Malays. Appl. Biol. (2022) 51(4): 119-126
https://doi.org/10.55230/mabjournal.v51i4.19

CHARACTERISATION OF ENDOPHYTIC BACTERIA FROM NAM-NAM PLANTS (*Cynometra cauliflora*) FOR ANTIBACTERIAL ACTIVITY AND PRODUCTION OF PLANT GROWTH PROMOTING FACTORS

RABIATUL ADAWIYAH KHALIL[1], SHARIFAH AMINAH SYED MOHAMAD[1,2*]
NUR RAHIMATUL HAYATI ABDUL RAHMAN[3], NURUL AIDA KAMAL IKHSAN[3],
NORFATIMAH MOHAMED YUNUS[1], OLAIDE OLAWUNMI AJIBOLA[4],
NURLIANA ABD MUTALIB[2] and MOHD CAIRUL IQBAL BIN MOHD AMIN[5]

[1]School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Selangor, Malaysia
[2]Atta-ur-Rahman Institute for Natural Products Discovery, Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Selangor, Malaysia
[3]Centre of Foundation Studies, Universiti Teknologi MARA Selangor Branch, Dengkil Campus, 43800 Dengkil, Selangor, Malaysia
[4]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[5]Centre for Drug Delivery Technology, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[*]E-mail: sharifah459@uitm.edu.my

Accepted 7 October 2022, Published online 31 October 2022

ABSTRACT

Plant-beneficial microorganisms also known as endophytes colonize the inside healthy tissues of living plants and form mutualistic relationships with them. Endophytes are important components of the plant microbiome and give a variety of benefits to their hosts. Nam-Nam plant (*Cynometra cauliflora*), is an indigenous tree to Peninsular Malaysia with various medicinal properties. This study aimed to isolate and characterize endophytic bacteria from different parts of Nam-Nam plants such as leaves, stems, and roots. The ethyl acetate extracts from the endophytic bacteria were tested for their antibacterial activity against 7 bacterial pathogens. Plant growth promotion traits including starch hydrolysis, phosphate solubilization, nitrogen fixation, and indole-3 acetic acid (IAA) production were screened among the endophytic bacteria isolates. Molecular identification by 16S rRNA gene sequencing was performed for isolates with good antibacterial activity and plant growth promotion traits. A total of 33 endophytic bacteria comprising 27 Gram-negative and 6 Gram-positive bacteria were isolated. The antibacterial activity was demonstrated by 7 isolates in which R1L3 and TKL2 extracts exhibited significant activity against *Bacillus cereus*, *Escherichia coli,* and *Proteus vulgaris*. Production of IAA was exhibited by 15 isolates wherein R1S4 produced the highest IAA (20.62 µg/mL). Analysis of the 16S gene sequence revealed that RIR2, TKS2/R1L3, and R1S4/R1S5 belonged to *Methylobacterium radiotolerans*, *Mycobacteroides abscessus,* and *Sphingomonas sp*, respectively. The findings from this study showed that Nam-Nam plants harbored endophytic isolates with the potential to be established as a source of natural compounds that can be used to develop new anti-infection agents in the future. This is the first study to report on antibacterial activity and IAA production by endophytic bacteria from the Nam-Nam plants.

Key words: Nam-Nam plants, *Cynometra cauliflora*, antimicrobial activity, indole-3-acetic acid

INTRODUCTION

Endophytes are plant-beneficial microorganisms that colonize the healthy internal tissues of living plants and develop mutualistic interaction with the plants. Endophytes are considered essential components of plant micro-bionetwork which provide several benefits to their host both directly and indirectly. The indirect growth-promoting attributes include suppressing plant diseases, induction of plant systemic resistance, protection against pests and herbivores, and phytoremediation. Several direct growth promotion effects are the production of phytohormones, alleviating abiotic stress, bio-fertilization, and plant nutrient availability (Eid *et al*., 2021). Endophytic bacteria have been identified from diverse types of

* To whom correspondence should be addressed

plant hosts. Nearly 300,000 existing species of plants are a host to one or more bacterial endophytes (Ryan *et al*., 2008). It is also believed that there is not a single plant species devoid of endophytes, including agronomic crops, prairie plants, and plants growing in extreme environment (Partida-Martinez & Heil, 2011; Yuan *et al*., 2014). Over the years, endophytic bacteria have been isolated from different parts of the plants, with roots as the main source of interest compared to above-ground tissues such as stems, leaves, nodules, and fruits (Senthilkumar *et al*., 2011). However, only a few of these plants are completely studied relative to their endophytic biology. Consequently, the opportunity to find new and beneficial endophytic microorganisms among the diversity of plants in different ecosystems is considerable.

