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Identification of rhizobacteria isolate from Bali Barat National Park, Indonesia and the potential as biological agents against soybean seed-borne pathogen

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Abstract. *Khalimi K, Pranatayana IBG, Yuliadhi KA, Gargita IWD, Yudha IKW. 2025. Identification of rhizobacteria isolate from Bali Barat National Park, Indonesia and the potential as biological agents against soybean seed-borne pathogen. Biodiversitas 26: 1799-1806.* The use of biological agents is a method of suppressing plant pathogenic fungi. Therefore, this study aimed to determine biochemical characteristics, identity, antifungal activity, and types of compounds produced by rhizobacteria isolates from *Taman National Bali Barat* (West Bali National Park/TNBB), Bali, Indonesia. Rhizobacteria was characterized using Microbact Biochemical Kits and molecular identification of rhizobacteria based on the 16S rRNA gene sequence analysis. Antifungal compounds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The results showed that rhizobacteria used gelatin and arabinose as carbon sources, producing indole, acetoin, urease, and tryptophan deaminase compounds. The identity of rhizobacteria isolate TNBB is *Bacillus thuringiensis*. Furthermore, rhizobacteria filtrate of TNBB isolate inhibited the growth of *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus aculeatus*, and *Rhizopus oryzae* fungi. The diameter of the inhibition zone formed was categorized as having very strong power. The filtrate of TNBB isolate rhizobacteria contained 16 antifungal compounds, namely 1,3,5-triazine-2,4,6-triamine, 2(1H)-pyridinone 6-hydroxy-, tridecanoic acid, N-acetyl-d-glucosamine, hexadecanoic acid, butanoic acid, pentyl ester, pentanoic acid butyl ester, pentanoic acid pentyl ester, linoleic acid, 1-naphthalene-sulfonic acid, 2-naphthalene-sulfonic acid, stearic acid, cyclotrisiloxane hexamethyl-, cyclotrisiloxane hexamethyl, 1,2-benzenedicarboxylic acid, diisooctyl ester, and benzo[h]quinoline, 2,4-dimethyl-. The results of this study provided information that TNBB isolate rhizobacteria are suitable for use as biological agents.

Keywords: *Bacillus thuringiensis*, biological agents, rhizobacteria, seed-borne pathogen, soybean

INTRODUCTION

Soybean is an important food commodity, necessitating an increased production need. The production of this food community can be increased using quality seed. However, the presence of seed-borne pathogen cause seed abortion, rot, necrosis, and decreased viability (Amza 2018). Some seed-borne pathogen that contribute significantly to the reduction of seed quality are *Aspergillus* species fungi such as flavus, parasiticus, niger, ochraceus, tubingensis, and aculeatus. *Aspergillus* species fungi contaminate soybean and bean seed, as well as decrease viability, vigor and produce mycotoxins that are harmful to human and animal health. Nnamani et al. (2021) reported that *Aspergillus flavus* and *A. parasiticus* fungi contaminate soybean seeds and were able to produce aflatoxin B1. According to Soesanto et al. (2020), *A. flavus* and *A. niger* were *Aspergillus* species that contaminate 8 varieties of soybean seed. Zhu et al. (2021) reported that *Aspergillus ochraceus* contaminated soybean seed and reduced protein levels in processed products made from soybean. Another study by Yao et al. (2024) confirmed that *Aspergillus tubingensis* produced aspergillopepsin I, which reduced protein levels in processed products made from soybeans. Ravn et al. (2015) reported that *Aspergillus aculeatus* contaminate soybean seed and the resulting processed products. In addition to *Aspergillus* species, *Alternaria alternata* and *Rhizopus oryzae* reduced the quality of soybean seed. Matniyazova et al. (2024) reported that *A. alternata* and *A. tenuissima* were pathogens that caused yield losses in soybean, chickpea, and mung bean plants. Furthermore, Souza et al. (2018) reported that *R. oryzae* fungus reduced the levels of amino and fatty acids in processed products made from soybeans.

An effort to suppress the population of pathogenic fungi is through the use of bacteria that act as biological agents. Several published studies examined the use of bacteria as biological agents against seed pathogenic fungi. Kazerooni et al. (2021) reported that *Bacillus amyloliquefaciens* inhibited the growth of *A. alternata* and caused damage to the hyphae through cell lysis mechanisms. Yuan et al. (2023) reported that *Bacillus subtilis* E11 was able to inhibit the growth of *A. flavus* with a power of 69.31% through in vitro testing. Santoso et al. (2020) reported that *Bacillus* sp. KRT inhibited the growth of *A. niger* with a power of 61.24% when compared to the control. Another study by Kryszczuk et al. (2023) reported that *Aureobasidium pullulans* PP3 and *Saitozyma podzolicus*

D10 inhibited the growth of *A. parasiticus* and *A. ochraceus* with an inhibitory power of 41.21% and 53.64%, respectively. According to Saleh et al. (2021), *Bacillus megaterium* BM344-1 was able to inhibit the growth of *Penicillium verrucosum* with an inhibitory power of 66.7% when compared to the control.

