ACADEMIA JOURNAL OF BIOLOGY 2021, 43(3): 125–135
DOI: 10.15625/2615-9023/16143

IN VITRO ANTIOXIDANT, α-AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITIES OF ENDOPHYTIC BACTERIA FROM THE ROOTS OF THE MANGROVE PLANT *Rhizophora stylosa* Griffith

Ton That Huu Dat*, Phung Thi Thuy Oanh

Mientrung Institute for Scientific Research, VAST, Vietnam

Received 17 June 2021, accepted 16 September 2021

ABSTRACT

Mangrove is one of the highly productive ecosystems and contains diverse plants and microbial communities. Bacterial endophytes from mangroves are considered as a prolific source of biological molecules with important functions in the protection of mangrove plants against herbivores, insects as well as pathogens. The present study aimed to isolate endophytic bacteria from the roots of mangrove plant *Rhizophora stylosa* and to screen antioxidant, α-amylase and α-glucosidase inhibitory activities of ethyl acetate extracts from the isolated endophytic bacteria. A total of 73 endophytic bacterial strains from *R. stylosa* roots were isolated, of which ethyl acetate extracts of 10 isolated endophytic strains at a concentration of 500 µg/mL showed antioxidant activity with ATBS and DPPH radical scavenging values ranging from 31.3 ± 2.5% to 62.2 ± 6.3% and 21.2 ± 3.2% to 56.7 ± 4.5%, respectively. Additionally, the ethyl acetate extracts of 11 isolated endophytic bacteria at a concentration of 500 µg/mL exhibited α-amylase and α-glucosidase inhibitory activities with values ranging from 31.4 ± 3.1% to 59.7 ± 6.4% and 17.3 ± 3.1% to 54.5 ± 6.1%, respectively. The identification of the five high antioxidant, α-amylase and α-glucosidase inhibitory endophytes by 16S rRNA sequences revealed that the bacterial endophytes belonged to three genera, including *Bacillus*, *Streptomyces*, and *Pseudovibrio* with the similarity of more than 99%. The obtained results suggest that the root endophytic bacteria of *R. stylosa* are a source of antioxidant and antidiabetic agents. To the best of our knowledge, this is the first report of antioxidant, α-amylase and α-glucosidase activities of the endophytic bacteria from the roots of Vietnam mangrove plants in general and *R. stylosa* in particular.

Keywords: *Rhizophora stylosa*, α-amylase inhibition, α-glucosidase inhibition, antioxidant activity, endophytic bacteria, mangrove.

Citation: Ton That Huu Dat, Phung Thi Thuy Oanh, 2021. *In vitro* antioxidant, α-amylase and α-glucosidase inhibitory activities of endophytic bacteria from the roots of the mangrove plant *Rhizophora stylosa* Griffith. *Academia Journal of Biology*, 43(3): 125–135. https://doi.org/10.15625/2615-9023/16143

**Corresponding author email*: huudat96@gmail.com

INTRODUCTION

Diabetics or diabetes mellitus is a chronic metabolic disorder that is characterized by high blood glucose levels. Currently, diabetics continue to be a crucial medical concern worldwide due to their high prevalence and potentially deleterious effects. Postprandial hyperglycemia is a key factor in the stimulation of type 2 diabetes and its complications. Therefore, one of the therapeutic approaches for diabetics, especially type 2, is to decrease postprandial hyperglycemia and maintain normal blood glucose levels (Chakrabarti & Rajagopalan, 2002). Alpha glucosidase and alpha amylase are the crucial enzymes involved in the digestion of carbohydrates in the body. These enzymes are involved in the breakdown of long-chain carbohydrates, starch, and disaccharides to glucose. As a result, they can cause an increase in glucose level in the blood and lead to diabetics. In such conditions, α-amylase and α-glucosidase inhibitory agents are considered as the potential targets for the development of lead compounds for the diabete treatment (Subramanian et al., 2008).

Free radicals, such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) are highly reactive oxygen species and naturally produced by cell metabolism in the body. However, excess production of free radicals leads to oxidative stress, which can stimulate a number of diseases (e.g., carcinogenesis, cardiovascular disease, inflammatory diseases, atherosclerosis, and ageing) (Pizzino et al., 2017). Furthermore, numerous experimental evidences have indicated that oxidative stress plays a pivotal role in the progression of diabetes and its complications, both microvascular and cardiovascular (Giacco & Brownlee, 2010; Maiese, 2015; Oguntibeju, 2019). In such conditions, antioxidants may provide protective effects against oxidative stress by scavenging free radicals and remove the harmful effects of oxidative stress (Neha et al., 2019).

The mangrove ecosystem is one of the highly productive ecosystems and provides unique environmental characteristics for developing diverse groups of microorganisms (Thatoi et al., 2013). Recent studies reveal that the endophytic bacteria from mangrove plants are an important source of bioactive secondary metabolites. The secondary metabolites derived from endophytes of mangrove plants are structurally diverse and pharmacologically active. These known compounds from mangrove plant endophytes include terpenes, chromones, coumarins, polyketides, alkaloids, and peptides (Kui-Wu et al., 2014; Xu, 2015; Ancheeva et al., 2018; Zhou et al., 2018; Manganyi et al., 2019). These compounds exhibit a broad spectrum of biological properties such as antioxidant, antidiabetic, cytotoxic, antifungal, antibacterial, acetylcholinesterase, antivirus (Kui-Wu et al., 2014; Xu, 2015; Ancheeva et al., 2018; Zhou et al., 2018; Manganyi et al., 2019; Dat et al., 2019a,b).

