 provides benefits in a long term and direct the development and employ those strains as biofertilizer to produce certain crops and reduce application of fertilizer.

Keywords: Plant growth promoting siderophore; 16s rDNA sequencing, endophytic bacteria; indole-3-acetic acid;

1. Introduction

Various kinds of plants communicate and interact with a huge microbe range. They synthesise hormones, sugars, organic acids, and various other metabolites, thereby protecting them against pathogen attacks or environmental stress, indicating that they have the potential for use in sustainable agriculture. Because this soil area contains more nutrients than bulk soil, the microflora is rich with microorganisms, resulting in greater microbial interactions

(Agathokleous et al., 2020, Bakker et al., 2020). Endophytic bacteria enhance plant growth, are capable of solubilising phosphate, and provide plants with assimilable nitrogen (Hardoim et al., 2015, Liu et al., 2017, Afzal et al., 2019). These mechanisms are crucial for using endophytic bacteria as environmentally friendly biofertilizers to improve food output and feed the world's population. This is critical for increasing agricultural productivity while minimizing the overuse of pesticides and chemical fertilizers. Because these practices are still in use and have resulted in severe environmental deterioration, they have become a critical concern.

While most studies have focused on endophytic bacteria associated with significant agricultural plants due to their beneficial impacts on the host trees, establishing effective strains from forest trees remains a challenge (Ji et al., 2014; Khan et al., 2019 and Rana et al., 2020). Microorganisms linked with forest trees may help hosts survive in severe environmental conditions. While numerous microorganisms are linked with forest trees, little is known about their bacterial counterparts' roles and variety. So far, among the most prevalent endophytic bacterial communities isolated from forest trees are Pseudomonas, Actinobacteria, Bacillus, Azotobacter, Burkholderia, Enterobacter and Serratia (Pirttilä et al. 2021). Endophytic *Paenibacillus polymyxa*, isolated from Lodgepole pine and capable of nitrogen fixation, is one of the studies reported on endophytic bacteria isolation from forest trees (Anand et al., 2013). In another study, *Enterobacter cloacae* strains isolated from Mediterranean pines were able to produce indole acetic acid in vitro (Madmony et al., 2005). These findings suggest that the discovery of endophytic bacteria from forest trees has the potential for future applications such as plant disease control and plant growth enhancement.

Uncaria borneensis, also known as ‘gambir,' refers to a native of forestal tree to Thailand, Sumatera, Malaya, and Borneo, across the woods of tropical Asia. Uncaria genus has gathered huge amount of attention from the researchers for vast number of therapeutic species, especially particularly Uncariagambir that is highly examined (Rauf and Siregar, 2015; Musdja et al., 2018 and Yunarto et al., 2021). The wood of Uncaria borneensis has utilized in heavy and medium structure, in musical instruments, and floors, although their function is not properly clear.

Plant growth-promoting bacteria isolated from *Uncaria borneensis* have received little attention. Due to the numerous reported benefits of Uncaria species, functional research on the endophytic bacteria associated with these species is critical. Some endophytes probably contribute to sustainable agriculture, forestry production, and many valuable metabolites of high biotechnological value (Kuźniar et al., 2019). Thus, this study aimed to isolate and characterise bacteria of endophytic from the roots of *Uncaria Borneensis* and evaluate their possible growth of bacteria in plant.

2. Methods and Materials

Sample collection along with isolation for endophytic bacteria from Uncaria borneensis tree’s roots

Root samples from healthy *Uncaria borneensis* trees were collected from the Borneo Base Forest at Universiti Putra Malaysia Bintulu Campus. The identification plant was done in the field with the help of a forest botanical expert. Figure 1 depicts the plant. Smaller pieces of the root samples were rinsed with tap water, sterilized for about 5 minutes with 5% NaCIO, or, sodium hypochlorite and washed about 4 – 5 times with distilled water for sterilization.

To confirm the success of surface sterilization and serve as a control, 100 µL of the final rinse was plated onto nutrient agar (NA) plates. The samples were then macerated in 5 mL of sterile distilled water with a mortar and pestle, and 100 mL of the macerated liquid was plated on King B agar media. All plates, including the control, were incubated at room temperature for 24 – 48 hours and were monitored regularly. Bacterial colonies with unique morphology were selected and purified on the same medium for further study.