Biochemical characterization and molecular identification of bacteria intended as biological agents are crucial for determining the activity and identity in controlling seed-borne pathogenic fungi. An important biochemical characteristic of bacteria includes the metabolic activity of bacterial cell in using nutrients in the environment. Each bacterium has the ability to use enzymes to degrade carbohydrates, fats, proteins, and amino acids which produce useful products for bacterial identification and characterization (Bonnet et al. 2019). According to Garbeva and Weisskop (2020), bacteria use various organic and inorganic compounds for the metabolic processes. Yang et al. (2024) reported that the 16S rRNA gene sequence found in the ribosome had high variability. This gene sequence can be used to distinguish between bacterial species. The data for the 16S rRNA sequence is ideal for taxonomic classification and identification of bacterial species. Therefore, this study aimed to characterize rhizobacteria of *Taman National Bali Barat* (West Bali National Park/TNBB), Bali, Indonesia, isolated biochemically and identify the bacteria based on the 16S rRNA gene sequence analysis. Antifungal activity tests were also conducted to identify compounds in the filtrate of rhizobacteria of TNBB isolate.

MATERIALS AND METHODS

Sampling site

Rhizobacteria isolates were isolated from rhizosphere of *Pterospermum javanicum* plant roots in *Taman National Bali Barat* (West Bali National Park/TNBB) (8°11'8.0"S 114°26'44.0"E), Bali, Indonesia. The isolates of the bacteria are shown in Figure 1.

Biochemical characterization and molecular identification of isolated rhizobacteria

Biochemical characterization of TNBB rhizobacteria isolate was carried out using Microbact Identification Kits, Thermo Scientific. In this test, Microplate 1 consists of 12 tests, namely lysine (1), ornithine (2), H2S (3), glucose (4), mannitol (5), xylose (6), o-nitrophenyl-ß-d-galactopyranoside or ONPG (7), indole (8), urease (9), V-P (10), citrate (11), TDA (12). Microplate 2 also consists of 12 tests, namely gelatin (13), malonate (14), inositol (15), sorbitol (16), rhamnose (17), sucrose (18), lactose (19), arabinose (20), adonitol (21), raffinose (22), salicin (23) and arginine (24). For the working procedure, one 24-hour-old bacterial colony was taken and dissolved in 3 ml of saline solution, then 100 µL of bacterial suspension was added to each well on Microplate.

Two drops of mineral oil were added into the black well, then the seal was closed and incubated for 24 hours at 35°C. The Microplate was then moved and 2 drops of Kovacs reagent were added to well 8 and observed after 2 minutes. In well 10, 100 µL of Voges-Proskauer reagent was added and observed after 30 minutes. In well 12, 100 µL of Tryptophan Deaminase reagent was added and observed immediately. Similarly, in well 7, nitrate reagent was added when ONPG was positive. Molecular identification of TNBB rhizobacteria isolate was carried out based on the analysis of the 16S rRNA gene sequence.

DNA was isolated using Genejet and the amplification was carried out through 2x Kappa PCR Ready Mix (Kappa Biosystem) with specific specifications (63F 5'-CAG GCC TAA CAC ATG CAA GTC-3' and 1387R 5'-GGG CGG WGT GTA CAA GGC-3'). The 16S rRNA gene obtained was sequenced using the Dye terminator with the V.3.1 cycle unification kit. The sequences were compared with the database available at National Center for Biotechnology Information (NCBI) using the BLAST search engine (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was developed using MEGA program version 6.0, PAUP program version 4.0b, and Maximum Parsimony Method.