With a length of coastline up to 3260 km, coastal mangroves are among the most productive and biologically important ecosystems in Vietnam. Regarding medicinal potential from mangrove microorganisms in Vietnam, a few studies on the antimicrobial and antioxidant activities of mangrove microorganisms have been reported (Dat et al., 2019a,b; Hong & Phuong, 2013), however, there is no report on antioxidant, α-amylase and α-glucosidase inhibitory activities of mangrove microorganisms. Herein, the present study isolated and screened antioxidant, α-amylase and α-glucosidase inhibitory bacterial endophytes from roots of *Rhizophora stylosa* for potential medicinal sources from Vietnamese mangrove endophytic bacteria.

MATERIALS AND METHODS

Sample plant collection

The plant *Rhizophora stylosa* was collected in the mangrove forest in Tan Xuan village, Gio Viet commune, Gio Linh district, Quang Tri province. The samples were contained in sterile bags and transferred immediately to the laboratory for isolation of bacterial endophytes.

Isolation of endophytic bacteria from roots of *R. stylosa*

The fresh plant roots were washed in running water to remove soil particles. The sample surface was then sterilized by sequential immersion in 70% ethanol for 5 min and sodium hypochlorite for 10 min. The samples were then washed three times in sterile distilled water to remove surface sterilizing agents before being soaked in 10% sodium bicarbonate (Dat et al., 2019). The samples were cut into small pieces (1–2 cm) and placed on Nutrient Agar (NA, Himedia, India) for isolation of bacteria and ISP medium No. 4 (ISP4, Himedia, India) for isolation of actinomycetes. The plates were incubated for 3–5 days at 37 ^{o}C for the growth of endophytic bacteria. Representative bacterial isolates with different colony morphotypes were selected, cultured, and stored with 20% glycerol (v/v) at -80 ^{o}C.

Preparation of ethyl acetate extracts

The endophytic bacterial strains were cultivated in 500 mL nutrient broth (NB, Himedia, India) at 37 ^{o}C, 150 rpm for 7 days and then the cultures were centrifuged at 10.000 rpm for 10 min. The cell-free supernatants were extracted with ethyl acetate (1:1 v/v, 5 times) overnight at room temperature, then ethyl acetate extractions were evaporated under reduced pressure (12–24 hours, 50 ^{o}C) to remove ethyl acetate and obtain the crude extracts.

***In vitro* antioxidant activity of the ethyl acetate extracts**

ABTS radical scavenging assay

The ABTS radical scavenging activity of the extracts was determined by measuring the decrease in absorbance of the ABTS radical solution in the presence of the extracts. Briefly, two solutions ABTS 7 mM (5 mL) and potassium persulfate 2.45 mM (88 µL) were mixed and allowed to stand in the dark at room temperature for 16 hours before use in order to produce ABTS radical solution. The ABTS radical solution was then diluted with ethanol (~100 times) to give an absorbance of 0.700 ± 0.02 at 734 nm. Ten microliters of each extract (500 µg/ml) were added to 190 µL of ABTS radical solution in 96 well plates. The solution was incubated at room temperature for 10 min and then the absorbance of the reaction was recorded at 734 nm on a microplate reader. Ascorbic acid was used as the positive control. The ABTS radical scavenging activity was calculated as follow: ABTS scavenging activity (%) = 100 × [(Ac – As)/(Ac – Ab)], where Ac is the absorbance of the control, As is the absorbance of the extract and Ab is the absorbance of the blank.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the extracts was determined by measuring the decrease in absorbance of DPPH radical solution in the presence of the extracts. Briefly, 10 µL of each extract (500 µg/mL) was added to 190 µL of DPPH (0.1 mg/mL) in 96 well plates. The solution was mixed for 1 min and incubated at room temperature for 30 min. Then the absorbance of the reaction mixture was recorded at 517 nm on a microplate reader. Ascorbic acid was used as the positive control. The DPPH radical scavenging activity was calculated as follow: DPPH scavenging activity (%) = 100 × [(Ac – As)/(Ac – Ab)], where Ac is the absorbance of the control, As is the absorbance of the extract and Ab is the absorbance of the blank.

***α*-Amylase inhibitory assay**

The α-amylase inhibitory activity was carried out by hydrolysis of starch in presence of the α-amylase enzyme. Briefly, the extract (50 µL of 500 mg/mL) and starch azure (50 µL of 10 mg/mL, suspended in 0.05 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$, boiled for 5 min and then pre-incubated at 37 ^{o}C for 5 min) were mixed in 30 µL of 0.1 M phosphate buffer (pH 7.0). After 5 min preincubation, 2 U/mL α-amylase solution (25 µL) was added, and the solution was incubated at 37 ^{o}C for 15 min. The reaction was stopped by adding 75 µL of acid acetic 50% to the solution. The absorbances were measured at 650 nm by a microplate reader. Acarbose was used as a positive control. The inhibition activity was calculated as follow: *α*-amylase

inhibitory activity (%) = 100 × [(Ac – As)/(Ac – Ab)], where Ac was the absorbance of the control, As was the absorbance of the extract and Ab was the absorbance of the blank.