The mutualistic relationship between endophytes and their hosts confers significant advantages for both parties and the environment where the impact of chemical fertilizers utilization can be reduced. A study done by Reay *et al*. (2012) revealed only 0.17% of chemical fertilizers were utilized in agriculture while the remainder accumulated in nature. Endophytic bacteria are believed to reduce the need for fertilizer in growing plants and potentially create a more sustainable farming method to combat the consequences of naturally damaging fertilizers. In addition, plant growth enhancement by endophytes was reported to be associated with the capability of the bacteria to synthesize phytohormones, specifically indole-3-acetic acid (IAA) (Khan *et al*., 2016). IAA increases the number of roots and improves root exudation, making them more accessible to soil nutrients.

Malaysia is a well-known country for its botanical diversity and ranks among the richest rainforest in the world. With 2,000 species of plants in the Malaysian rainforest (Lockard *et al*., 2021), the exploitation of flora for mainly economic growth has been robust. *Cynometra cauliflora* also commonly known as Nam-Nam or *katak puru* (Malay name) is one of the indigenous fruit trees found in Peninsular Malaysia. Interestingly, different parts of the Nam-Nam plant have different bioactive compounds with distinct bioactivities including leaves and fruits, revealing promising medicinal effects. The leaves contain active compounds such as flavonoids, glycosides, saponins, and terpenoids and are traditionally used to treat hyperlipidemia and diabetes (Aziz *et al*., 2013). Nam-Nam fruits possess antiproliferative activity by inhibiting the cytotoxic effect of leukemia cells (Tajudin *et al*., 2012). Moreover, fruit extracts have been suggested to possess antioxidant effects (Rabeta & Faraniza, 2013) and antifungal activity against the human pathogen (Ong *et al*., 2018). Although Nam-Nam plants have been well-studied concerning their phytochemical constituents and pharmacological properties, their microbiome and the physiological interactions between host and microbes remain poorly understood. In addition, the pharmacological properties of endophytic bacteria specifically from Nam-Nam plants are still not well-established and reports on the diversity of endophytic bacteria from Nam-Nam plants have not been documented to date. Thus, the present study aimed to determine the antibacterial activity of the ethyl acetate extracts and the production of plant growth promotion traits of endophytic bacteria isolated from different parts of Nam-Nam plants. The potential endophytes with significant bioactivities were further identified by 16S ribosomal RNA (rRNA) sequencing analysis and the respective identity was determined by Basic Local Alignment Search Tool (BLAST) analysis.

MATERIALS AND METHODS

Isolation and characterization of endophytic bacteria

Three samples of Nam-Nam plants were used in this study comprising one plant from Tanjong Karang, Selangor (3º25’40.7” N 101º10’44.9” E) and two plants from Rompin, Pahang (2º58’05.8” N 102º59’22.5” E). The plant specimens were authenticated by Forest Research Institute Malaysia (FRIM) and deposited at the FRIM herbarium. The voucher specimen number is PID 060422-05 for Tanjong Karang and PID 050422-05 for the Rompin plant respectively. Each plant part including leaves, stems, and roots was detached from the original tree with a sterile knife. The samples were then transferred into sterile bags and kept at 4 °C before being processed.

The Nam-Nam plant parts were washed with sterile distilled water before surface sterilization was performed on 2–3 cm pieces of tissue as described by Akinsanya *et al*. (2015). The samples were immersed in 90% ethanol for 5 min, followed by sodium hypochlorite (3%) for 2 min, and 75% ethanol for 3 min. Finally, the samples were thoroughly rinsed three times with double distilled water. Complete disinfection was checked after streaking 50 µL of the last rinsing water on Nutrient Agar (NA) at 30 °C for 48 h. Four different growth media including nutrient agar (NA), Reasoner’s 2A agar (R2A), tryptone soy agar (TSA), and Luria-Bertani agar (LB) were used for the isolation of endophytic bacteria. The media were incubated for 3-7 days at 30 °C. The selected colonies were subcultured repeatedly until pure cultures were established. The isolated endophytes were examined for their macroscopic and microscopic characteristics including colony morphology, cell morphology, and Gram reaction by standard method.