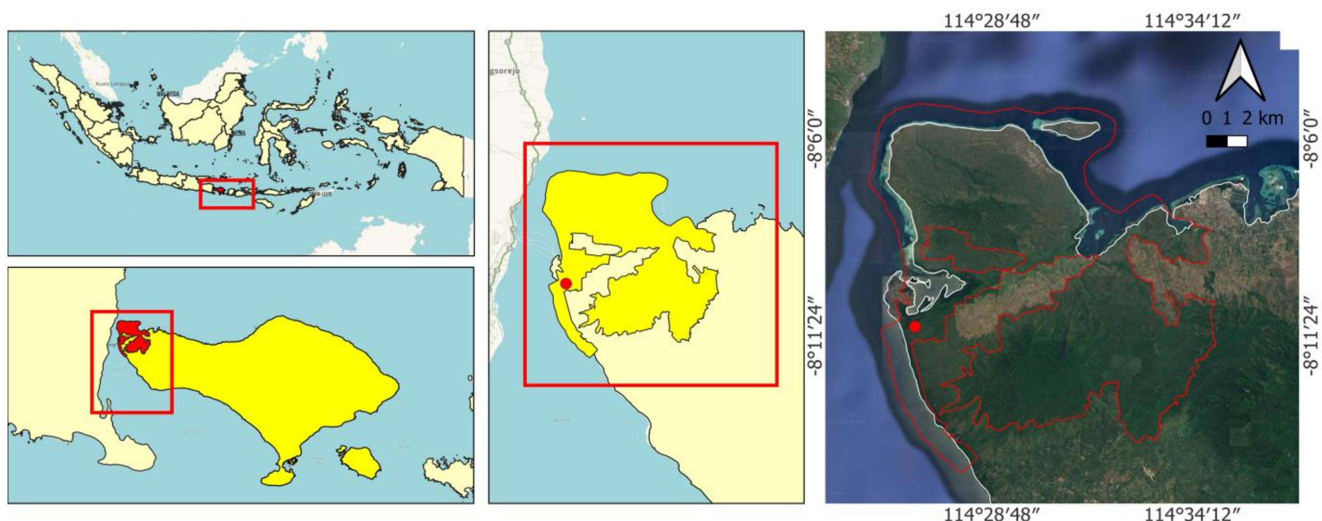

Figure 1. Sampling site, Balai Taman Nasional Bali Barat, Bali, Indonesia

Antifungal activity test of TNBB isolate rhizobacterial filtrate against soybean seed-borne pathogenic fungi

Antifungal activity test of TNBB rhizobacterial filtrate against the growth of seed-borne pathogenic fungi was carried out using the paper disc diffusion method. Seed-borne pathogenic fungi used were *A. alternata*, *A. flavus*, *A. parasiticus*, *R. oryzae*, *A. ochraceus*, *A. niger*, *A. tubingensis*, and *A. aculeatus*. This test was carried out by pouring 1 mL of each seed-borne pathogenic fungal suspension into a petri dish. Furthermore, 10 ml of PDA media was poured into the petri dish. After the media solidified, a 6 mm diameter paper disc that had been previously soaked in rhizobacterial filtrate of TNBB isolate was inoculated. The filtrate was obtained from rhizobacterial culture of TNBB isolate that had been shaken for 14 days and filtered with a 0.45 µm Millipore membrane (Nihon Millipore Ltd. Yonezawa). The area not overrun by fungi was designated as the inhibition zone. According to Paudel et al. (2014), the inhibition zones formed in the media can be categorized into four categories. The inhibition zones of less than 10 mm were categorized as having weak inhibition. Meanwhile, 10-15, 15-20, and > 20 mm were categorized as having moderate, strong, and very strong inhibition, respectively.

Identification of antifungal compounds using Gas Chromatography-Mass Spectrometry (GC-MS)

Antifungal compounds from rhizobacterial filtrate of TNBB isolate were identified using GC-MS (7890A GC-system 5975C inert XL E1/C1 MSD model G3174A, Agilent Technologies, Wilmington, DE, USA). A total of 2 µL of rhizobacterial filtrate extract sample solution of TNBB isolate was injected into GC-MS. The injector temperature was maintained at 240°C for 26 minutes. Identification of antifungal compounds was carried out through comparison with the WILLEY09TH.L library and the chemical names of the results of this analysis followed the database nomenclature.

RESULTS AND DISCUSSION

Biochemical characterization of TNBB isolated rhizobacteria

The results of the antagonistic test of 100 rhizobacterial isolates obtained from the roots of *P. javanicum* identified one isolate with the highest percentage of inhibition against the growth of Soybean Seed-borne Pathogenic Fungi. This isolate is designated as TNBB (Data not included). Subsequently, the TNBB rhizobacterial isolate was biochemically characterized. The results of biochemical characterization test, conducted using Microbact Biochemical Identification Kits, showed that TNBB isolate rhizobacteria did not use lysine, ornithine, or arginine as nitrogen sources. Additionally, these bacteria did not use glucose, mannitol, xylose, citrate, malonate, inositol, sorbitol, rhamnose, sucrose, lactose, adonitol, raffinose, and salicin as carbon sources in the metabolic processes, as shown in Table 1. TNBB isolate rhizobacteria can use gelatin and arabinose as carbon sources in the metabolism. This shows that bromothymol blue does not form the specific cadaverine amine. TNBB Rhizobacteria isolate did not use ornithine, as shown by the absence of a color change from yellow to light blue. This result suggested that there was no change in pH because bromothymol blue experienced less decarboxylation of lysine compared to the formation of the specific amine putrescine.

TNBB rhizobacteria isolate H_2S from the decomposition of thiosulfate. H_2S production can be determined by the presence of metal sulfite or black sediment in the test well, showing a reaction of H_2S with iron salts. TNBB isolate rhizobacteria do not use glucose, mannitol, xylose, citrate, inositol, sorbitol, rhamnose, sucrose, lactose, adonitol, raffinose, and salicin as carbon sources in the metabolic process. This is evidenced by the absence of a color shift to yellow, showing the pH remains alkaline rather than acidic, which prevents bromothymol blue from turning yellow. Meanwhile, TNBB rhizobacteria isolate using arabinose as a carbon source in the metabolic process, evident by a color change from blue to yellow. This suggests a change in pH from alkaline to acidic, thereby turning bromothymol blue to yellow, as shown in Figure 2.

TNBB rhizobacteria isolate produces galactosidase enzyme that hydrolyzes o-nitrophenyl-ß-d-galactopyranoside (ONPG) into galactose and nitrophenol. Consequently, the color changes from clear to yellow, showing the release of ortho-onitrophenol from the hydrolysis of the galactosidase enzyme. This isolate also produces proteolytic enzymes that melt gelatin as well as indole compounds, shown by a change in color from clear to pink. Tryptophan deaminase (TDA) enzyme is also produced, shown by a color change from clear to cherry red. This change shows that the tryptophan deaminase enzyme hydrolyzes tryptophan into indole pyruvic acid.