α-Glucosidase inhibitory assay

The α-glucosidase inhibitory assay was performed using the substrate p-nitrophenyl-α-D-glucopyranoside. Briefly, the extract (50 µL of 500 mg/mL) and 0.5 U/mL α-glucosidase (40 µL) were mixed in 70 µL of phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mM p-nitrophenyl-α-D-glucopyranoside solution (40 µL) was added and the solution was incubated at 37 ºC for 30 min. The absorbance of released p-nitrophenol was measured at 405 nm by using a microplate reader. Acarbose was used as the positive control. The inhibition activity was calculated as follow: α-glucosidase inhibitory activity (%) = 100 × [(Ac – As)/(Ac – Ab)], where Ac is the absorbance of the control, As is the absorbance of the extract and Ab is the absorbance of the blank.

Identification of the isolates by the 16S rRNA sequence

The most potential antioxidant, α-amylase and α-glucosidase inhibitory isolates were identified using 16S rRNA gene sequencing. Genomic DNA of isolates was extracted according to the protocol described by Sambrook & Russel (2001). The 16S rRNA gene of the isolates was amplified with universal primers: 27f (5′- AGAGTTTGATC CTGGCT CAG-3′) and 1492r (5′- GGTTAC CTTGTTACGACTT-3′). The PCR cycling parameters: an initial denaturation at 94 ºC for 5 min followed by 30 cycles of denaturation at 94 ºC for 1 min, annealing at 56 ºC for 50 s, amplification at 72 ºC for 1.5 min and a final extension at 72 ºC for 7 min. The PCR products were confirmed by electrophoresis in 1.5% (w/v) agarose gel. The 16S rRNA gene sequencing was carried out by using DNA Analyzer (ABI PRISM 3100, Applied Bioscience). The obtained DNA sequences were removed poor quality ends using BioEdit software v.2.7.5, and then were blasted to sequences in the GenBank database using the Blast search programme (http://www.ncbi.nlm.nih.gov/) to find their highest similarity sequences. The sequences were aligned using the ClustalW algorithm. The phylogenetic tree of 16S rRNA sequences was created by the Neighbor-Joining algorithm with 1000 bootstraps using MEGA v.7.0.0.

The 16S rRNA gene sequences of isolates are available in the NCBI database under accession numbers: MZ618710-MZ618714.

RESULTS

Isolation of endophytic bacteria from roots of *R. stylosa*

From roots of the plant *R. stylosa* collected from mangrove forest Gio Linh district, Quang Trị province, 73 endophytic bacterial strains were isolated and subcultured to get the pure isolates of which 38 strains were isolated from NA medium and 35 strains were isolated from ISP4 medium. Among them, 40 representative strains with different colony morphotypes (Table 1) were selected for preparing ethyl acetate extracts and screening antioxidant, α-amylase and α-glucosidase inhibitory activities.

Antioxidant activity of the ethyl acetate extracts of the endophytic bacteria

The antioxidant activity of ethyl acetate extracts of the endophytic bacteria was determined by the ABTS and DPPH radical scavenging assays (Fig. 1). The ABTS and DPPH radical scavenging assays showed the extracts of 10 endophytic bacteria at a concentration of 500 µg/mL exhibited ABTS and DPPH radical scavenging activities ranging from 31.3 ± 2.5% to 62.2 ± 6.3% and 21.2 ± 3.2% to 56.7 ± 4.5%, respectively. Among them, the ethyl acetate extracts of three bacterial endophytes (RSR-N9, RSR-N11, RSR-N33) showed both ABTS and DPPH radical scavenging activities more than 50%, and two bacterial endophytes (RSR-I15, RSR-I25) showed the ABTS radical scavenging activity more than 50%. The positive control (ascorbic acid at a concentration of 50 µg/mL) showed ABTS and DPPH radical scavenging activities of 88.5 ± 5.9% and 85.6 ± 6.3%, respectively.