Extraction of extracellular metabolite compound

The extraction of extracellular secondary metabolites was performed as described by Akinsanya *et al*. (2015). Endophytic bacteria isolates were

cultured in 30 mL nutrient broth and incubated at 30 °C for 24 h at 150 rpm followed by centrifugation at 8000 g for 5 min. The cell-free supernatant was collected and an equal volume of ethyl acetate (ratio 1:1) was added. The solvent mixture was shaken at 150 rpm for 2 h before allowing the phases to separate at room temperature. The organic layer (top layer) containing secondary metabolites was transferred into a pre-weighed universal bottle and concentrated in a rotatory evaporator at 37 °C to dryness. The dried ethyl acetate extracts were re-dissolved in 5% DMSO and filtered with 0.22 µm filtration membrane to obtain a final concentration of 20 mg/mL.

Antibacterial activity of ethyl acetate bacterial extracts

The antibacterial activities of ethyl acetate extracts were tested against seven bacterial pathogenic strains such as *Staphylococcus aureus* ATCC 35556, *Bacillus cereus* ATCC 11778, *Salmonella Typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 6380, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli ATCC* 1129 and *Staphylococcus epidermidis* ATCC 12228 using Kirby-Bauer disc diffusion method (Akinsanya *et al*., 2015). An overnight culture of the bacterial pathogens was prepared in Mueller-Hinton broth at 35 °C and 5 mL of the culture was centrifuged at 6000 ×*g* for 5 min. The pellets were re-suspended in sterile distilled water and the density was adjusted to 0.5 McFarland standards. The suspension was seeded onto Mueller-Hinton agar plates for antibacterial testing. The extract (20 mg/mL) was impregnated onto sterile discs and placed on seeded agar plates. The plates were then incubated at 35 °C for 24-48 h and the zone of inhibition was determined by measuring the diameter of the annular clear zone. The experiment was performed in triplicates. Ampicillin (100µg/mL) and 5% DMSO were used as positive and negative controls, respectively.

Determination of Plant Growth-Promoting (PGP) traits

The PGP traits of the endophytic bacteria, including phosphate solubilization, nitrogen fixation, extracellular amylase activity, and IAA production were qualitatively determined according to Xu *et al*. (2019) and Bambharolia *et al*. (2020).

One purified endophytic bacteria colony on LB agar was inoculated into LB medium and incubated at 30 °C for 3 days at 180 rpm. An amount of 10 µL of each culture was spotted on Pikovskaya's (PVK) agar medium containing tricalcium phosphate (Ca3[PO4]2) and nitrogen-free (NFM) agar medium for evaluation of phosphate solubilization and nitrogen fixation, respectively. These activities were qualitatively evaluated by the presence of a transparent zone around the bacterial colony after 7 days of incubation at 30 °C.

The amylase activity was evaluated by a starch hydrolysis test. The consumption of starch by endophytic bacteria was assessed as described by Minotto *et al*. (2014) with modification. In general, the isolate was streaked onto a starch agar media containing 0.2% soluble starch. After incubation for 5 days at 35 °C, 10 mL of Gram's iodine solution was poured onto bacterial colonies. Amylase production was detected as a transparent halo around the colony that typically signals starch hydrolysis.

The production of IAA was assayed according to Tashi-Oshnoei *et al*. (2017) with slight modification. A colony of the endophytic bacterial isolate was inoculated into yeast mannitol broth with tryptophan medium and incubated in the dark at 30 °C for 7 days. The culture was then centrifuged at 3000 rpm for 30 min and 2 mL of supernatant was mixed with 2 drops of orthophosphoric acid before adding 4 mL of Salkowski's reagent. The mixture was allowed to stand for 15 min. The intensity of the rose color produced was measured using a spectrophotometer at 530 nm.

Molecular identification by 16S rRNA gene sequence

The endophytic bacteria isolate with the best antimicrobial activity and plant growth-promoting (PGP) traits were sent for 16S rRNA gene sequencing to a third-party service provider. Sequences were analyzed and compared to online databases by using the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). As for nucleotide sequence, the database used was non-redundant nucleotide collection (nr). Sequences with high homology (more than 98%), low E value (less than E^{-10}), and possessed conserved regions in the multi-sequence alignments were selected to establish the closest sequence matches.