Table 1. Biochemical test results with microbact biochemical identification kits

Substrate	Reaction results
Lysine	Negative
Ornithine	Negative
H_2S	Positive
Glukosa	Negative
Mannitol	Negative
Xylosa	Negative
ONPG	Positive
Indole	Positive
Urease	Positive
VP	Positive
Citrate	Negative
TDA	Positive
Gelatin	Positive
Malonate	Negative
Inositol	Negative
Sorbitol	Negative
Rhamnosa	Negative
Sukrosa	Negative
Laktosa	Negative
Arabinosa	Positive
Adonitol	Negative
Raffinosa	Negative
Salicin	Negative
Arginine	Negative

Furthermore, rhizobacteria isolate produces acetoin, resulting from glucose decomposition. This acetoin production test identifies the presence of 2,3-butanediol by detecting acetoin, a precursor, thereby confirming 2,3-butanediol as a fermentation product. Acetoin added with alpha-naphthol and 40% KOH will become diacetyl and keratin, ensuring that the test well is pink. TNBB isolate rhizobacteria do not use malonate as a carbon source in the metabolic process, as shown by the absence of a color change from green to blue. The use of Na malonate combined with ammonium sulfate as a nitrogen source produces sodium hydroxide, thereby increasing alkalinity and a blue color. TNBB isolate rhizobacteria hydrolyze urea, which is determined by the change in phenol color from yellow to red. This shows that TNBB rhizobacteria isolate releases ammonium from the urea separation process.

Molecular identification of TNBB rhizobacteria isolate

The amplification results of the 16S rRNA gene in the TNBB rhizobacterial isolate show a DNA fragment measuring 1500 bp. This amplification is presented in the electropherogram, as shown in Figure 3. Rhizobacteria are included in *Bacillus thuringiensis* group because TNBB isolate is homologous to several strains with a maximum identity level of 100%. Some of these strains include *B. thuringiensis* RII2-97 (LT604455.1), *B. thuringiensis* strain RII2-66 (LT604435.1), *B. thuringiensis* RII2-56 (LT604428.1), RI2-29 (LT604374.1), RII1-32 (LT604314.1), RII1-31 (LT604313.1), RII2-100 (LT604457.1), RII2-65 (LT604434.1), RII2-16 (LT604412.1), and S2-104 (LT604346.1), as shown in Table 2.

The results of the phylogenetic tree analysis using Maximum Parsimony method with 1,000 Bootstrap repetitions showed that rhizobacteria of TNBB isolate were *B. thuringiensis.* Based on the results of the phylogenetic analysis using the Maximum Parsimony method with 1,000 bootstrap replications, the TNBB isolate exhibits a close relationship with the *B. thuringiensis* group. However, in the obtained dendrogram, the TNBB isolate forms a distinct branch that is slightly separated from the main clade of *B. thuringiensis,* as shown in Figure 4.

Although it shares 100% similarity in the BLAST analysis with several *B. thuringiensis* strains, its phylogenetic position in the tree suggests the possibility of genetic differences that have not been clearly identified. This may indicate that the TNBB isolate is a variant or sub-population of *B. thuringiensis* that has undergone slight differences in its 16S rRNA sequence. Thus, while the TNBB isolate is classified within the *B. thuringiensis* group, the phylogenetic results indicate that it occupies a slightly distinct branching position, which may reflect variations in certain genetic aspects. Therefore, additional analyses, such as whole-genome sequencing or phenotypic characterization**,** are required to ensure more accurate classification.

Table 2. Comparison of the percentage of similarity of the 16S rRNA gene of rhizobacteria isolates TNBB with several DNA sequences in Genbank using the BLAST program

Bacillus thuringiensis	Similarity percentage (%)	Accession number
B. thuringiensis strain RII2-97	100	LT604455.1
B. thuringiensis strain RII2-66	100	LT604435.1
B. thuringiensis strain RII2-56	100	LT604428.1
B. thuringiensis strain RI2-29	100	LT604374.1
B. thuringiensis strain RII1-32	100	LT604314.1
B. thuringiensis strain RII1-31	100	LT604313.1
B. thuringiensis strain RII2-100	100	LT604457.1
B. thuringiensis strain RII2-65	100	LT604434.1
B. thuringiensis strain RII2-16	100	LT604412.1
B. thuringiensis strain S2-104	100	LT604346.1

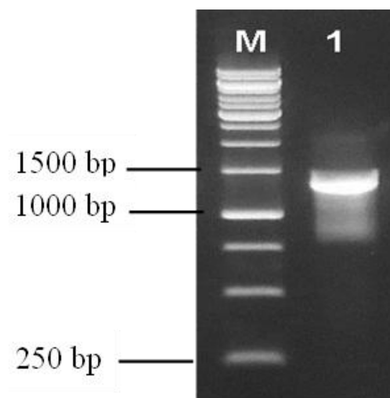

Figure 3. Amplicon of the 16S rRNA gene of TNBB rhizobacteria isolate. M. 1 Kb DNA marker, 1. TNBB rhizobacteria isolate DNA