Table 1. Colony morphology of the isolated bacteria

Strain ID	Isolation media	Colony morphology				
		Shape	Surface	Color	Margin	Elevation
RSR-I5	ISP4	Circular	Dry	White	Entire	Raised
RSR-I6	ISP4	Circular	Smooth	Cream	Entire	Flat
RSR-I8	ISP4	Circular	Rough	Dirty white	Lobate	Raised
RSR-N9	NA	Irregular	Rough	Cream	Irregular	Flat
RSR-N11	NA	Circular	Rough	Off-white	Irregular	Flat
RSR-N12	NA	Circular	Smooth	Yellow	Entire	Raised
RSR-I15	ISP4	Irregular	Dry	White	Undulate	Raised
RSR-N21	NA	Irregular	Dry	White	Filamentous	Raised
RSR-N22	NA	Circular	Smooth	Yellow	Entire	Flat
RSR-N23	NA	Irregular	Smooth	Dirty white	Entire	Raised
RSR-N24	NA	Circular	Rough	Cream	Entire	Flat
RSR-N25	NA	Circular	Smooth	Opaque	Entire	Raised
RSR-N27	NA	Circular	Smooth	Yellow	Entire	Raised
RSR-N32	NA	Circular	Wrinkled	Light green	Entire	Raised
RSR-N33	NA	Irregular	Rough	Slightly yellow	Irregular	Raised
RSR-I2	ISP4	Circular	Smooth	Yellow	Entire	Flat
RSR-I35	ISP4	Circular	Smooth	Light pink	Entire	Raised
RSR-I38	ISP4	Circular	Dry	Dirty white	Lobate	Flat
RSR-N40	NA	Irregular	Smooth	White	Entire	Raised
RSR-N41	NA	Circular	Smooth	Yellow	Entire	Raised
RSR-N42	NA	Irregular	Smooth	White	Lobate	Raised
RSR-N45	NA	Circular	Smooth	Cream	Entire	Raised
RSR-N47	NA	Circular	Wrinkled	Light green	Entire	Raised
RSR-N48	NA	Irregular	Dry	White	Lobate	Raised
RSR-N51	NA	Circular	Smooth	Yellow	Entire	Flat
RSR-N52	NA	Circular	Rough	Dirty white	Entire	Raised
RSR-I53	ISP4	Circular	Smooth	Light green	Entire	Flat
RSR-I54	ISP4	Circular	Dry	White	Entire	Raised
RSR-I55	ISP4	Circular	Smooth	Yellow	Entire	Flat
RSR-N57	NA	Circular	Rough	Dirty white	Entire	Raised
RSR-N58	NA	Circular	Smooth	Cream	Entire	Flat
RSR-N60	NA	Irregular	Dry	White	Filamentous	Raised
RSR-I61	ISP4	Circular	Smooth	Yellow	Entire	Raised
RSR-N62	NA	Circular	Wrinkled	Light green	Entire	Raised
RSR-N64	NA	Circular	Dry	White	Entire	Raised
RSR-N66	NA	Circular	Smooth	Yellow	Entire	Flat
RSR-N67	NA	Circular	Smooth	Cream	Entire	Raised
RSR-N68	NA	Circular	Dry	Dirty white	Entire	Flat
RSR-N70	NA	Irregular	Smooth	White	Filamentous	Raised
RSR-N73	NA	Circular	Smooth	Yellow	Entire	Raised

Notes: NA: Nutrient agar (Himedia, India), ISP4: ISP medium No. 4 (Himedia, India).

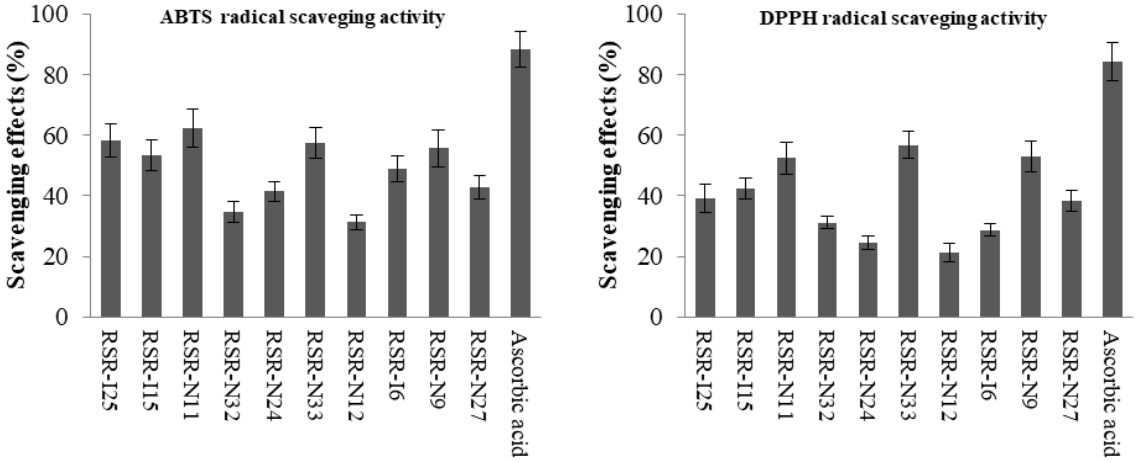

Figure 1. DPPH and ABTS radical scavenging activities of the ethyl acetate extracts

α-Amylase and α-glucosidase inhibitory activities of the ethyl acetate extracts of the endophytic bacteria

The antidiabetic properties of ethyl acetate extracts of the endophytic bacteria were determined by the α-amylase and α-glucosidase inhibitory assays (Fig. 2). The α-amylase and α-glucosidase inhibitory assays showed the extracts of 11 endophytic bacteria at a concentration of 500 µg/mL exhibited α-amylase and α-glucosidase inhibitory activities with inhibitory values ranging from 31.4 ± 3.1% to 59.7 ± 6.4% and 17.3 ± 3.1% to 54.5 ± 6.1%, respectively. Among them, the ethyl acetate extracts of three bacterial endophytes (RSR-I15, RSR-N9, RSR-N33) showed the inhibitory activity against both α-amylase and α-glucosidase enzymes more than 50% and one bacterial endophyte (RSR-I25) showed the inhibitory activity against α-glucosidase enzyme more than 50%. The positive control (acarbose at a concentration of 100 µg/mL) showed α-amylase and α-glucosidase inhibitory activities with inhibition values of 91.3 ± 5.5% and 85.4 ± 5.1%, respectively.