Figure 1: Main parts of *Uncaria borneensis* tree

Molecular identification of endophytic bacteria

Bacterial identification was based on the analysis of the 16S rRNA gene sequence. Each isolate's genomic DNA was extracted using the method described by Freschi et al. 2005, with some modifications. Individual colonies were picked up from an agar plate with a sterile toothpick and resuspended in 10 mL of sterile deionized water. The cell suspension was centrifuged (10,000 rpm, 5 minutes) to recover the DNA supernatant. The 16S rRNA gene was amplified using the primers pA (30-AGAGTTTGATCCTGGCTCAG-50, forward) and pH. (50-AAGGAGGTGATCCAGCCGCA-30, reverse). The PCR products were sent for sequencing for validation, and the sequences were identified using the EzTaxon database (Yoon et al., 2017). Two sequences were considered from the same species or same genus if they showed at least 98% and 94.8% similarity, respectively (Moreira and López-Garca, 2014; Yarza et al., 2014).

Plant growth-promoting potential of endophytic bacteria

Phosphate solubilization

Bacterial endophytic isolates were tested for phosphate solubilization using Pikovskaya medium (containing 10 g L-1 glucose, 5 g L-1 Ca3(PO4)2, 0.2 g L-1 KCl, 0.5 g L-1 (NH4)2SO4, 0.2 g L-1 NaCl, 0.1 g L-1 MgSO4.7H2O, 0.002 g L-1 MnSO4.H2O, 0.002 g L-1 FeSO4 7H2O and 15 g L-1 Bacto agar) and bromophenol blue as an indicator, as described by Jasim et al. [78]. Strains that produced a clear zone surrounding the colonies after 5 - 7 days of incubation at 35°C were considered positive.

Indole-3-acetic-acid (IAA) production

The efficiency of the isolated bacterial endophytes for IAA synthesis was quantified using a method described by Gordon and Weber (1951). The bacterial isolates were cultured in NB overnight on an orbital shaker set to 120 rpm. A loopful of bacteria culture was inoculated in 5 mL of NB containing 0.1% of L-tryptophan (TRP) and incubated at 30°C for 24 hours, shaking at 120 rpm. The culture was centrifuged at 12,000 rpm for 10 minutes the next day. A total of 1 mL of the supernatant was mixed with 2 mL of Salkowski's reagent (50 mL 35% sulphuric acid and 0.5M FeCl3), gently vortexed and incubated in the dark for 30 min. The development of pink or red colour indicated IAA production. Optical density at 530 nm was measured using a spectrophotometer (Lambda 25, Perkin Elmer). IAA concentration was determined by comparing OD to the standard calibration curve obtained using IAA solution ranging from 0 to 100 μg mL-1 (Sasirekha and Shivakumar, 2012). All the IAA production assays were performed in triplicates.

Siderophore production

According to Louden et al. (2011) CAS or, blue medium of chrome azurol has been prepared to test the formation of siderophores. A loopful of overnight bacterial culture was inoculated onto the CAS plate and incubated for 5 days at 28°C. This study The experiment was carried out in triplicate, and an uninoculated plate was used as a control. Following incubation, the development of the orange zone around the colony indicated siderophore production.

Effect of endophytic bacteria on root formation

Using the paper towel method, a seed germination assay was used to determine whether the isolated bacteria could promote root development (Abdul Baki and Anderson, 1973). The five most potent IAA-producing and phosphate-solubilizing isolates were inoculated in NB and incubated overnight with shaking at 120 rpm. All treated seeds were placed in a petri dish moistened with sterile distilled water and germinated in the dark. After 3 days of germination, uniform seeds were selected and inoculated for 2 hours in a separate tube containing diluted bacterial isolates at a dilution of 108 cells mL-1. The seeds were then placed on a dampened paper towel in a sterile container, grown at room temperature and observed until day 10. Seeds inoculated with distilled water were used as a control.

3. Results

Isolation and identification of endophytic bacteria

The findings of the study indicates that the *Uncaria borneensis* trees exhibit a huge culturable proportion of endophytic bacteria. About 61 bacteria that were endophytic been identified from their roots. The plates of control showed the absence of colonies among the bacteria that isolated the endophytes. It had found that every isolates were identified molecularly. (Figure 2) these had evaluated for the parameters that were crucial (Table 1).