Statistical analysis

The data from the antibacterial activity of endophytic bacterial extracts against selected pathogens were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Version 25. The significance level was set at $p<0.05$. The values were expressed as the mean standard deviation (SD) of three replicates.

RESULTS AND DISCUSSION

Isolation of endophytic bacteria from Nam-Nam plants

A total of 33 isolates were obtained in this study comprising 11 isolates from Tanjong Karang (TK), 12 isolates from Rompin 1 (R1), and 10 isolates from Rompin 2 (R2) plant samples respectively (Table 1). No colonies were observed from the final

rinse of the sterilization process indicating that the surface sterilization effectively removed all surface-adhering microorganisms (Coombs & Franco, 2003). The isolates were further classified according to their macroscopic and microscopic characteristics and their Gram stain and cell shape were determined (Table 2).

In the present study, endophytic bacteria were successfully isolated from R2A (16/33, 48.5%), followed by NA and TSA (6/33, 18.2%), and lastly LB (5/33,15%). This shows that R2A agar is the best media for the isolation of bacterial endophytes from Nam-Nam plants. According to Parray *et al*. (2021), R2A agar contains casein, dextrose, starch, and other ingredients that stimulate the growth of stressed and fastidious bacteria. In terms of plant parts, roots produced the highest endophytic bacteria (13/33, 39.4%) followed by stems (11/33, 33.3%) and leaves (9/33, 27.3%) respectively. Several other studies also demonstrated that the population density of the endophytic bacteria was more significant in roots than in other plant parts (Akinsanya *et al*., 2015; Gupta *et al*., 2015; Li *et al*., 2018). Hence, the plant's root is favored for the colonization of associated endophytic bacteria. It is not surprising that the population of endophytic bacteria in leaves and shoots overlaps with those in roots because bacterial endophytes ascend from roots to upper parts of the plant via the apoplast in xylem vessels.

Table 2 showed that the population of Gram-negative bacteria was higher (27/33, 81.8%) than Gram-positive (6/33, 18.2%) which deduced that endophytic bacteria from Nam-Nam plants are dominantly comprised of Gram-negative bacteria. The high population of Gram-negative endophytic bacteria was also reported by Gagne-Bourgue *et al*. (2013), Akinsanya *et al*. (2015), and Afzal *et al*. (2019).

Antibacterial activity of endophytic bacteria extracts from Nam-Nam plants

Only seven of the 33 isolates showed positive antibacterial activities toward at least one pathogen at 20 mg/mL concentration of ethyl acetate extracts (Table 3). Crude extracts from TKL1, TKS3, and TKR2 isolates showed inhibition activity against *B. cereus* only whereas extracts from the R1L2 isolate were only capable to inhibit *E. coli* growth (8.67 ± 0.29 mm). None of the crude extracts from Nam-Nam plants inhibit *K. pneumoniae* and *S. epidermidis* in this study (results not shown). The best antibacterial activity was observed in the crude extract of R1L3 isolate which inhibited five different pathogens with the highest activity against *S. typhimurium* (10.83 ± 1.04 mm) and *S. aureus* (10.67 ± 0.58 mm) respectively.

Table 1. Distribution of endophytic bacteria from different parts of Nam-Nam plants based on different growth media

Plant parts	Nutrient agar (NA)	Luria Bertani agar (LB)	Tryptone soy agar (TSA)	Reasoner's 2A agar (R2A)	Total Isolates
Tanjong Karang (TK)					
Leaves (L)	1	1	-	1	3
Stems (S)	1	1	1	1	4
Roots (R)	1	1	1	1	4
Rompin 1 (R1)					
Leaves (L)	1	1	1	-	3
Stems (S)	-	1	-	4	5
Roots (R)	-	-	1	3	4
Rompin 2 (R2)					
Leaves (L)	-	-	-	3	3
Stems (S)	-	-	1	1	2
Roots (R)	2	-	1	2	5
Total Isolates	6	5	6	16	33

Table 2. Distribution of endophytic bacteria of Nam-Nam plants based on Gram reaction and cell morphology

