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Research Article

Screening and Molecular Identification of Endophytic Bacteria from *Calamus caesius* Blume with Potential as Antioxidant and α-Glucosidase Inhibitors

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ABSTRACT

Calamus caesius, traditionally used in Asian medicine for managing diabetes mellitus, is a promising source of endophytic bacteria due to its bioactive potential. This study aims to identify the potential of endophytic bacteria isolated from "Pakkat" (*Calamus caesius* Blume), a traditional food of North Sumatra, as a source of α-glucosidase inhibitors. These inhibitors, such as acarbose, are used to manage type 2 diabetes mellitus but have significant gastrointestinal side effects. This study focuses on isolating and characterizing endophytic bacteria that can produce natural inhibitors, potentially safer alternatives. Twenty endophytic bacterial isolates were successfully isolated, of which five were excluded due to hemolytic activity. Antioxidant activity was determined using the DPPH method, and isolates with the highest activity proceeded to α-glucosidase inhibitor testing. Molecular identification of potential isolates was performed through 16S rRNA gene amplification, and genomic DNA sequencing utilized the Oxford Nanopore Technologies platform. Secondary metabolite analysis was performed using antiSMASH, and gene cluster comparison with CAGECAT. Of 15 isolates, LTUP1.3 (*Bacillus cereus*) exhibited 86.6% α-glucosidase inhibitor activity, close to the positive control (88%). Although in *Bacillus cereus* LTUP1.3 no secondary metabolites were found that act as α-glucosidase inhibitors, three enzymes have similarities with enzymes found in Actinoplanes that play a role in acarbose biosynthesis namely dTDP-glucose 4,6-dehydratase RffG, glucose-1-phosphate thymidylyltransferase rmlA and glucose-1-phosphate adenylyltransferase glgC. These results suggest that Bacillus cereus strain LTUP1.3 has a potential biosynthetic pathway similar to the acarbose biosynthetic pathway in Actinoplanes. However, further confirmation is required to establish its overall acarbose production capability.

1. Introduction

The number of people who have diabetes has increased worldwide as a result of rapid economic growth, urbanization, unhealthy lifestyles, and physical inactivity (Riastawaty *et al.* 2023). Diabetes causes high morbidity and premature mortality and has recently raised concerns as more than 75% of deaths from diabetes occur in individuals under the age of sixty years of age (Khan *et al.* 2020). According to the WHO, between 2000 and 2019, there was a 3% increase in the age-adjusted death rate from diabetes. The WHO also reports that more than 95% of people with diabetes have type 2 diabetes. Until recently, this type of diabetes was only seen in adults, but it is now also increasingly common in children. Metformin is the first-line pharmacotherapy for type 2 diabetes mellitus (T2DM). In addition to reducing glucose levels, the drug has an insulin-sensitizing effect with multiple actions on tissues such as the liver, skeletal muscle, endothelium, adipose tissue, and ovaries. Unfortunately, metformin

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has several side effects (from mild to severe) that lead to a lack of adherence and is, therefore, the lowest adherence to antidiabetic oral therapy (Artasensi *et al.* 2020).

Acarbose is the first α-glucosidase inhibitor used in the clinical treatment of T2DM. It can delay carbohydrate absorption in the intestine to reduce postprandial blood glucose (Zhang *et al.* 2022). The disadvantage of acarbose is that it must be used three times a day, and gastrointestinal side effects, such as gas, bloating, and diarrhea, are relatively common (Altay 2022). Natural materials are a potential source of secondary metabolites to be used as drug candidates. A previous study reported that betel leaf has the potential to be an analgesic, anti-inflammatory, antioxidant, and antidiabetic (Ginting *et al.* 2021). However, the overexploitation of natural materials can damage the ecosystem. One promising approach is the use of natural microbial bioactive compounds. Microbes, especially bacteria, are one of the potential sources of bioactive compounds or secondary metabolites (Ginting *et al.* 2023).

Endophytic microbes can act as a biological defense for plants by producing various bioactive compounds similar to the bioactive compounds of their host plants (Gultom *et al.* 2023). "Pakkat" is a traditional food from the Mandailing region of North Sumatra made from young rattan shoots (*Calamus caesius* Blume). The local people of North Sumatra believe that consuming *C. caesius* can help reduce various symptoms of diseases such as heart gout, diarrhea, and diabetes. Previous studies reported that young rattan extract has antibacterial activity against *S. aureus* (Umar *et al.* 2023), *Klebsiella penumoniae*, and *Propionibacterium acnes* (Padena *et al.* 2023). Concerning antidiabetes, *C. caesius* is also reported to contain various secondary metabolites, including flavonoids, terpenoids, steroids, tannins, and glucosides, which have the potential to reduce blood glucose levels, as well as to be able to increase germ epithelial cells in alloxan-induced male rats (Lubis *et al.* 2023).

Research on the potential of endophytic microbes as producers of antidiabetic bioactive compounds has a strong background because it can contribute to developing alternative therapies for diabetes mellitus. In addition, using natural bioactive compounds from endophytic microbes can provide several advantages compared to conventional treatments, such as lower side effects and the potential to overcome drug resistance in patients with diabetes mellitus (Kaur *et al.* 2018). An equally important benefit is exploring the potential of North Sumatra's local wisdom, Pakkat (*C. caesius*), in the health sector. The objectives of this study were to isolate endophytic bacteria from *C. caesius*, characterize endophytic bacteria, test antioxidant activity and α-glucosidase inhibitor activity, and identify potential endophytic bacteria.

2. Materials and Methods

2.1. Isolation of Endophytic Bacteria

The stems of *C. caesius* were washed using running water to remove dust and dirt. *C. caesius* was then cut sterile using a scalpel with a size of 1 cm × 1 cm. The explants were disinfected in 70% ethanol for 1 minute, followed by immersion in 1% sodium hypochlorite (NaOCl). These pieces were then washed in sterile distilled water for 1 minute and dried using sterile filter paper. To validate the effectiveness of sterilization, the water from the last wash was inoculated onto a Nutrient Agar medium and incubated for 1 week. Surface sterilization was validated if no bacterial growth was found. Pakkat pieces were inoculated onto the surface of Nutrient Agar, to which ketoconazole was added to inhibit the growth of endophytic fungi. Petri dishes were incubated for 7 days. Bacteria that grew were inoculated onto fresh media without the addition of antifungal agents (Pierre *et al.* 2022).