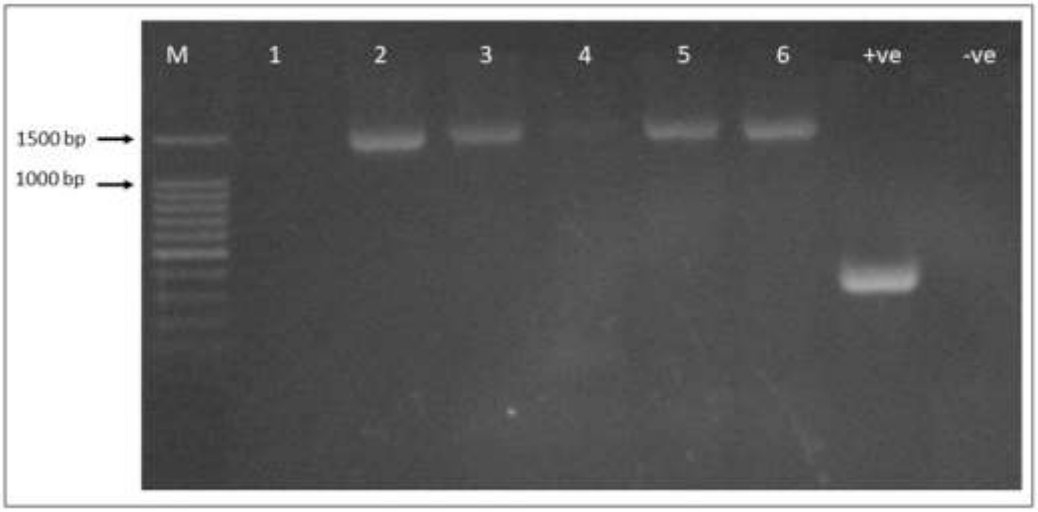

Figure 2: Agarose gel analyses (1% agarose, w/v) of amplified 16S RNA of the isolates used in the study. (Lane M = marker; lane 6 = L-112; lane 5 = K-311; lane 4 = J-221; lane 3 = J-122; lane 2 = J-112; lane 1 = J-111 lane -ve = negative control and ; +ve = positive control without any colony).

Table 1: Identification of the endophytic bacteria isolated from root

Bacterial Strains	***Blast Matches***	***Top Hit Strain (Accession Number)***	***Similarity (%)***
J-111	*Pantoea rwandensis*	*CP009454.1*	*99.37*
J-112	*Pantoea rwandensis*	*CP009454.1*	*99.44*
J-122	*Pantoea rwandensis*	*CP009454.1*	*99.37*
J-221	*Enterobacter lignolyticus*	*CP002272.1*	*98.99*
K-311	*Enterobacter sp.*	*MW375575.1*	*99.79*
L-112	*Enterobacter bugandensis*	*CP083403.1*	*98.74*

Plant growth-promoting activity

Each isolate's ability to produce indole acetic acid (IAA), solubilize phosphate and form a siderophore were evaluated. There are about 61 isolates of bacteria and from there 35 phosphate are solubilized and are indicated by the development of distinct zone of halo growing around the every colony in the medium of Pikovskaya (Figure 3a). Strains of bacteria with highest solubilizing phosphate efficacy had been found among the strains of bacterial strain named J11-1, with an index of a solubilizing phosphate 4.1. (Figure 3b). About 70% of the 35 isolates could produce IAA in the 99 – 220 µg/ml range. The IAA produced by these bacteria were compared with the IAA standard curve, and the most potent bacteria strain in producing IAA was found in bacteria strains J-112 and L-112 (Figure 4). A total of 8 isolates were positive for siderophore production; however, only the best 6 isolates were presented in Figure 5.