Gram Reaction and Cell Morphology	Isolates	Total
Gram-negative, rod	TKL1, TKL3, TKR4, R1L1, R1S4, R2S2, R2R2, TKS2, TKR3, R1L2, R1S1, R1R1, R2S1, R2R3, TKL2, TKS4, TKR1, R1S3, R1R4, R2L2, R2R4, R1S2, R1R3, R2L1, R2R1	25
Gram-negative, coccus	R1S5, TKS1	2
Gram-positive, rod	TKS3, TKR2, R1L3, R1R2, R2L3, R2R5	6

Plant growth promotion traits of endophytic bacteria from Nam-Nam plants

The results revealed that 18/33 (54.5%) isolates were capable of producing extracellular amylase enzymes and diffusing into the starch agar. Four isolates (12.1%) possessed phosphate solubilization, whereas nitrogen fixation activity was detected in 7 isolates (21.2%) (Figure 1). A total of 15 IAA-producing bacterial endophytes are listed in Table 4 with their corresponding plant growth-promoting traits included. The concentration of IAA by these isolates ranged between 0.14 – 20.62 µg/mL and R1S4 isolate yielded the highest concentration (20.62 µg/mL), followed by TKS2 (14.44 µg/mL) and R2R1 (12.05 µg/mL). Among the 15 isolates with IAA production, only R1S4 and R1S5 isolates showed all three plant growth promotion traits. Xu *et al*. (2019) reported a higher nitrogen fixation (63.6%) and phosphate solubilization (48.5%) activity from endophytic bacteria isolated from mulberry plants. The results revealed that mulberry endophytic bacteria possess high PGP potential and might be good candidate strains as biofertilizers.

Identification of bacterial endophytes by 16S rRNA gene sequence and phylogenetic tree analysis

In this study, five isolates with promising antimicrobial activities and plant growth-promoting traits (TKL2, TKS2, R1L3, R1S4, & R1S5) were

Table 3. Antibacterial activity of ethyl acetate crude extracts of endophytic bacteria from Nam-Nam plants

	Zone of Inhibition (mm)				
Isolates	*B. cereus*	*E. coli*	*P. vulgaris*	*S. typhimurium*	*S. aureus*
TKL1	6.67 ± 0.58	-	-	-	-
TKL2	8.83 ± 0.76	7.00 ± 0.50	8.00 ± 0.87	-	-
TKS2	6.67 ± 1.15	10.33 ± 0.58	-	-	-
TKS3	7.33 ± 1.15	-	-	-	-
TKR2	8.33 ± 0.58	-	-	-	-
R1L2	-	8.67 ± 0.29	-	-	-
R1L3	8.83 ± 0.29	7.00 ± 0.50	9.00 ± 0.00	10.83 ± 1.04	10.67 ± 0.58
AMP	15.33 ± 0.58	33.67 ± 1.53	10.5 ± 1.32	16.00 ± 0.00	21.83 ± 1.61

The zone of inhibition diameter was measured in means of three replicates and expressed as mean ± SD. All values of inhibition zone (mm) are significantly different at p<0.05. Ampicillin (AMP) (100 ug/mL) is used as the positive control and "-"denotes no activity.