2.2. Characterization of Endophytic Bacteria

Purified endophytic bacteria were characterized morphologically and biochemically, including Gram staining, catalase, motility, TSIA test, gelatinase test, Simmon citrate test, and hemolysis.

2.3. Preparation of Ethyl Acetate Extract

Endophytic bacterial strains were cultured in 500 ml nutrient broth (NB, Himedia, India) at 37°C, 150 rpm for 7 days. Then, the culture was centrifuged at 10,000 rpm for 10 minutes. The cell-free supernatant was extracted with ethyl acetate (1:1 v/v, 5 times) overnight at room temperature. Then, the ethyl acetate extraction was evaporated under reduced pressure (12-24 h, 50°C) to remove the ethyl acetate and obtain the crude extract (Dat & Phung Thi Thuy 2021).

2.4. Antioxidant Activity

Antioxidant activity was determined using the DPPH free radical scavenging method. Briefly, 10 µL of each extract (500 µg/ml) was added to 190 µL DPPH (0.1 mg/ml) in 96 wells. 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 a positive control. The DPPH radical scavenging activity was calculated as follows.

DPPH scavenging activity (%) = 100 × [(Ac - As) / (Ac - Ab)]

Where Ac is the control absorbance, As is the extract absorbance, and Ab is the blank absorbance (Dat *et al.* 2019).

2.5. α-glucosidase Inhibitor

The endophytic bacteria that exhibited the strongest antioxidant activity were further tested for their alpha-glucosidase inhibitor activity. The reaction test was conducted in a 96-well microplate with four mixtures (A0, A1, AI0, and AI1). Briefly, 2 µL of metabolite extract in the supernatant was mixed with 48 µL of phosphate buffer (100 mM, pH 7) and, for mixtures A1 and AI1, 25 µL of α-glucosidase enzyme (0.25 units/ml). The mixtures were incubated at 37°C for 5 minutes. Subsequently, 25 µL of substrate p-nitrophenyl α-D-glucopyranoside (Sigma Aldrich®, N1377) (20 mM) was added to all mixtures, followed by further incubation for 15 minutes at 37°C. The reaction was terminated with 100 µL of sodium carbonate (200 mM). The absorbance was measured using a microplate reader at a wavelength of 415 nm. Acarbose solution (1%) prepared by dissolving acarbose powder in phosphate buffer (1:100, pH 7) was used as a positive control for the enzymatic reaction.

The percentage inhibition of α-glucosidase enzyme was calculated using the formula:
% inhibition = (A1 absorbance - A0 absorbance) - (AI1 absorbance - AI0 absorbance) / (A1 absorbance - A0 absorbance) (Susilowati *et al.* 2019).

2.6. Molecular Identification of Endophytic Bacteria

The genomic DNA of potential bacteria was isolated using the Fungal/Bacterial DNA miniprep kit. DNA concentration was quantified using Nanodrop. Primer 27F/1492% was used to amplify the 16SrRNA gene using Polymerase Chain Reaction (PCR). Pre-denaturation was performed at 95°C for 90 seconds, followed by 30 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 55°C, extension for 90 seconds at 72°C and final extension for 3 minutes at 72°C. PCR products were sequenced, and the sequences were submitted to http://www.ncbi.nlm.nih.gov/BLAST to determine the homology of the microorganisms (Fachrial *et al.* 2023). Phylogenetic tree construction was performed using the Maximum Likelihood method based on the Tamura-Nei model using Mega X software (Tilahun *et al.* 2018).

2.7. Whole Genomic Sequencing

The genomic DNA of prospective isolates was sequenced using the Oxford Nanopore Technologies sequencing platform, which produced 1GB of data. gDNA samples were used as input for library preparation using Oxford Nanopore Technologies' Library Preparation Kit (ONT). gDNA was repaired with the final enzyme mixture. The corrected DNA was ligated with ONT-compatible adapters. Prior to insertion into the Flow Cell, the library was measured using a Qubit Fluorometer. GridION (Oxford Nanopore Technologies) was used to carry out sequencing until the necessary findings were obtained. MinKNOW software was used to carry out GridION sequencing. Guppy was used for base calling in high accuracy mode (HAC). Filtering was accomplished using filtlong. The data was transformed using Samtools. Nanoplot was used to evaluate read quality. Canu and Flye were used for readout correction and assembly. Racon and Medaka were used four and three times, respectively, to refine the constructed sequences. Minimap2 was used for mapping.

2.8. Genomic Information and Secondary Metabolites Analysis

The visualization of the assembly results of Pediococcus acidilactici strain LBSU8 was analyzed using Proksee-Genome Analysis (https://proksee.ca/). Secondary metabolites were predicted using AntiSMASH 7.0 software (Blin *et al.* 2023). Detection strictness on the AntiSMASH website was set to "loose." The databases used were KnownClusterBlast, MIBiG cluster comparison, ClusterBlast, Known Cluster Blast, Sub Cluster Blast, TFBS Finder, Pfam, and TIGRFam domains. To visualize homologous gene clusters, we used the Comparative Gene Cluster Analysis (CAGECAT) tool (https://cagecat.bioinformatics.nl/). The entire genome of Actinoplanes was obtained from the NCBI database and utilized as the query dataset to identify homologous sequences in other genomes. We utilized the clinker module in CAGECAT for a comparative visual analysis of these clusters. It produced publication-quality figures that

depict gene organization and sequence identity inside and among gene clusters from Actinoplanes, which are acarbose-producing bacteria.

3. Results

3.1. Isolation of Endophytic Bacteria

We succeed to isolated 15 endophytic bacterial isolates and coded LTUP1.1; LTUP1.2; LTUP1.3; LTUP1.4; LTUP1.5; LTUP2.1; LTUP2.2; LTUP2.3; LTUP2.4; LTUP2.5; LTUP2.6; LTUP2.7; LTUP2.8; LTUP2.9; LTUP2.10. Some of the bacterial isolates are shown in Figure 1.