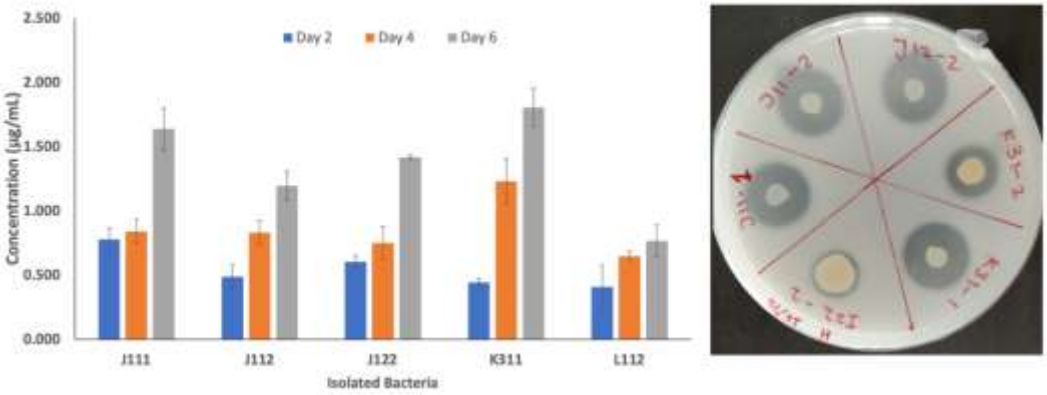

Figure 3: Phosphorous solubilisation activity of the isolated bacteria

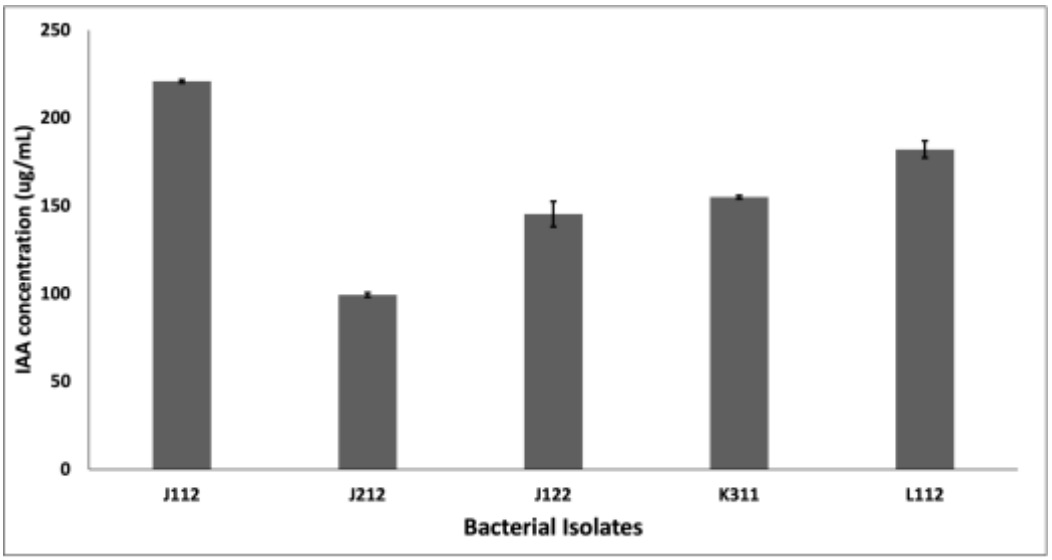

Figure 4: IAA production of the selected bacterial isolates. Data represent average standard error.

There found some potent strains of bacteria counted as 5 had been tested for knowing the ability to promote the growth of tomato plant. Plants of tomato inoculated with J-112 and J-111 strains, and produced better formation of root, fresh weight, and shoot length and been inoculated with L-112 strains, K-311 and J-122. This demonstrates that strains J-111 and J-112 had the greatest effect and greatly increased root biomass compared to a control plant without bacteria (Figure 6).