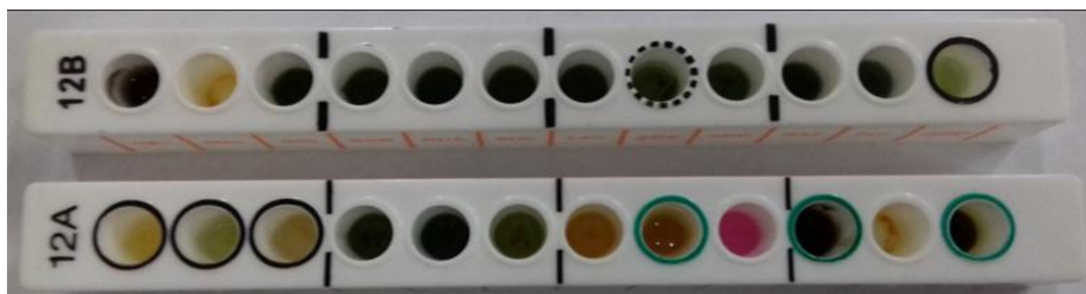

Figure 2. Reaction results in the microbact test

Antifungal activity test of TNBB isolate rhizobacterial filtrate against soybean seed-borne pathogenic fungi

The results of antifungal activity test showed that rhizobacterial filtrate of TNBB isolate inhibited the growth of *A. alternata*, *A. flavus*, *A. parasiticus*, *R. oryzae*, *A. ochraceus*, *A. niger*, *A. tubingensis*, and *A. aculeatus* fungi (Table 3). In this case, the inhibition zone diameter ranging from 22.75 mm to 27.62 mm was categorized as very strong, according to Paudel et al. (2014).

Rhizobacterial filtrate of TNBB isolate has antifungal activity, shown by the formation of an inhibition zone around the disc paper (Figure 5). Kadjo et al. (2023) reported that *B. thuringiensis* strain ATCC 10792 had antifungal activity against *Aspergillus carbonarius*. Furthermore, He et al. (2020) reported that *B. thuringiensis* BCN10 had antifungal activity against *Fusarium oxysporum*, *Botryosphaeria* sp., *Trichoderma atroviride*, *Colletotrichum gloeosporioides*, and *Penicillium expansum*. Hashem et al. (2022) also stated that *B. thuringiensis* MAE6 has antifungal activity against *A. niger* RCMB 02724, *A. terreus* RCMB 02574, *A. flavus* RCMB 02782, and *A. fumigatus* RCMB 02568.

Based on the results of GC-MS analysis, rhizobacterial filtrate of TNBB isolates contains 16 compounds, namely 1,3,5-triazine-2,4,6-triamine, 2(1H)-pyridinone, 6-hydroxy-, tridecanoic, N-acetyl-d -glucosamine, hexadecanoic, butanoic, pentyl ester, pentanoic acid butyl ester, pentanoic acid pentyl ester, linoleic, 1-naphthalene-sulfonic, hexamethyl -, 2-naphthalene-sulfonic, stearic, cyclotrisiloxane, and 1,2-benzenedicarboxylic acide, as well as hexamethyl-, cyclotrisiloxane, diisooctyl ester, and benzo[h]quinoline, 2,4-dimethyl- (Table 4).

Compound 1,3,5-triazine-2,4,6-triamine was detected in peak 1 at a retention time of 4.94 minutes with an area percentage of 0.35%, as shown in Figure 6. Chadotra and Baldaniya (2019) reported that this compound had antifungal activity against *Candida albicans*, *A. niger,* and *A. clavatus*. Compound 2(1H)-pyridinone, 6-hydroxy- was detected in peak 2 at a retention time of 5.24 minutes with an area percentage of 0.33%. Similarly, compound 2(1H)-pyridinone had antifungal activity against *C. albicans* (Lin et al. 2022; Mena et al. 2022). Tridecanoic acid compound was detected in peak 3 at a retention time of 5.65 minutes with an area percentage of 4.22%.

Table 3. Results of antifungal activity test of filtrate of TNBB rhizobacteria isolate against soybean seed-borne pathogenic fungi

Seed-borne pathogenic fungi	Inhibition zone diameter (mm)
Alternaria alternata	22.75 ± 0.25
Aspergillus flavus	26.37 ± 0.121
Aspergillus parasiticus	25.72 ± 0.122
Rhizopus oryzae	27.62 ± 0.131
Aspergillus ochraceus	26.5 ± 0.229
Aspergillus niger	23.33 ± 0.241
Aspergillus tubingensis	24.42 ± 0.141
Aspergillus aculeatus	25.92 ± 0.162