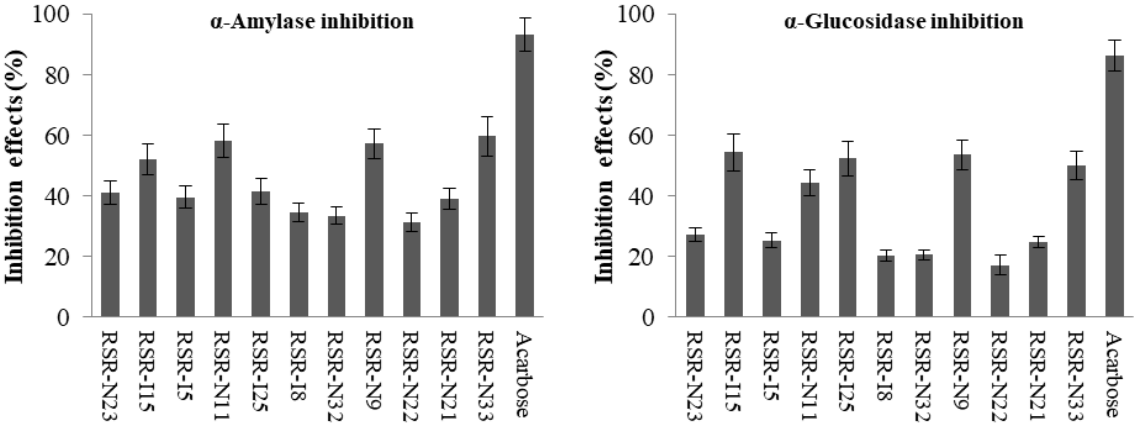

Figure 2. α-Amylase and α-glucosidase inhibitory activities of the ethyl acetate extracts

Molecular identification of active bacterial endophytes

The 16S rRNA genes of five endophytic bacteria (RSR_N9, RSR_N11, RSR_I15,

RSR_I25, RSR_N33) with high antioxidant, α-amylase and α-glucosidase inhibitory activities were amplified and sequenced to identify their taxa. The agarose gel electrophoresis of DNA samples and PCR products (Fig. 3) indicated that genomic DNA from isolates was extracted and the 16S rRNA gene (~1.5 kb) of the isolates was also amplified successfully from the extracted genomic DNA samples. The 16S rRNA genes of strains were sequenced with lengths ranging from 1330 bp to 1400 bp and had high similarity (99–100%) to that of bacteria on GenBank (Table 2). The phylogenetic analysis revealed that five endophytic bacteria belonged to 3 genera, including *Bacillus* (RSR_N9, RSR_N11, RSR_N33), *Pseudovibrio* (RSR_I25), and *Streptomyces* (RSR_I15) (Fig. 4).

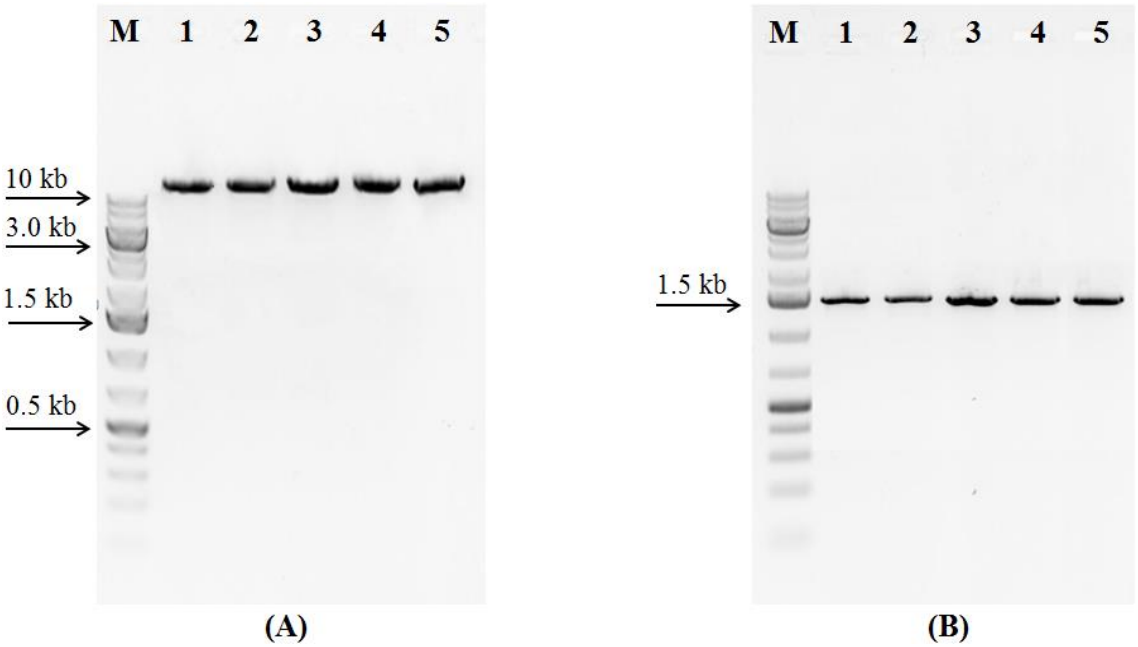

Figure 3. Agarose gel electrophoresis of DNA samples (A) and PCR products (B) from the isolates RSR_N9, RSR_N11, RSR_I15, RSR_I25, RSR_N33 (lanes 1–5), lane M: 1 kb plus DNA ladder (Thermo Scientific)

Table 2. The closest sequences of 16S rRNA sequences of isolates obtained in NCBI

Isolates	Media	16S length (nt)	Closest homologs	Query cover (%)	Similarity (%)
RSR-N33	NA	1400	*Bacillus subtilis* WE2, AB862124	100	99.9
RSR-N9	NA	1400	*Bacillus licheniformis* MSWS30, KX785167	100	100
RSR-I15	ISP4	1330	*Streptomyces* sp. YIM_KMY48, DQ358662	100	99.8
RSR-I25	ISP4	1330	*Pseudovibrio denitrificans* AP3, HE584768	100	99.4
RSR-N11	NA	1400	*Bacillus aerophilus* Zn-B, MN922745	100	99.7

Notes: NA: Nutrient agar (Himedia, India), ISP4: ISP medium No. 4 (Himedia, India).