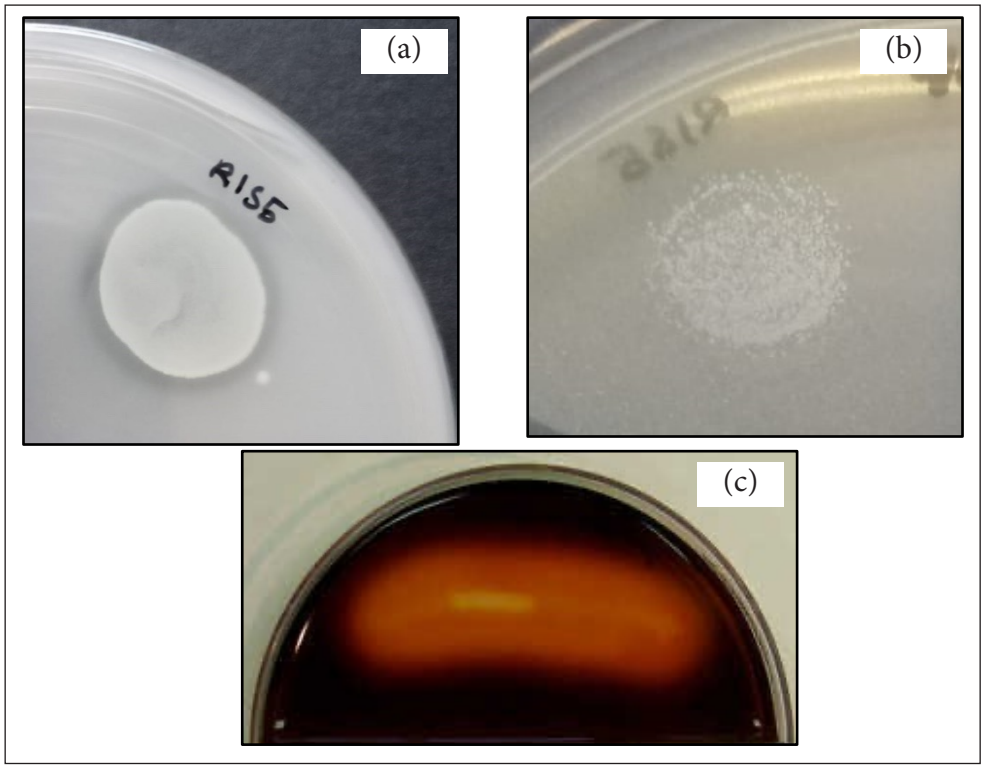

Fig. 1. Representative reactions for starch hydrolysis test and plant growth promotion traits of endophytic bacteria from Nam-Nam plants. (a) Growth on Pikovkaya's agar, (b) Growth on nitrogen-free agar, and (c) Starch hydrolysis test.

selected for identification by 16S rRNA gene sequencing, and three identifications of endophytic bacteria were obtained (Table 5). The BLAST identified TKL2 as *Methylobacterium radiotolerans*. Studies by Aleynova *et al*. (2021) and Darkazanli and Kiseleva (2020) also isolated *Methylobacterium* sp. from wild grapes and beans, respectively. Both isolates TKS2 and R1L3 were identical and belonged to *Mycobacteroides abscessus* strain. A study by Fang and Hsu (2012) also reported *Mycobacteroides abscessus* isolated from Aglaonema plants. A similar identity result for R1S4 and R1S5 showed that they belong to *Sphingomonas* sp. An endophytic bacteria identified as *Sphingomonas dokdonensis* had been isolated from french bean (*Phaseolus vulgaris*) plants by de Oliveira Costa *et al*. (2018). Both isolate R1S4 and R1S5 in this study produced the highest level of IAA. A study by Khan *et al*. (2014) recorded high production of IAA by *Sphingomonas* sp. LK11 (11.23 + 0.93 µm/mL), which was lower than the present study. Tomato plants inoculated with *Sphingomonas* sp. LK11 resulted in significantly enhanced growth. The same strain of *Sphingomonas* sp. was also observed to promote the shoot and root length of the soybean plant (Asaf *et al*., 2017). Higher production of IAA was reported in a study by Sukweenadhi *et al*. (2015), where *Sphingomonas panaciterrae* was found to produce 33.73 + 4.66 µg/mL of IAA.

CONCLUSION

In conclusion, this study revealed that endophytic bacteria from Nam-Nam plants were mostly Gram-negative bacteria and these isolates produced bioactive compounds with good antimicrobial and PGP traits. Three bacterial identities known as *Methylobacterium radiotolerans*, *Mycobacteroides abscessus,* and *Sphingomonas sp*. have the most potential for the development of effective antimicrobial and PGP compounds using endophytic strains for pharmaceutical and agricultural applications in the future.

Table 4. Characterization of extracellular amylase enzyme production and plant growth-promoting (PGP) traits of endophytic bacteria from Nam-Nam plant

Isolate	Starch Hydrolysis	Growth on Pikovkaya's Agar	Growth of Nitrogen Free Agar	IAA Production (µg/mL)
R1S4	+	+	+	20.62
TKS2	-	-	-	14.44
R1S5	+	+	+	12.05
TKL2	-	+	+	11.97
R1S3	+	-	-	10.25
R1R1	-	-	-	9.46
TKS3	+	-	-	7.44
R1S1	-	-	-	6.39
R1L3	+	-	+	4.74
R1S2	-	-	-	4.14
R2S1	-	-	-	3.32
R1L2	-	-	-	2.57
R1R3	-	-	-	1.86
R2R4	+	-	-	1.63
R1L1	-	-	-	0.14

Summary of activities in which "+" denotes the presence of the specific activity and "-" denotes no activity