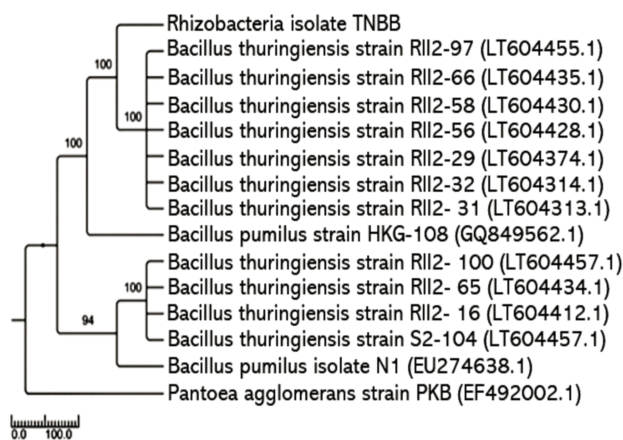

Figure 4. The position of TNBB isolate rhizobacteria in the dendrogram is included in *B. thuringiensis* group and *Pantoea agglomerans* as the outgroup

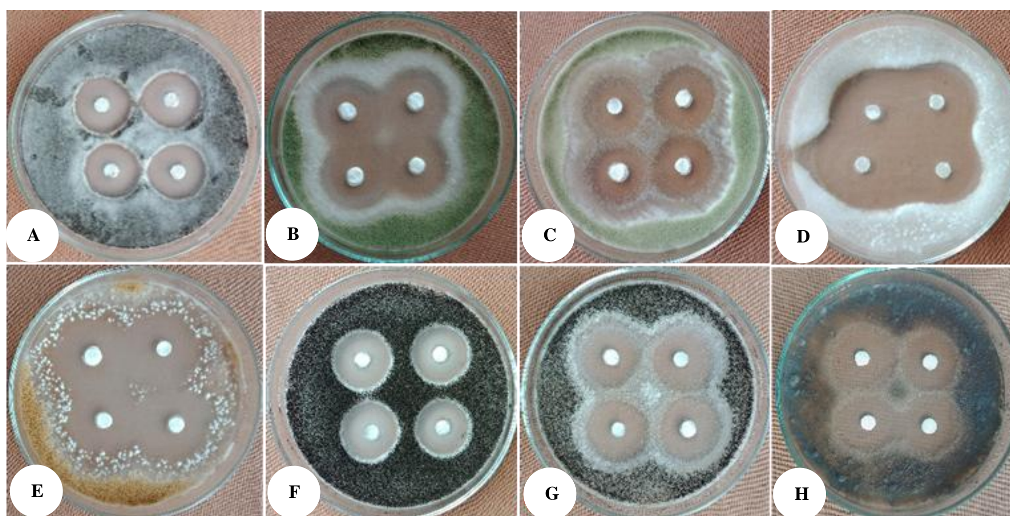

Figure 5. Results of antifungal activity test of TNBB isolate rhizobacteria filtrate. A. *Alternaria alternata*; B. *Aspergillus flavus*; C. *Aspergillus parasiticus*; D. *Rhizopus oryzae*; E. *Aspergillus ochraceus*; F. *Aspergillus niger*; G. *Aspergillus tubingensis*; H. *Aspergillus aculeatus*

Table 4. Results of compound identification in TNBB isolate rhizobacterial filtrate

Peak	Identified compounds	Retention time (minutes)	Area (%)	Chemical formula
1	1,3,5-Triazine-2,4,6-triamine	4.94	0.35	$C_3H_6N_6$
2	2(1H)-Pyridinone, 6-hydroxy-	5.24	0.33	$C_5H_5NO_2$
3	Tridecanoic acid	5.65	4.22	$C_{13}H_{26}O_2$
4	N-acetyl-d-glucosamine	5.95	5.57	$C_8H_{15}NO_6$
5	Hexadecanoic acid	6.23	5.36	$C_{16}H_{32}O_2$
6	Butanoic acid, pentyl ester	6.72	4.61	$C_9H_{18}O$
7	Pentanoic acid, butyl ester	6.86	3.54	$C_9H_{18}O$
8	Pentanoic acid, pentyl ester	7.15	3.27	$C_{10}H_{20}O$
9	Linoleic acid	7.63	4.14	$C_{18}H_{32}O_2$
10	1-Naphthalene-sulfonic acid	8.12	1.47	$C_{10}H_8O_3S$
11	2-Naphthalene-sulfonic acid	9.55	0.54	$C_{10}H_8O_3S$
12	Stearic acid	14.98	0.14	$C_{18}H_{36}O_2$
13	Cyclotrisiloxane, hexamethyl-	16.73	26.15	$C_6H_{18}O_3Si_3$
14	Cyclotrisiloxane, hexamethyl-	19.88	21.02	$C_6H_{18}O_3Si_3$
15	1,2-benzenedicarboxylic acid, diisooctyl ester	21.28	14.15	$C_{24}H_{38}O_4$
16	Benzo[h]quinoline, 2,4-dimethyl-	22.45	5.15	$C_{15}H_{13}N$