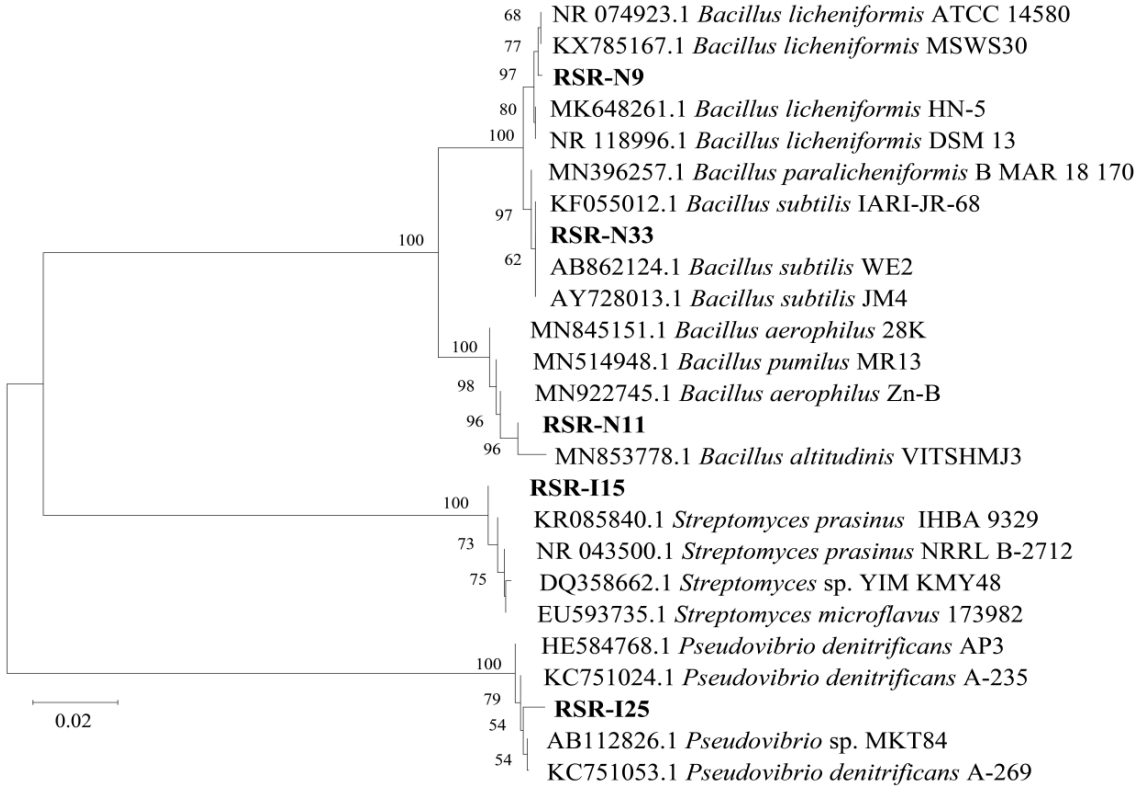

Figure 4. Phylogenetic tree based on 16S rRNA gene sequences of isolates and their closest sequences derived from NCBI. The phylogenetic tree of 16S rRNA sequences was created by the Neighbor-Joining algorithm with 1000 bootstraps using MEGA v.7.0.0

DISCUSSION

Mangrove plants are considered an ecological niche for diverse bacterial endophytes and are potential sources of bioactive compounds. The antioxidant, α-amylase and α-glucosidase inhibitory assays in our study revealed root endophytic bacteria of *R. stylosa* as potential producers of antioxidant and antidiabetic agents. Among ethyl acetate extracts of 40 isolated root endophytic bacteria, 10 extracts showed antioxidant activity and 11 extracts showed α-amylase and α-glucosidase inhibitory activities. Previous studies have reported that endophytic microorganisms from mangrove plants exhibit a wide range of biological activity, including antioxidant, α-amylase and α-glucosidase inhibitory activities. In regard to the antioxidant activity, Guo et al. (2015) isolated six compounds (penipyrols A-B, peniamidones A-B, peniamidones C-D) from the extract of a fungus *Penicillium solitum* GWQ-143 from rhizosphere soil of the mangrove plant *R. stylosa*. The antioxidant assays showed that all six isolated compounds exhibited great radical scavenging activities against DPPH with IC_{50} values ranged from 4.7 µM to 15.0 µM. Zhoe et al. (2018) isolated 225 fungal strains from two mangrove plants *R. stylosa* and *Rhizophora mucronata*. Antioxidant assays revealed that the crude extracts of 40 isolated endophytic fungi showed the DPPH and ABTS radical scavenging activities with IC_{50} values from 0.33 ± 0.02 to 14.36 ± 0.68 mg/mL. In another study, Rahmawati et al. (2019) isolated six fungi and three bacteria from two mangrove plants *Avicennia marina* and *Xylocarpus granatum*. Of these, six fungi and two bacteria showed DPPH radical scavenging activity with IC_{50} values from 1–19 ppm. Dat et al.