Table 5. BLAST analysis results for identification of endophytic bacteria from Nam-Nam plants

Isolates	Description	Query Coverage	E Value	Identity
TKL2	*Methylobacterium radiotolerans* strain KCOM 1463 (= ChDC B635) 16S ribosomal RNA gene, partial sequence MT261799.1	100%	0	99.87%
TKS2 &R1L3	*Mycobacteroides abscessus* 16S ribosomal RNA, partial sequence NR_074427.1	100%	0	100%
R1S4 & R1S5	*Sphingomonas* sp. MG49 partial 16S rRNA gene, isolate MG49 AJ746106.1	100%	0	100%

ACKNOWLEDGEMENTS

This research is funded by Universiti Teknologi MARA under Lestari Grant (600-IRMI 5/3/LESTARI (063/2019)). We would like to thank Dr. Suhaidi Ariffin for providing the plant samples for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Afzal, I., Khan, Z.S., Sikandar, S. & Shahzad, S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiological Research*, **221**: 36–49. https://doi.org/10.1016/j.micres.2019.02.001

Akinsanya, M.A., Goh, J.K., Lim, S.P. & Ting, A.S.Y. 2015. Diversity, antimicrobial and antioxidant activities of culturable bacterial endophyte communities in *Aloe vera*. *FEMS Microbiology Letters*, **362(23)**: 1–8. https://doi.org/10.1093/femsle/fnv184

Aleynova, O.A., Nityagovsky, N.N. & Kiselev, K.V. 2021. Biodiversity of endophytic bacteria and fungi of wild grapes *Vitis amurensis* Rupr. *BIO Web of Conferences*, **39**: 1-8. https://doi.org/10.1051/bioconf/20213905001

Asaf, S., Khan, M.A., Khan, A.L., Waqas, M., Shahzad, R., Kim, A.Y., Kang, S.M. & Lee, I.J. 2017. Bacterial endophytes from arid land plants regulate endogenous hormone content and promote growth in crop plants: An example of *Sphingomonas* sp. and *Serratia marcescens*. *Journal of Plant Interactions*, **12(1)**: 31–38. https://doi.org/10.1080/17429145.2016.1274060

Aziz, A., Farina, A. & Iqbal, M. 2013. Antioxidant activity and phytochemical composition of *Cynometra cauliflora*. *Journal of Experimental and Integrative Medicine*, **3(4)**: 337–341. https://doi.org/10.5455/jeim.250813.or.086

Bambharolia, R.P., Khunt, M.D., Deshmukh, A.J., Prajapati, V.P. & Vavdiya, P.A. 2020. Isolation, screening and characterization of endophytic bacteria from root of finger millet (*Eleusine coracana* (L.) for different plant growth promotion (PGP) activities: An *in-vitro* study. *Journal of Pharmacognosy and Phytochemistry*, **9(5)**: 539-545

Darkazanli, M. & Kiseleva, C.I. 2020. The effects of inoculation beans by endophytic bacteria *Methylobacterium* sp., and *Bacillus subtilis*. *Актуальные вопросы органической химии и биотехнологии. Екатеринбург*, **2020**: 525-527.

de Oliveira Costa, L.E., Correa, T.L.R., Teixeira, J.A., de Araujo, E.F. & de Queiroz, M.V. 2018. Endophytic bacteria isolated from *Phaseolus vulgaris* produce phytases with potential for biotechnology application. *Brazilian Journal of Biological Sciences*, **5(11)**: 657–671. https://doi.org/10.21472/bjbs.051105

Eid, A.M., Fouda, A., Abdel-Rahman, M.A., Salem, S.S., Elsaied, A., Oelmüller, R., Hijri, M., Bhowmik, A., Elkelish, A. & Hassan, S.E.D. 2021. Harnessing Bacterial Endophytes for Promotion of Plant Growth and Biotechnological Applications: An Overview. *Plants*, **10(5)**: 935. https://doi.org/10.3390/plants10050935

Fang, J.Y. & Hsu, Y.R. 2012. Molecular identification and antibiotic control of endophytic bacterial contaminants from micropropagated *Aglaonema* cultures. *Plant Cell, Tissue and Organ Culture*, **110**: 53–62.