3.2. Isolates Characterization

Endophytic bacteria were characterized based on Gram staining, catalase reaction, TSIA (Triple Sugar Iron Agar) test, Simmon Citrate test, gelatinase test, and hemolysis reaction test. The characterization is shown in Table 1.

3.3. Antioxidant Test

The antioxidant activity of endophytic bacterial isolates is shown in Figure 2.

From Figure 2 above, it can be seen that LTUP1.3 isolate showed the highest antioxidant activity of 29.9%. This isolate is then analyzed further.

3.4. α- glucosidase Inhibitor Activity

Based on the antioxidant activity value, LTUP1.3 isolate was analyzed for its α-glucosidase inhibitor activity. Based on the calculation, the α-glucosidase inhibitor activity was 86.6%, while the positive control, 1,000 ppm ascorbic acid, showed 88% activity.

The α-glucosidase inhibitor activity of isolate LTUP1.3 and acarbose is shown in Table 2.

3.5. Molecular Identification of LTUP1.3

Basic Local Alignment Search Tool (BLAST) results of LTUP1.3 isolate against the NCBI database showed that isolate LTUP 1.3 has a percent identification of 99.37 with *Bacillus cereus* with query cover identity reach 100%.The sequence was registered to GenBank with accession number PQ409425. Phylogenetic analysis of isolate LTUP1.3 (Figure 3) was determined using Neighbor-Joining by NCBI Blast Tree Method.

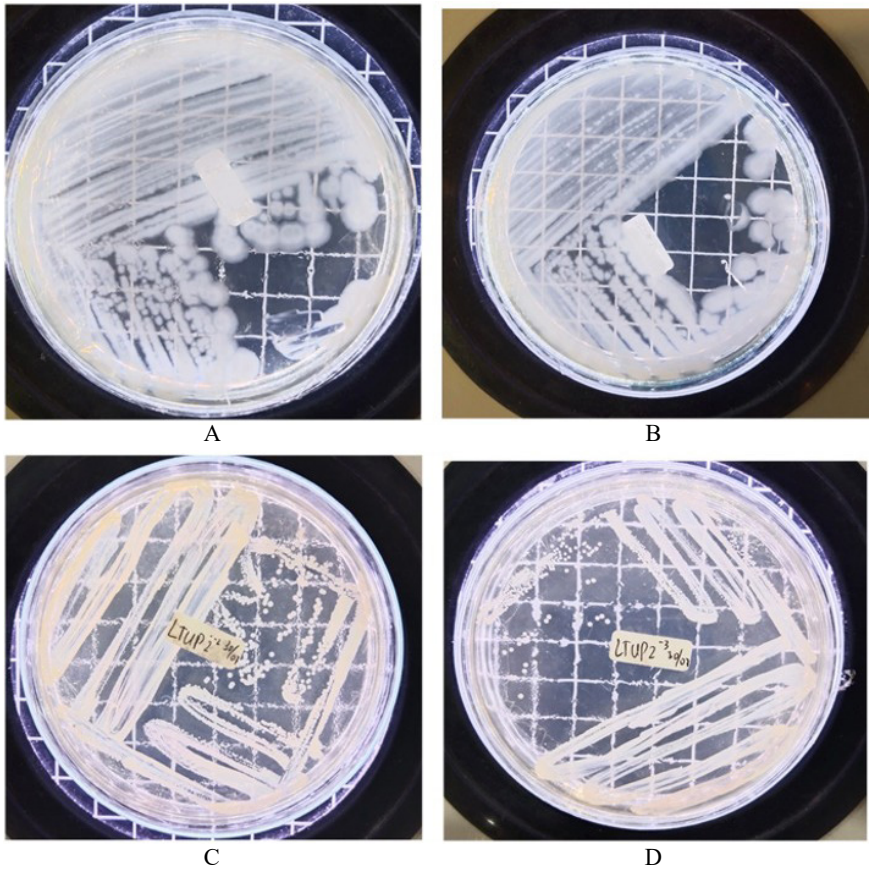

Figure 1. (A) isolate LTUP1.2, (B) isolate LTUP1.3, (C) isolate LTUP2.2, (D) isolate LTUP2.3

Table 1. Characterization isolate endophyte bacteria

Isolates	Characterization				
	Gram staining	Catalase test	TSIA	gelatinase	hemolysis
LTUP1.1	+	+	K/M	-	β
LTUP1.2	+	+	K/M	-	γ
LTUP1.3	+	+	K/M	-	γ
LTUP1.4	+	+	K/M	-	γ
LTUP1.5	+	+	K/M	-	β
LTUP2.1	+	+	K/K	-	β
LTUP2.2	+	+	K/K	-	γ
LTUP2.3	+	+	M/K	-	γ
LTUP2.4	+	+	M/K	-	γ
LTUP2.5	+	+	M/M	-	B
LTUP2.6	+	+	M/K	-	γ
LTUP2.7	+	+	M/K	-	γ
LTUP2.8	+	+	M/M	-	γ
LTUP2.9	+	+	K/M	-	β
LTUP2.10	+	+	K/K	-	γ