(2019b) also isolated antioxidant endophytic bacteria from leaves of *R. stylosa* with ABTS and DPPH scavenging activities of ethyl acetate extracts at a concentration of 500 µg/mL ranging from 36.3 ± 2.6% to 71.5 ± 6.6% and from 26.2 ± 3.3% to 57.4 ± 5.8%, respectively. The antioxidant strains isolated from leaves of *R. stylosa* belonged to genera *Bacillus*, *Streptomyces*, *Pseudovibrio*, and *Pseudomonas* which are similar to antioxidant bacterial genera isolated from roots of *R. stylosa*. These results imply that these genera are common active taxa among endophytic bacterial communities from leaves and roots of *R. stylosa*. Especially, the genus *Streptomyces* is often reported as the main producer of lead bioactive compounds from mangroves (Ancheeva et al., 2018).

In respect of α-amylase and α-glucosidase inhibitory activities, Pujiyanto et al. (2018) also isolated 11 endophytic bacteria from *Annona muricata*. The free cell supernatant of these endophytes showed inhibitory activity against α-amylase with 28.9–72.2% of inhibition. He et al. (2019) isolated 25 secondary metabolites from the culture of a mangrove root endophytic fungus *Penicillium pinophilum* SCAU037, of which three compounds (vermistatin, penicillide, and Sch725680) showed significant inhibitory activity against α-glucosidase with IC_{50} of 51.9, 78.4 and 33.8 µM, respectively. Lopez et al. (2019) reported that the crude extract of mangrove leaves endophytic fungus *Zasmidium* sp. EM5-10 showed significant inhibitory activity against α-glucosidase with 91.3% of inhibition. Additionally, Qiu et al. (2019) isolated 11 compounds from a mangrove fungus *Mycosphaerella* sp. SYSU-DZG01. Among them, three compounds (asperchalasine I, epicoccolide B, asperchalasine A) exhibited significant α-glucosidase inhibitory activity with IC_{50} values ranging from 15.7 µM to 26.7 µM, and four compounds (asperchalasine I, 2-methoxycarbonyl-4,5,6-trihydroxy-3-methyl-benzaldehyde, 1,3-dihydro-5-methoxy-7-methylisobenzofuran, epicoccolide B) showed antioxidant activity by scavenging DPPH with EC_{50} values ranging from 16.3 µM to 85.8 µM. Thus, the obtained results in our study and the previous studies show that the endophytic microorganisms from mangrove plants are one of the sources of antioxidant and antidiabetic agents for pharmaceutical applications. The strains with high antioxidant, α-amylase and α-glucosidase inhibitory activities may be targeted for further studies on chemical structure, the genome as well as gene clusters related to the biosynthesis of bioactive secondary metabolites.

CONCLUSION

The present study isolated endophytic bacteria from roots of the mangrove plant *R. stylosa* and evaluated antioxidant, α-amylase and α-glucosidase inhibitory activities of ethyl acetate extracts of the isolated endophytic bacteria. From roots of *R. stylosa*, 73 endophytic bacterial strains were isolated, of which the ethyl acetate extracts of 10 isolated endophytic strains at a concentration of 500 µg/mL showed antioxidant activity with ABTS and DPPH radical scavenging values from 31.3 ± 2.5% to 62.2 ± 6.3% and 21.2 ± 3.2% to 56.7 ± 4.5%, respectively. In addition, the ethyl acetate extracts of 11 isolated endophytic bacteria exhibited α-amylase and α-glucosidase inhibitory activities with values from 31.4 ± 3.1% to 59.7 ± 6.4% and 17.3 ± 3.1% to 54.5 ± 6.1%, respectively. Notably, the ethyl acetate extracts of two isolated endophytic bacteria (RSR-N9, RSR-N33) exhibited both antioxidant, α-amylase and α-glucosidase inhibitory activities more than 50%. The identification of five promising endophytic bacteria by 16S rRNA sequences revealed that the endophytes belonged to 3 genera, including *Bacillus* (RSR_N9, RSR_N11, RSR_N33), *Pseudovibrio* (RSR-I25), and *Streptomyces* (RSR-I15). The obtained results suggest that mangrove endophytes are a source of antioxidant and antidiabetic agents, and the strains with high antioxidant, α-amylase and α-glucosidase inhibitory activities may be targeted for further studies on chemical structure, the genome as well as gene clusters related to the

biosynthesis of bioactive secondary metabolites. To the best of our knowledge, this is the first report of antioxidant, *α*-amylase and *α*-glucosidase inhibitory activities of the endophytic bacteria from the roots of Vietnam mangrove plants in general and *R. stylosa* in particular.

Acknowledgements*:* This work was supported by the Vietnam Academy of Science and Technology under grant number: ĐL0000.02/19–20.

REFERENCES

Ancheeva E., Daletos G., Proksch P., 2018. Lead compounds from mangrove-associated microorganisms. *Mar. Drugs*, 16: 319. https://doi.org/10.3390/md16090319

Chakrabarti R., Rajagopalan R., 2002. Diabetes and insulin resistance associated disorders: Disease and the therapy. *Curr. Sci.*, 83(12): 1533–1538. https://www.jstor.org/stable/24108177

Dat T. T. H., Oanh P. T. T., Tam V. T. T., Anh H. L. T., 2019a. Antimicrobial activity of bacteria isolated from the coastal mangrove sediment in Thua Thien Hue. Proceedings of National Scientific Forum 2019: Marine Biology and Sustainable Development: 971–980. Publishing House of Natural Science and Technology, Hanoi, Vietnam.