(a)

Bacterial Strains	Siderophore Production	
	Colony Diameter (cm)	Halozone Diameter (cm)
J-111	1.6 ± 0.05	2.5 ± 0.14
J-112	1.8 ± 0.08	2.5 ± 0.14
J-122	1.7 ± 0.07	2.4 ± 0.13
J-221	2.0 ± 0.12	2.5 ± 0.14
K-311	1.9 ± 0.10	2.7 ± 0.05
L-112	1.8 ± 0.08	2.5 ± 0.18

(b)

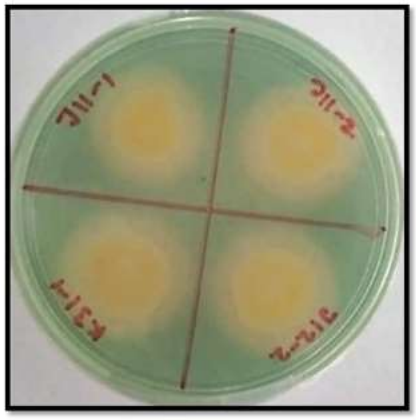

Figure 5: The isolated strains' ability to produce siderophores. (a) Colony and halozone diameter measurement; (b) A variety of colonies growing on a CAS plate.\

Figure 6: Germination of Root in plants l;ike tomato inoculated with selected bacterial strain. C = control; T1 = J-111; T2 = J-112; T3 = J122; T4 = K-311; T5 = L-112.

4. Discussion

Bacteria of culturable endophytic were isolated from *Uncaria borneensis* roots as a step called preliminary toward understanding of their role of potentiality as biofertilizers in enhanced condition of agriculture that is sustainable. In total, 61 endophytic bacteria representing four phyla were identified. Additionally, 40 novel species and 3 new genera had been identified from wild *U. borneensis* plant's root. Most of the research in present involves

the endophytic bacteria's isolation. A single medium of isolation need to cultivate microorganisms in diverse array(e.g., TSA or, nutritional agar), a constant temperature of 28°C, and an incubation time of 48–72 h. (Borah et al., 2019; Soares et al., 2020 and Rat et a., 2021). Higher diversity has shown in this study as B medium of King can be utilized. Based on the result obtained in the present study, we selected 6 most potent strains and found a similarity to Enterobacter lignolyticus(J-221), Enterobacter sp.(K-311), Enterobacter bugandensis(L-112), and 3 strains had similarity to Pantoearwandensis (J-111,J-112,J-122). A similar report isolated Entrobecter, pontoea, bacillus and Burkholderia from Tylosema esculentum (Chimwamurombe and Sinyinza, 2022). Other than that, bacteria belonging to γ Proteobacteria, such as Enterobacter sp. Serratia and Pontoea were found from the root of Salicornia brachiate. Pantoea and Enterobacter are also reported to be commonly found in rice plants (Zhang et al. 2013).

In this study, every IAA has produced in various amounts. It is considered to be the most common characteristic found in endophytic bacteria. Some bacteria isolated from the U. borneensis roots produce a high concentration of IAA phytohormone that help in root and stem development. IAA phytohormone also influences the ability to increase nutrient uptake by encouraging lateral root hair development (Mohite, 2013). Phosphate is one of the major elements needed for overall plant development, such as photosynthesis and root development. There are 57% of the bacteria isolated from the U. borneensis root are able to solubilized Calcium Phosphate (Ca3(PO4)2) on Pikovskaya (PVK) agar with solubilizing index varying 2 – 4.1. In the study carried out by Silva et al. (2020), the strain of Pontoea and entrobacter isolated from Aloe Vera plants showed ability to solubilized different sources of phosphate such as s Calcium Phosphate (Ca3(PO4)2) and Iron Phosphate (FePO4). However, according to Rodrigues et al. (2016) reports, rhizobacteria exhibited the highest solubilization indices. Siderophore-producing characteristic is one of the unique traits found in endophytic bacteria that is important for plant development and indirectly help in defense against pathogen. No significant differences were found in the ability to produce siderophore from 8 strains isolated from the root of U. borneensis. Selected strain show increases in tomato growth in terms of root formation and the weight of the stem.

5. Conclusions

In conclusion, 5 strains of endophytic bacteria from *U. borneesis* show improvement in growth and successful colonization in the tomato plant despite having a different host. It is possible for these bacteria to be multiple host bacteria and may be beneficial for the agricultural crop because the biological properties of the forest tree are different compared to agricultural plants,which may affect beneficial traits obtained by the forest bacteria. According to results obtained inthis study, strain J-111 and J-112, similar to Pantoearwandensis is a suitable candidate that can potentially be used as agents to promote plant growth. However, further experiments on its PGP characteristic and the performance of their promoting potential in natural environments are needed to confirm this hypothesis.

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