Isolates that showed hemolysis γ reaction were excluded in the next test

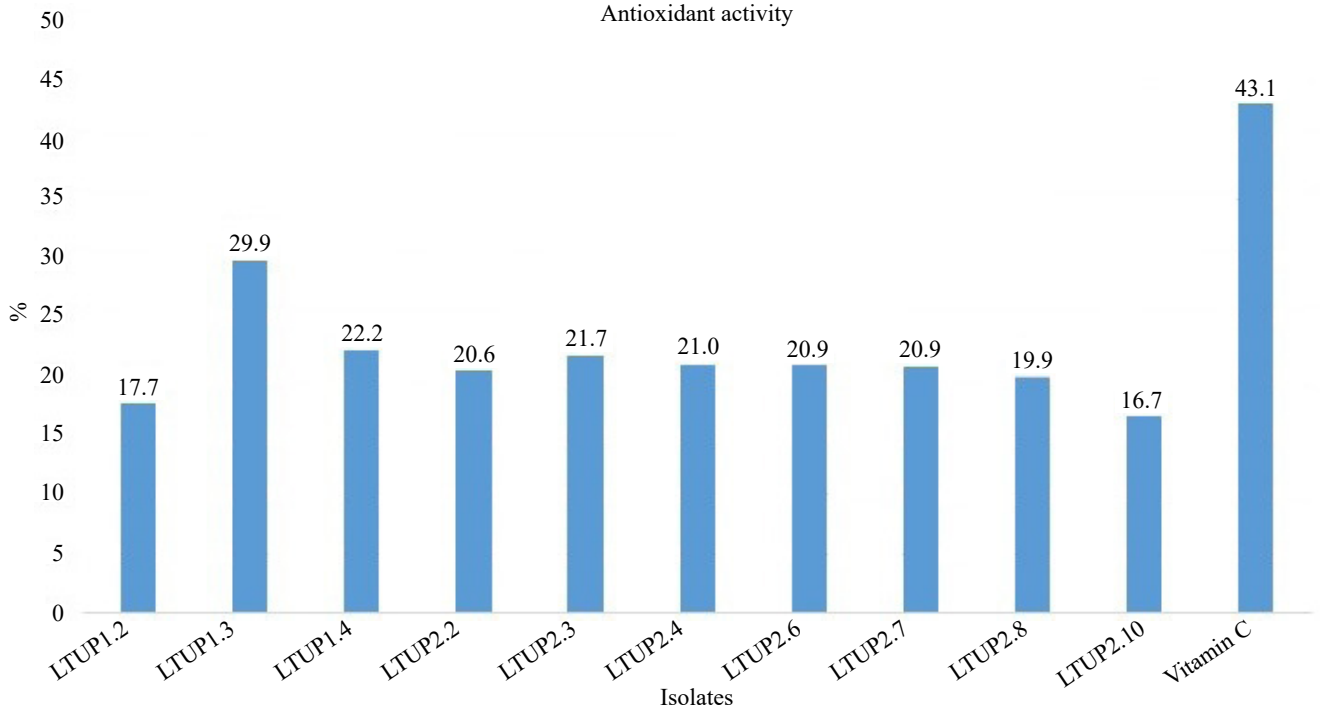

Figure 2 Antioxidant activity of endophytic bacterial isolates

Table 2. Inhibitory α-Glucosidase Activity of LTUP1.3 and acarbose

Samples	Concentration of samples (ppm)	% Inhibition
LTUP1.3	500	86.6
Acarbose	10,000	88

3.6. Analysis of Whole Genome Sequence Assembly

Figure 4 represents the circular whole-genome map of *Bacillus cereus* strain LTUP1.3 obtained from whole-genome sequencing. The genome size is approximately 5.5 Mbp, as indicated by the concentric rings showing the cumulative genome positions from 0 to 5.5 Mbp. The outermost ring displays annotated coding sequences (CDS) marked in blue, while structural RNAs, including tRNA, rRNA, and tmRNA, are labeled in green, red, and magenta, respectively. Important genes and loci of interest, such as dnaE, cas9, leuS, and gltB, are annotated around the genome map, highlighting key functional elements. The quality

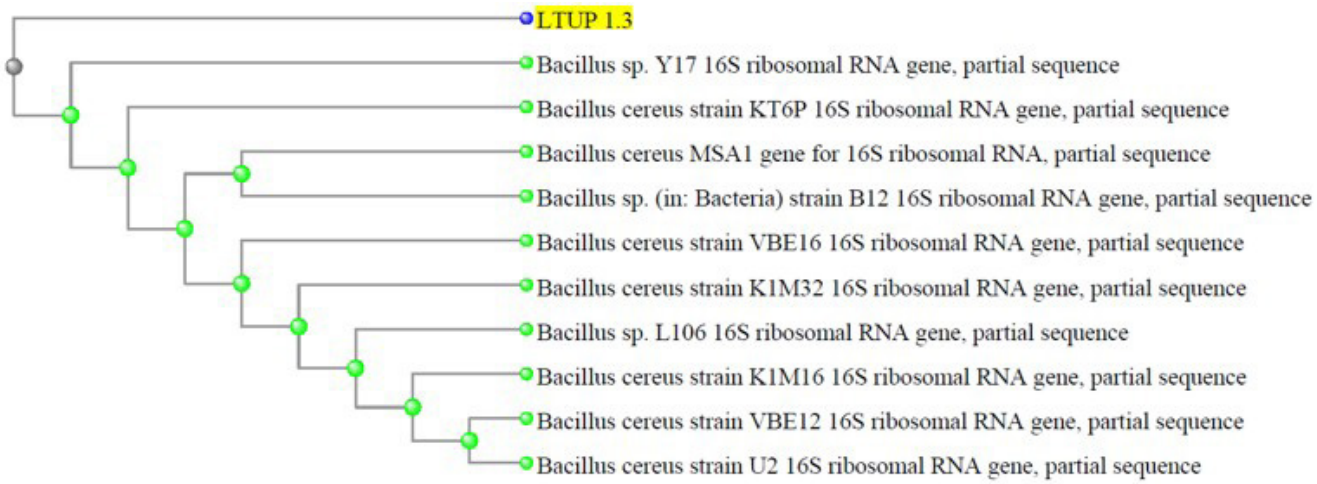

Figure 3. Phylogenetic analysis of LTUP 1.3. A phylogenetic tree showing the evolutionary relationship of isolate LTUP1.3 with other related bacterial species. This phylogenetic analysis is based on the 16S rRNA gene sequence, which shows the evolutionary proximity between the tested strains and species that are in the same group