Dat T. T. H., Oanh P. T. T., Tam V. T. T., Anh H. L. T., 2019b. Antimicrobial and antioxidant activity of bacterial endophytes isolated from leaves of the mangrove plant *Rhizophora stylosa*. *Acad. J. Biol.*, 41(4): 91–99. https://doi.org/10.15625/0866-7160/v41n4.14675

Giacco F., Brownlee M., 2010. Oxidative stress and diabetic complications. *Circ. Res*., 107(9): 1058–1070. https://doi.org/10.1161/CIRCRESAHA.110.223545

Guo W., Kong X., Zhu T., Gu Q., Li D., 2015. Penipyrols A-B and peniamidones A-D from the mangrove *Derived Penicilli*. *Arch. Pharm. Res*., 38(8): 1449–1454. https://doi.org/10.1007/s12272-014-0513-3

He F., Li X., Yu J. H., Zhang X., Nong X., Chen G., Zhu K., Wang Y. Y., Bao J., Zhang H., 2019. Secondary metabolites from the mangrove sediment-derived fungus *Penicillium pinophilum* SCAU037. *Fitoterapia*, 136: 104177. https://doi.org/10.1016/j.fitote.2019.104177

Hong H. T., Phuong N. N., 2013. The isolation and selection of actinomycete species from Can Gio tropical swamp for their antifungal feature against *Fusarium* sp. *J. Sci., Ho Chi Minh City University of Education*, 51: 59–71.

Kui-Wu W., Shi-Wei W., Bin W., Ji-Guang W., 2014. Bioactive natural compounds from the mangrove endophytic fungi. Mini-Rev. *Med. Chem*., 14: 370–391. https://doi.org/10.2174/1389557514666140220122829

Lopéz D., Cherigo L., Mejia L. C., Loza-Mejía M. A., Martínez-Luis S., 2019. α-Glucosidase inhibitors from a mangrove associated fungus, *Zasmidium* sp. strain EM5-10. *BMC Chem*., 13(1): 22. https://doi.org/10.1186/s13065-019-0540-8

Maiese K., 2015. New insights for oxidative stress and diabetes mellitus. *Oxid. Med. Cell. Longev.*, 2015: 875961. https://doi.org/10.1155/2015/875961

Manganyi M. C., Tchatchouang C. D. K., Regnier T., Bezuidenhout C. C., Ateba C.N., 2019. Bioactive compound produced by endophytic fungi isolated from *Pelargonium sidoides* against selected bacteria of clinical importance. *Mycobiology*, 47: 335–339. https://doi.org/10.1080/12298093.2019.1631121

Neha K., Haider M. R., Pathak A., Yar M. S., 2019. Medicinal prospects of antioxidants: A review. *Eur. J. Med.*

Chem., 178: 687–704. https://doi.org/10.1016/j.ejmech.2019.06.010

Oguntibeju O. O., 2019. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int. J. Physiol. Pathophysiol. Pharmacol*., 11(3): 45–63.

Pizzino G., Irrera N., Cucinotta M., Pallio G., Mannino F., Arcoraci V., Squadrito F., Altavilla D., Bitto A., 2017. Oxidative stress: harms and benefits for human health. *Oxid. Med. Cell. Longev.*, 2017: 8416763. https://doi.org/10.1155/2017/8416763

Pujiyanto S., Resdiani M., Raharja B., Ferniah R. S., 2018. α-Amylase inhibitor activity of endophytic bacteria isolated from *Annona muricata* L. *J. Phys.: Conf. Ser.*, 1025: 012085. https://doi.org/10.1088/1742-6596/1025/1/012085

Qiu P., Liu Z., Chen Y., Cai R., Chen G., She Z., 2019. Secondary metabolites with α-glucosidase inhibitory activity from the mangrove fungus *Mycosphaerella* sp. SYSU-DZG01. *Mar. Drugs*, 17(8): 483. https://doi.org/10.3390/md17080483

Rahmawati S. I., Izzati F. N., Hapsari Y., Septiana E., Rachman F., Bustanussalam, Simanjuntak P., 2019. Endophytic microbes and antioxidant activities of secondary metabolites from mangroves *Avicennia marina* and *Xylocarpus granatum*. *IOP Conf. Ser.: Earth Environ. Sci*., 278: 012065. https://doi.org/10.1088/1755-1315/278/1/012065

Subramanian R., Asmawi M. Z., Sadikun A., 2008. *In vitro* alpha-glucosidase and alpha-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta. Biochim. Pol*., 55(2): 391–398.

Thatoi H., Behera B. C., Mishra R. R., Dutta S. K., 2013. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. *Ann. Microbiol*., 63: 1–19. https://doi.org/10.1007/s13213-012-0442-7

Xu J., 2015. Bioactive natural products derived from mangrove-associated microbes. *RSC Adv*., 5: 841–892. https://doi.org/10.1039/C4RA11756E

Zhou J., Diao X., Wang T., Chen G., Lin Q., Yang X., Xu J., 2018. Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China Sea. *PloS one*, 13: e0197359. https://doi.org/10.1371/journal.pone.0197359