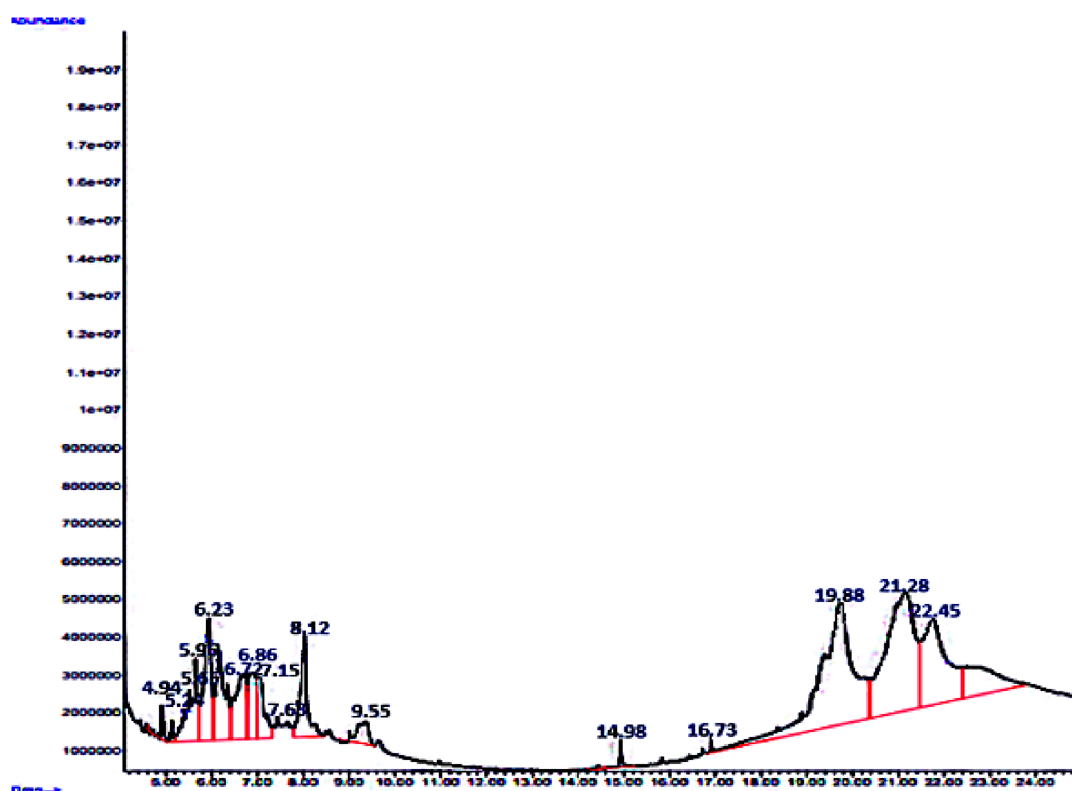

Figure 6. Representative GC-MS chromatography data on rhizobacterial filtrate of TNBB isolate

According to Patel et al. (2024), the tridecanoic acid methyl ester compound produced by *Bacillus* spp. had antifungal activity against *Fusarium graminearum* and *Macrophomina phaseolina*. N-acetyl-d-glucosamine was detected in peak 4 at a retention time of 5.95 minutes with an area percentage of 5.57%. Seyfarth et al. (2008) reported that this compound had antifungal activity against *C. albicans*, *C. krusei,* and *C. glabrata*. The hexadecanoic acid compound was detected in peak 5 at a retention time of 6.23 minutes with an area percentage of 5.36%. Prasath et al. (2020) reported that the hexadecanoic acid compound had antifungal activity against *C. tropicalis*.

Butanoic acid, pentyl ester compounds were detected in peak 6 at a retention time of 6.72 minutes with an area percentage of 4.61%. Meanwhile, Minervini et al. (2019) reported that *Lactobacillus plantarum* A2 produced butanoic acid, pentyl ester compounds which were antimicrobial against *Micrococcus luteus* and *Listeria monocytogenes*. Pentanoic acid, butyl ester compounds were also detected in peak 7 at a retention time of 6.86 minutes with an area percentage of 3.54%. In peak 8, pentanoic acid, pentyl ester compounds were detected at a retention time of 7.15 minutes with an area percentage of 3.27%. A previous study by Minervini et al. (2019)

reported that *Lactobacillus paraplantarum* produced pentanoic acid compounds, which were antimicrobial against *M. luteus* and *L. monocytogenes*. The linoleic acid compound was detected in peak 9 at a retention time of 7.63 minutes with an area percentage of 4.14%. According to Guimaraes and Venancio (2022), linoleic acid has antifungal activity against *A. niger*, *C. albicans*, *C. valida*, *Pichia membranaefaciens*, *P. roqueforti* and *Saccharomyces cerevisiae*. Compound 1-Naphthalene-sulfonic and Naphthalene-sulfonic acid were detected in peaks 10 and 11. The retention time of these compounds was 8.12 and 9.55 minutes with an area percentage of 1.47% and 0.54%, respectively.