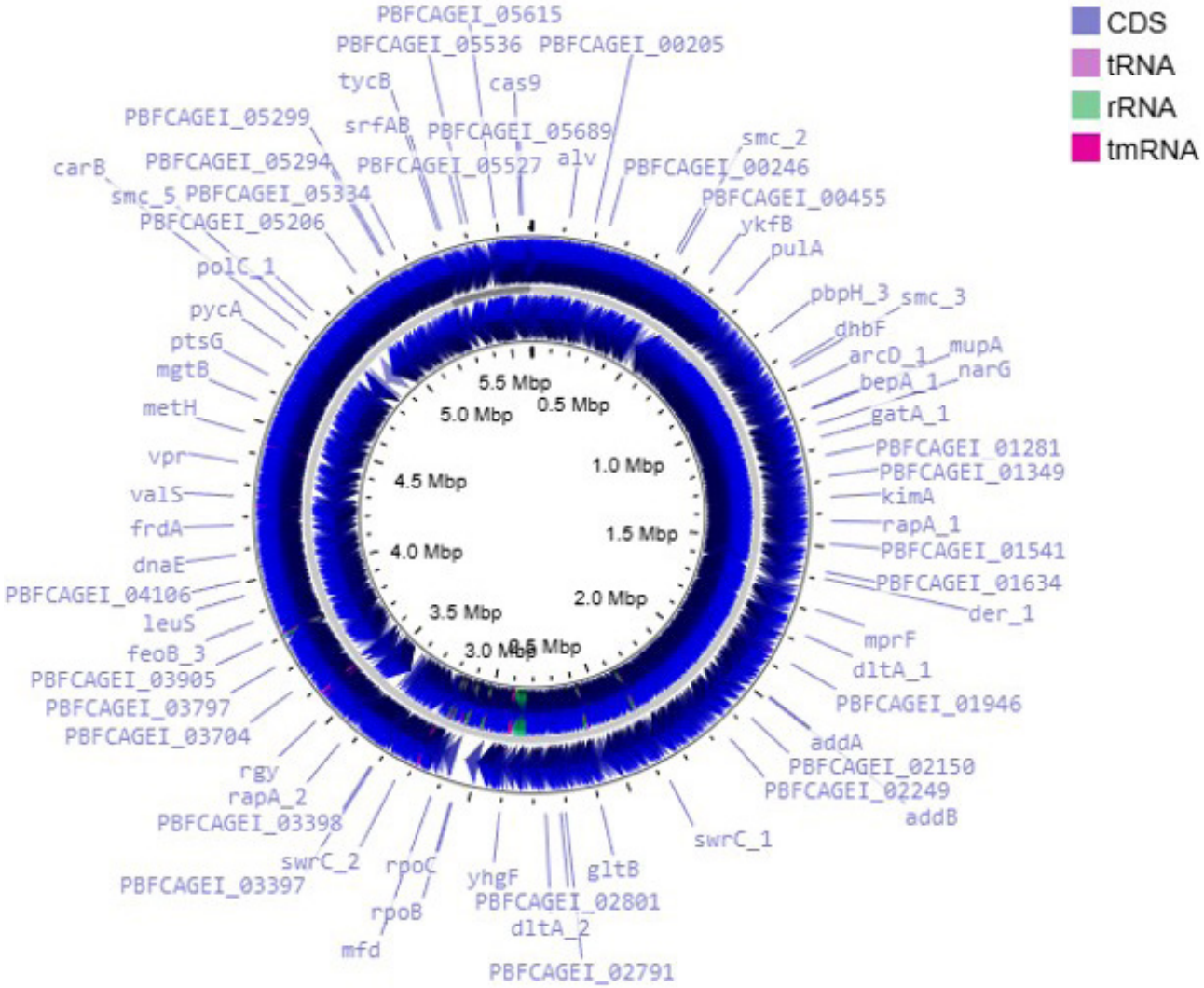

Figure 4. Circular genome map of *Bacillus cereus* strain LTUP1.3 based on whole-genome sequencing results. The map shows the locations of coding sequences (CDS), tRNA, rRNA, and tmRNA, as well as some important genes identified along the genome

of the assembled sequence was determined using Qualimap, as shown in Table 3.

3.7. Secondary Metabolites Analysis

Secondary metabolite analysis on *Bacillus cereus* strain LTUP1.3 was performed using antiSMASH software to identify and characterize potential biosynthetic regions. The results of antiSMASH analysis show the presence of various biosynthetic regions that produce various types of compounds. The overview of identifying secondary metabolites is shown in Table 4.

AntiSMASH analysis showed the presence of various biosynthetic regions that have the potential to produce a variety of secondary metabolites. Based on identification using antiSMASH, various clusters associated with metabolite types such as saccharides, peptides, betalactones, NRPS, fatty acids, and terpenes were found. Some of them showed similarities with known metabolite clusters, such as *bacillibactin*, which has antimicrobial activity, or siderophores, respectively. The diverse similarity percentages indicate that some clusters have strong functional relationships with known metabolites, while others may produce new compounds with uncharted functions. In this study, the results of MIBiG comparison (data not shown) analysis using antiSMASH showed that the endophytic bacteria studied had some similarities with various biosynthetic gene clusters (BGCs) identified in the MIBiG database. Based on the MIBiG database, some of the antioxidant secondary metabolites predicted to be synthesized by *Bacillus cereus* strain LTUP1.3 are indigoidine, kasugamycin, xanthan, and huperzine A, cryptophycin-327 and merochlorin.

Table 3. Assembly statistic based on qualimap

Category	Value
Reference size	5.608.434
Number of reads	189,954
Mapped reads	189,954 / 100%
Unmapped reads	0%
Mapped paired reads	0%
Secondary alignment	8,695
Supplementary alignments	12,727 / 6,7%
Read min/max/mean length	0/86,032 / 6,582.42
Duplicated reads (estimated)	21,749 / 11.45%
Number/percentage of A's	385,351,016 / 32.28%
Number/percentage of C's	210,401,562 / 17.63%
Number/percentage of T's	382,518,586 / 32.04%
Number/percentage of G's	215,492,460 / 18.05%
Number/percentage of N's	0 / 0%
GC Percentage	35.68%

3.8. Gene Cluster Comparison

Actinoplanes were chosen as a comparator because this genus is known as an acarbose-producing bacterium. the results of the gene cluster comparison analysis between *Bacillus cereus* strain LTUP1.3 and Actinoplanes are presented in the following Figure 5.