Gagne-Bourne, F., Aliferis, K.A., Seguin, P., Rani, M., Samson, R. & Jabaji, S. 2013. Isolation and characterization of indigenous endophytic bacteria associated with leaves of switchgrass *(Panicum virgatum* L.) cultivars. *Journal of Applied Microbiology*, **114(3)**: 1–18. https://doi.org/10.1111/jam.12088

Gupta, R.M., Kale, P.S., Rathi, M.L. & Jadhav, N.N. 2015. Isolation, characterization and identification of endophytic bacteria by 16S rRNA partial sequencing technique from roots and leaves of *Prosopis cineraria* plant. *Asian Journal of Plant Science and Research*, **5(6)**: 36–43.

Khan, A.L., Halo, B.A., Elyassi A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A. & Lee, I-J. 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. *Electronic Journal of Biotechnology*, S0717345816000245. https://doi.org/10.1016/j.ejbt.2016.02.001

Lockard, C.A., Ahmad, Z., Ooi, J.B. & Leinbach, T.R. 2021. Plant and animal life. In *Encyclopaedia Britannica*. Retrieved from https://www.britannica.com/place/Malaysia (accessed 25.08.2021)

Minotto E., Milagre L.P., Oliveira M.T. & Van Der Sand S.T. 2014. Enzyme characterization of endophytic actinobacteria isolated from tomato plants. *Journal of Advanced Scientific Research*, **5(2)**: 16-23.

Ong, C.W., Chan, Y.S., Khoo, K.S., Ong, H.C. & Sit, N.W. 2018. Antifungal and cytotoxic activities of extracts obtained from underutilised edible tropical fruits. *Asian Pacific Journal of Tropical Biomedicine*, **8(6)**: 313–319. https://doi.org/10.4103/2221-1691.235326

Partida-Martinez, L. & Heil, M. 2011. The microbe-free plant: Fact or artifact? *Frontiers in Plant Science*, **2**: Article 100. https://doi.org/10.3389/fpls.2011.00100

Rabeta, M.S. & Faraniza, N. R. 2013. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis*

and *Cynometra cauliflora*. *International Food Research Journal*, **20(4)**: 1691–1696.

Reay, D.S., Davidson, E.A., Smith, K.A., Smith, P., Melillo, J.M., Dentener, F. & Crutzen, P.J. 2012. Global agriculture and nitrous oxide emissions. *Nature Climate Change*, **2(6)**: 410–416. https://doi.org/10.1038/NCLIMATE1458

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J. & Dowling, D.N. 2008. Bacterial endophytes: Recent developments and applications. *FEMS Microbiology Letters*, **278(1)**: 1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Senthilkumar, M., Anandham, R., Madhaiyan, M., Venkateswaran, V. & Sa, T. 2011. Endophytic bacteria: Perspectives and applications in agricultural crop production. In: *Bacteria in Agrobiology: Crop Ecosystems*. D.K. Maheshwari (Ed.). Springer-Verlag, Berlin, Heidelberg. pp. 61–96. https://doi.org/10.1007/978-3-642-18357-7_3

Tajudin, T-J.S.A., Mat, N., Abu Bakar, S-A., Yusran, A.A.M., Alwi, A. & Ali, A.M. 2012. Cytotoxicity, antiploriferative effects, and apoptosis induction of methanolic extract of *Cynometra cauliflora* Linn. whole fruit on human promyelocytic leukemia HL-60 Cells. *Evidence-Based Complimentary and Alternative Medicine*, **2012**: Article ID 127373. https://doi.org /10.1155/2012/127373

Tashi-Oshnoei, F., Harighi, B., & Abdollazadeh, J. 2017. Isolation and identification of endophytic bacteria with plant growth promoting and biocontrol potential from oak trees. *Forest Pathology*, **47(5)**: e12360. https://doi.org/10.1111/efp.12360

Xu, W., Wang, F., Zhang, M., Ou, T., Wang, R. & Strobel, G. 2019. Diversity of cultivable endophytic bacteria in mulberry and their potential for antimicrobial and plant growth-promoting activities. *Microbiological Research*, **229**: 126328. https://doi.org/10.1016/j.micres.2019.126328

Yuan, M., He, H., Xiao, L., Zhong, T., Liu, H., Li, S., Deng, P., Ye, Z. & Jing Y. 2014. Enhancement of Cd phytoextraction by two *Amaranthus* species with endophytic *Rahnella* sp . JN27. *Chemosphere*, **103**: 99–104. https://doi.org/10.1016/j.chemosphere.2013.11.040