Saleh et al. (2022) reported that spiro-naphthalene-1,2'-[1,3,4] oxadiazol-4-ones was a naphthalene derivative compound that has antifungal activity against *C. albicans*. The stearic acid compound was detected in peak 12 at a retention time of 14.98 minutes with an area percentage of 0.14%. According to Hawar et al. (2023), *Paecilomyces* sp. (JN227071.1) produced a stearic acid compound that had antifungal activity against *Rhizoctonia solani*. Cyclotrisiloxane, hexamethyl- compound was detected in peaks 13 and 14 at retention times of 16.73 minutes and 19.88 minutes with an area percentage of 26.15% and 21.02%, respectively. *Bacillus velezensis* CE100 produced cyclotrisiloxane, a hexamethyl- compound that had antifungal activity against *Colletotrichum gloeosporioides* (Choub et al. 2022). The compound 1,2-benzenedicarboxylic acid, diisooctyl ester was detected in peak 15 at a retention time of 21.28 minutes with an area percentage of 14.15%. According to Zeatar et al. (2022), the compound 1,2-benzenedicarboxylic acid, diisooctyl ester produced by *Streptomyces hypolithicus* HSM had antifungal activity against *C. albicans* ATTC 90028, *A. niger*, *Fusarium equiseti*, and *F. proliferatum*. Benzo[h]quinoline, 2,4-dimethyl- was detected in peak 16 at a retention time of 22.45 minutes with an area percentage of 5.15%. Antoci et al. (2021) reported that the compound benzo[f]quinoline had antifungal activity against *C. albicans* ATCC10231.

Sixteen antifungal compounds produced by TNJ isolate rhizobacteria worked synergistically to inhibit the growth of soybean seed-borne pathogenic fungi. Compounds in the fatty acid group, such as tridecanoic, hexadecanoic, butanoic pentyl ester, pentanoic butyl ester, pentyl ester, linoleic, 1-naphthalene-sulfonic, 2-naphthalene-sulfonic, stearic, and 1,2-benzenedicarboxylic diisooctyl ester work simultaneously with 1,3,5-triazine-2,4,6-triamine, 2(1H)-pyridinone, 6-hydroxy-, N-acetyl-d-glucosamine, cyclotrisiloxane hexamethyl-, and benzo[h]quinoline, 2,4-dimethyl- in inhibiting the growth of pathogenic fungi transmitted to soybean seeds. The mechanism of fatty acid compounds in inhibiting fungal growth is by disrupting the permeability of cell membranes, specifically in those with low sterol content. This process inhibits the work of the topoisomerase enzyme which plays an important role in DNA replication in the nucleus and the translation process in the ribosome. Consequently, the formation of the N-myristoyltransferase enzyme plays an important role in the translation process in the ribosome is inhibited. This process inhibits β-oxidation in mitochondria (Guimaraes and Venancio 2022) as well as the formation of triacylglycerol and sphingolipids in mitochondria, causing damage (Prasath et al. 2020; Guimaraes and Venancio 2022). Apoptosis was also induced, causing DNA damage (Prasath et al. 2020), and inhibiting ergosterol biosynthesis (Lee et al. 2020).

The mechanism of the compounds 1,3,5-triazine-2,4,6-triamine and 2(1H)-pyridinone 6-hydroxy-, N-acetyl-d-glucosamine in inhibiting fungal growth is by disrupting the formation of chitin and α-mannan in the cell walls of *C. albicans* (Mena et al. 2022). Meanwhile, Xie et al. (2022) reported that the 1,3,5-triazine derivative compound inhibited the formation of *C. albicans*, *C. neoformans*, and *C. parapsilosis* biofilms. Benzo[h]quinoline 2,4-dimethyl- also inhibits the process of fungal DNA replication by disrupting the activity of enzymes topoisomerase II and ATP synthase (Antoci et al. 2021). Pippi et al. (2017) reported that 8-Hydroxyquinoline and the derivatives induced cell death in fungal by inhibiting the activity of enzymes in DNA and RNA synthesis. The mechanism of action is to inhibit spore germination and the growth of fungal mycelium (Choub et al. 2022).

In conclusion biochemical characteristics of TNBB rhizobacteria isolate in cell metabolism use gelatin and arabinose as carbon sources, producing indole compounds, acetoin, urease enzymes, and tryptophan deaminase enzymes. TNJ Rhizobacteria isolate has been identified as *B. thuringiensis*, given the homology to *B. thuringiensis* sequences in GenBank, which exhibit a maximum identity of 100%. The filtrate of TNBB rhizobacteria isolate inhibits the growth of *A. alternata*, *A. flavus*, *A. parasiticus*, *R. oryzae*, *A. ochraceus*, *A. niger*, *A. tubingensis*, and *A. aculeatus* fungi. In this case, the diameter of the inhibition zone formed was categorized as very strong. In conclusion, TNBB isolate rhizobacteria filtrate contained 16 antifungal compounds, namely 1,3,5-triazine-2,4,6-triamine, 2(1H)-pyridinone 6-hydroxy-, tridecanoic acid, N-acetyl-d-glucosamine, hexadecanoic acid, butanoic acid, pentyl ester, pentanoic acid butyl ester, pentanoic acid pentyl ester, linoleic acid, 1-naph thalene-sulfonic acid, 2-naphthalene-sulfonic acid, stearic acid, cyclotrisiloxane hexamethyl-, cyclotrisiloxane hexamethyl-, 1,2-benzenedicarboxylic acid, diisooctyl ester, and benzo[h]quinoline, 2,4-dimethyl-.

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