The results of gene cluster comparison analysis showed enzyme similarities between Actinoplanes, which is an acarbose producer, and *Bacillus cereus* strain LTUP 1.3, namely in genes related to the enzymes dTDP-glucose 4,6-dehydrase and dTDP-glucose synthase. In Actinoplanes, these two enzymes play a role in the biosynthesis of acarbose, an α-glucosidase

Table 4. Overview of identified secondary metabolite regions

Region	Type	From	To	Most similar known cluster		Similarity (%)
Region 1.1	saccharide	42,408	65,619			
Region 1.2	saccharide	654,949	677,495			
Region 1.3	RiPP-like	695,482	705,748			
Region 1.4	beta lactone	791,319	816,557	fengycin	NRP	40
Region 1.5	NRP-metallophore,NRPS	905,571	957,297	*bacillibactin*	NRP	71
Region 1.6	saccharide	1,273,217	1,296,928			
Region 1.7	fatty_acid	1,389,406	1,410,389			
Region 1.8	saccharide	1,606,973	1,639,014	dipeptide aldehydes	NRP	11
Region 1.9	LAP	1,870,352	1,893,859			
Region 1.10	saccharide	1,904,475	1,934,698	S-layer glycan	Saccharide	19
Region 1.11	fatty_acid	1,945,032	1,967,234	oryzanaphthopyran A/ oryzanaphthopyran B/ oryzanaphthopyran C/oryzanthrone A/oryzanthrone B/chlororyzanthrone A/chlororyzanthrone B		6
Region 1.12	saccharide	2,460,112	2,497,017			
Region 1.13	saccharide	2,600,913	2,631,219			
Region 1.14	saccharide	2,699,354	2,730,845			

Table 4. Continued

Region	Type	From	To	Most similar known cluster		Similarity (%)
Region 1.15	NRPS	2,741,822	2,785,010	thailanstatin A	NRP+Polyketide	10
Region 1.16	saccharide	2,848,901	2,870,832			
Region 1.17	saccharide	3,196,648	3,220,578	micrococcin P1	RiPP: Thiopeptide	8
Region 1.18	saccharide	3,225,929	3,274,277	micrococcin P1	RiPP: Thiopeptide	8
Region 1.19	saccharide	3,388,130	3,422,847	polysaccharide B	Saccharide	6
Region 1.20	saccharide	3,478,206	3,502,329	micrococcin P1	RiPP: Thiopeptide	8
Region 1.21	RiPP-like	3,591,507	3,602,044	toyoncin	RiPP	27
Region 1.22	saccharide	3,768,506	3,849,927	micrococcin P1	RiPP: Thiopeptide	8
Region 1.23	saccharide	3,978,924	4,006,458			
Region 1.24	saccharide	4,441,302	4,469,309			
Region 1.25	saccharide	4,661,170	4,690,941			
Region 1.26	saccharide	4,762,994	4,787,702			
Region 1.27	terpene	5,146,476	5,168,329	molybdenum cofactor	Other	17
Region 1.28	saccharide	5,264,787	5,283,18			
Region 2.1	arylpolyene,NRPS	11,528	73,078	micrococcin P1	RiPP:Thiopeptide	8
Region 2.2	phosphonate	245,681	260,915			

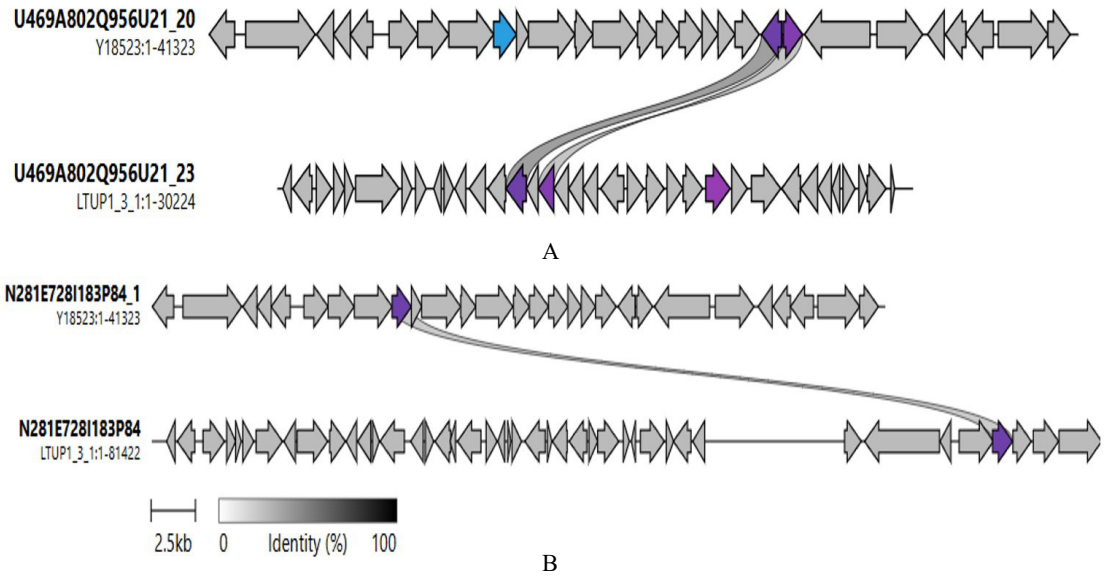

Figure 5. Comparison of Actinoplanes gene cluster (above) and Bacillus cereus strain LTUP1.3. The results of analysis using CAGECAT show that there are similarities between (A) dTDP-glucose 4,6-dehydratase AcbB and dTDP-glucose synthase AcbA in Actinoplanes with dTDP-glucose 4,6-dehydratase RffG and glucose-1-phosphate thymidylyltransferase rmlA in *Bacillus cereus* strain LTUP 1.3, with similarity 47% and 33% respectively and (B) putative 1-epi-valienol-1,7-biphosphate-1-adenylyltransferase AcbR in Actinoplanes and glucose-1-phosphate adenylyltransferase glgC with similarity 31%

which is used to control blood sugar levels in diabetics. The similarity of these enzymes suggests that Bacillus cereus strain LTUP 1.3 may have biosynthetic pathways similar to Actinoplanes, especially those associated with metabolites containing dTDP-glucose as a precursor. Figure 6 illustrates the genes in *Bacillus cereus* LTUP 1.3 that are anticipated to show similarities with genes in Actinoplanes in acarbose biosynthesis.

4. Discussion

A total of 15 endophytic bacterial isolates were successfully isolated from "pakkat" (*C. caesius*), and the characterization results showed that 5 isolates showed β hemolysis reaction. Hemolysis α and β reactions in bacteria are closely related to pathogenicity(Almwafy 2020). Therefore, all bacterial isolates that showed beta

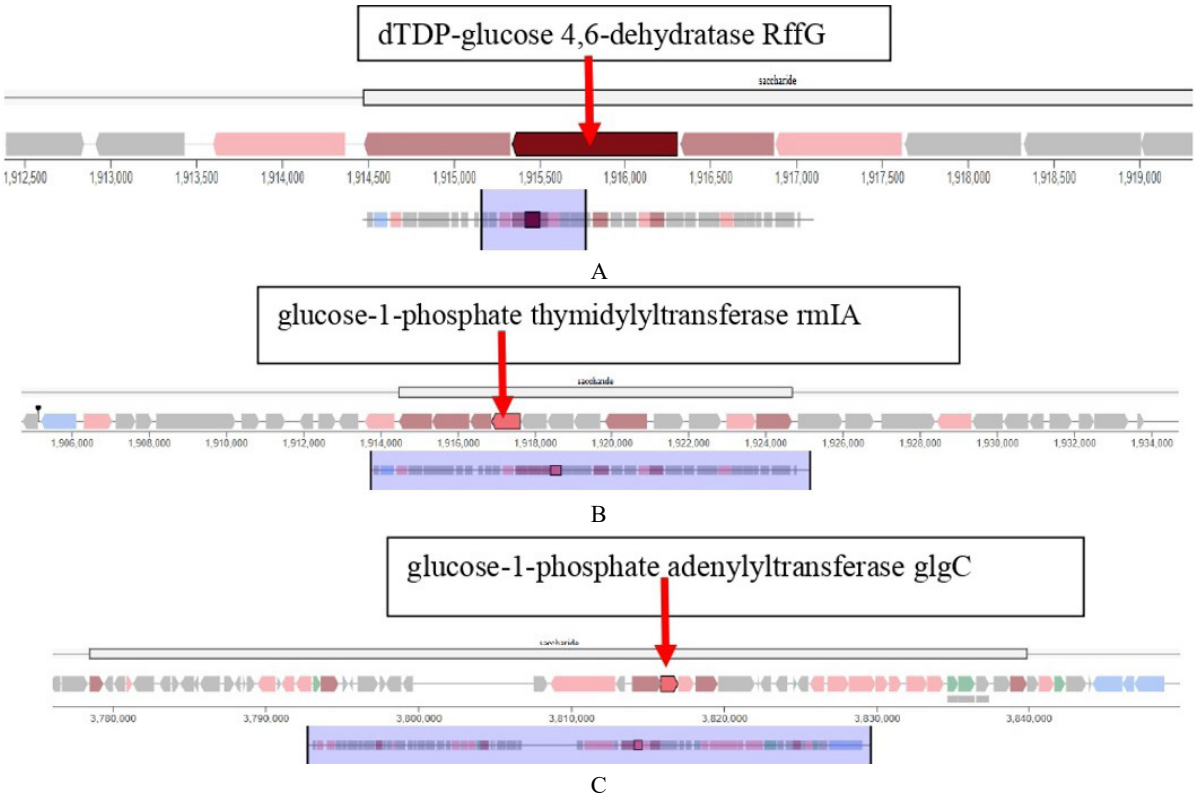

Figure 6. Red arrow indicates: (A) dTDP-glucose 4,6-dehydratase RffG, a core biosynthetic gene, located in 1,915,341-1,916,309, (total: 969 nt), (B) Glucose-1-phosphate thymidylyltransferase rmIA, an additional biosynthetic gene, located in 1,916,879-1,917,616, (total: 738 nt), (C) glucose-1-phosphate adenylyltransferase glgC, an additional biosynthetic gene, located in 3,815,876-3,817,006, (total: 1131 nt)

hemolysis reaction did not continue to the next stage. The antioxidant test results showed that the percentage activity of the bacterial isolates ranged from 17.7% to 24.5% at a sample concentration of 500 µg/ml. The positive control used was 1,000 ppm ascorbic acid, which showed a 43% antioxidant activity. Endophytic bacteria isolated from various plants have shown significant antioxidant activity. In a previous study, it was reported that endophytic bacteria isolated from Girang (*Leea indica*) leaves showed high antioxidant activity. The n-butanol extract of the bacteria showed an IC_{50} value of 59,021±0.541 µg/ml (Arivo *et al.* 2023).

In another study, it was mentioned that endophytic bacteria isolated from Noni fruit (*Morinda citrifolia*) showed antioxidant activity of 68.90% (Rabima *et al.* 2020). Endophytic microorganisms isolated from various plants have shown potential as a source of antioxidant and antidiabetic compounds. Several studies have shown that endophytic fungi and bacteria can produce metabolites with significant α-glucosidase inhibitory and antioxidant activities. Recent studies have explored the potential of endophytic microorganisms as a source of antioxidants and α-glucosidase inhibitors for diabetes management. Endophytic fungi and bacteria isolated from various plants, including *Ficus deltoidea, Cassia siamea,* and *Rhizophora stylosa*, have shown significant α-glucosidase inhibitory activity, often exceeding that of acarbose (Janatiningrum *et al.* 2022). The relationship between antioxidants and α-glucosidase inhibitory activity has been observed in several studies, which suggests a potential dual benefit for the treatment of diabetes (Naveen *et al.* 2023).

From the antioxidant activity, LTUP1.3 isolate was selected for further analysis because it showed the highest antioxidant activity compared to other isolates. Molecular identification results showed that LTUP1.3 isolate showed 99.37% similarity with *Bacillus cereus. Bacillus cereus* exhibits significant antioxidant activity through various mechanisms, primarily due to its

production of bioactive compounds such as enzymes, exopolysaccharides, and other metabolites. A study reported that exopolysaccharides (EPS) produced by endophytic *B. cereus* SZ1 have shown significant antioxidant activity, including DPPH radical scavenging and protection against DNA damage. These EPS can protect cells from oxidative stress-induced cytotoxicity, suggesting potential applications in pharmaceuticals (Zheng *et al.* 2016). β-Glucan extracted from *B. cereus* LVK13 also demonstrated notable antioxidant properties comparable to synthetic antioxidants, highlighting its potential as a natural antioxidant source (Yamuna *et al.* 2016). Another study reported that *Bacillus cereus* EW5 exhibited high antioxidant activity, achieving 83% DPPH, 99.6% ABTS, and 51% hydroxyl radical scavenging activities. The culture supernatant's antioxidant properties are primarily attributed to the production of N-acetylglucosamine (GlcNAc) and chitobiose during shrimp-shell waste degradation (Azam *et al.* 2014).

Based on the MIBiG database, *Bacillus cereus* LTUP1.3 is predicted to produce secondary metabolites that are thought to exhibit antioxidant activity, including indigoidine, huperzine A, and merochlorin. Indigoidine, a non-ribosomal peptide pigment, exhibits significant antioxidant activity, making it a promising candidate for various industrial and therapeutic applications. In a previous study, it was reported that indigoidine is produced by genetically engineered strains of *Aspergillus oryzae*, which overexpress the indigoidine synthetase gene. This production process is growth-associated and can be optimized by adjusting environmental factors such as pH, temperature, and nutrient concentrations. The antioxidant activity of indigoidine is linked to its ability to neutralize free radicals, thereby preventing oxidative damage to cells and tissues (Panchanawaporn *et al.* 2022). Huperzine A (HupA) is an alkaloid known for its antioxidant properties, which contribute to its neuroprotective and therapeutic potential. In various studies, HupA has been shown to decrease malondialdehyde (MDA) levels, a marker of lipid peroxidation, and increase the activity of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Sui & Gao 2014; Mei *et al.* 2021).

The measurement results of the α-glucosidase inhibitor of *Bacillus cereus* LTUP1.3 showed an activity of 86.6% at a sample concentration of 500 µg/ml, with positive control (acarbose) showing an activity of 88%. Endophytic bacteria have shown significant potential in producing bioactive α-glucosidase inhibitor compounds, which are crucial for managing conditions like type 2 diabetes by delaying carbohydrate digestion and absorption. These inhibitors are derived from various microbial sources, including endophytic bacteria. The aqueous extract of *Streptomyces* sp. IPBCC.a.29.1556, an endophyte of Indonesian *Ficus deltoidea*, demonstrated significant α-glucosidase inhibitory activity. The extract's fraction 1 exhibited an IC_{50} value of 58.8 µg/ml, outperforming the standard drug acarbose (IC_{50} = 90.4 µg/ml) (Janatiningrum *et al.* 2022). Actinoplanes is known as a microbe that synthesizes acarbose, a bioactive compound that inhibits α-glucosidase. Acarbose biosynthesis in Actinoplanes is a complex process involving multiple genes and regulatory mechanisms. Acarbose, an α-glucosidase inhibitor, is produced as a secondary metabolite by *Actinoplanes* sp., primarily *Actinoplanes* sp. SE50/110, and is used in the treatment of type II diabetes.

Based on the MiBIG database, we did not find any secondary metabolites that showed α-glucosidase inhibitor activity. Based on the MiBIG database, we did not find any secondary metabolites that showed α-glucosidase inhibitor activity. Still, we found 2 enzymes that may be involved in the biosynthesis of α-glucosidase inhibitors, namely dTDP-glucose 4,6-dehydratase RffG, glucose-1-phosphate thymidylyltransferase rmlA and glucose-1-phosphate adenylyltransferase glgC. These three enzymes show similarities with dTDP-glucose 4,6-dehydratase AcbB, dTDP-glucose synthase AcbA, and putative 1-epi-valienol-1,7-biphosphate-1-adenylyltransferase AcbR in Actinoplanes respectively. The roles of dTDP-glucose 4,6-dehydratase AcbB and dTDP-glucose synthase AcbA in acarbose biosynthesis are crucial for the formation of nucleotide sugar precursors, which are essential for the synthesis of acarbose, an α-glucosidase inhibitor used in diabetes treatment. The acbA gene encodes a protein that functions as dTDP-D-glucose synthase. This enzyme is essential for the initial steps in the biosynthetic pathway of acarbose, as it catalyzes the conversion of glucose-1-phosphate to dTDP-D-glucose, a critical precursor in the pathway. The acbB gene encodes an enzyme known as dTDP-D-glucose 4,6-dehydratase. This enzyme is crucial for converting dTDP-D-glucose into dTDP-4-dehydro-6-deoxy-D-glucose, a key intermediate in the acarbose biosynthetic pathway. This step is essential for the formation of the sugar moieties that are part of the acarbose structure (Stratmann *et al.* 1999). The putative enzyme 1-epi-valienol-1,7-biphosphate-1-adenylyltransferase AcbR plays a crucial role in the biosynthesis of acarbose, a clinically significant α-glucosidase inhibitor. AcbR is integral to the acarbose biosynthetic pathway, as it catalyzes a key step that leads to the formation of GDP-V, which is a precursor in the synthesis of acarbose (Tsunoda

et al. 2022).

In conclusion, this study successfully identified the potential of *Bacillus cereus* LTUP1.3, an endophytic bacterium from "Pakkat" (*C. caesius*), as a source of natural α-glucosidase inhibitors with high activity. Based on genomic analysis, *Bacillus cereus* LTUP1.3 showed enzymatic similarity to Actinoplanes, an acarbose producer, with similarity percentages of 47% and 33% for dTDP-glucose 4,6-dehydratase AcbB and dTDP-glucose synthase AcbA, respectively, and 31% for putative 1-epi-valienol-1,7-biphosphate-1-adenylyltransferase AcbR. However, additional studies are needed to confirm the production capability of this metabolite. These findings suggest that endophytic bacterial isolates from local plants could potentially serve as candidate sources of bioactive compounds for diabetes therapy, reducing dependence on synthetic drugs. Furthermore, such exploration of local endophytic bacteria also strengthens local wisdom in the utilization of traditional plants for public health.

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