JURNAL KIMIA VALENSI
p-ISSN: 2460-6065; e-ISSN: 2548-3013
Journal homepage: https://journal.uinjkt.ac.id/index.php/valensi
Research Article

Isolation, Characterization, and Identification of Root-Associated Endophytic Bacteria Producing Chitinase from Strawberry Plants

Nur Aulia[1*], Hasnah Natsir[2], Ahyar Ahmad[2], Nunuk Hariani Soekamto[2], Abd. Wahid Wahab[2], Rugaiyah Andi Arfah[2], Harningsih Karim[3]

[1]*Master's Program, Chemistry Department, Mathematics and Natural Sciences Faculty, Hasanuddin University, Makassar, 90245, Indonesia*
[2]*Chemistry Department, Mathematics and Natural Sciences Faculty, Hasanuddin University, Makassar, 90245, Indonesia*
[3]*Pharmacy Department, School of Pharmacy Yayasan Ma'bullo Sibatang, Makassar, 90222, Indonesia*

[*]*Email: aulianur934@gmail.com*

Article Info
Received: Oct 01, 2025
Revised: Dec 07, 2025
Accepted: Feb 01, 2026
Online: May 31, 2026

Citation:
Aulia, N., Natsir, H., Ahmad, A., Soekamto, N, H., Wahab, A. W., Arfah, R.A., Karim, H. (2026). Isolation, Characterization, and Identification of Root-Associated Endophytic Bacteria Producing Chitinase from Strawberry Plants. *Jurnal Kimia Valensi*, 12(1), 14-25.

Doi: 10.15408/jkv.v12i1.46618

Abstract

Endophytic bacteria inhabiting root tissues represent a promising resource for biotechnological applications, particularly as chitinase producers for biological control. This study aimed to isolate, characterize, and identify chitinase-producing endophytic bacteria from strawberry (*Fragaria* spp.) roots. Root samples underwent surface sterilization and serial dilution before being plated on selective media containing a chitin colloidal substrate. They were then characterized morphologically and identified molecularly through analysis of the 16S rRNA gene. The initial screening on agar medium with a chitin colloidal substrate at 37 °C yielded four bacterial isolates. Two of the bacterial isolates showed high chitinolytic index (CI), where isolate RS-A1 had a CI (1.55) and isolate RS-A2 had a CI (1.65). The electrophoresis of the 16S rRNA PCR product showed an amplicon of approximately 1300 bp. BLAST analysis against GenBank showed that isolate RS-A1 had 98.06% similarity to *Acinetobacter baumannii*, whereas isolate RS-A2 had 97.21% similarity to *Bacillus aerius*. The quantitative result of specific chitinase activity was highest in the RS-A2 isolate at 0.01146 U/mg, while that of the RS-A1 isolate was 0.00781 U/mg. These results indicate that endophytic bacteria from strawberry plant roots have the potential to act as biocontrol agents.

Keywords: *Acinetobacter baumannii, Bacillus aerius*, chitinase, endophytic bacteria

1. INTRODUCTION

One of the biggest challenges in agriculture today is finding ways to increase crop productivity and reduce losses without compromising the quality of agricultural products due to pathogen attacks. The use of synthetic pesticides is still often chosen as a solution in modern agriculture to tackle pathogen issues in crops; however, the use of synthetic pesticides has side effects on health, such as increasing oxidative stress, disrupting the endocrine system, and modulating gene expression, which can lead to health problems, including cancer [1]. In addition to causing negative health effects, the use of synthetic pesticides also leads to environmental issues such as soil and water pollution[2]. Consequently, environmentally sustainable approaches are required to support long-term agricultural practices and address pathogen- or disease-related issues in crops. One potential strategy is the utilization of endophytic bacteria that inhabit internal plant tissues and contribute to plant growth and health as biological control agents.

Recent research shows that endophytic bacteria hold substantial promise as biofungicides, biofertilizers, and biostimulators against pest and disease attacks in future agricultural applications [3]. Several studies found that endophytic bacteria in tomato plants have potential as biocontrol agents [4]. This is because isolated endophytic bacteria can produce secondary metabolites and chitinase, which have antifungal activity and promote plant growth. This is in line with research reporting that volatile organic compounds were successfully produced by

Pseudomonas sp. isolates, including 2-undecanone, methyl thiocyanate, 2-undecanol, and 2-nonanol. Among these four compounds, 2-undecanone showed antifungal activity against *V. dahliae*, while bacterial isolates of *P. simiae* produced several volatile organic compounds, including tridecane, 2,5-dimethylpyrazine, 1-decene, and 2-decyloxirane, and it has been reported that these compounds have antifungal activity against several phytopathogens such as *Pseudomonas syringae* pv., *Penicillium italicum*, *Sclerotinia minor*, *Pythium ultimum*, *R. solani*, or *B. cinerea* (Montes-Osuna et al., 2022). Chitinase, also produced by *Bacillus pumilus,* has been genetically introduced into *Bacillus subtilis* using protein coating techniques to naturally inhibit the growth of plant pathogens, making it highly suitable for development as a new biopesticide [5].

Chitinase is an enzyme that hydrolyzes chitin into its monomers. The main component of fungal cell walls is chitin compounds[6]. Therefore, in the context of plant disease control, chitinase acts as an antifungal agent by degrading chitin in fungal cell walls. Chitinase can be found in various organisms, including bacteria, fungi, plants, and animals, where chitinase is part of the plant's natural defense mechanism that helps activate responses to pathogen attacks [7–9].

Strawberries (*Fragaria spp*.) are horticultural plants in the genus *Fragaria*, whose fruits are widely favored by the public but are highly susceptible to diseases caused by pathogenic fungi, making them an interesting subject for this study. Strawberry fruits contain volatile organic compounds (VOCs), phenolic compounds, vitamin C, and also have antioxidant activity [10,11]. Research found that endophytic bacteria were also present in the roots of strawberry plants and have potential as antifungal agents [12].

The issues outlined above form the background of this study. This study aims to isolate and identify endophytic bacteria from strawberry plant roots and to characterize the chitinase produced by the endophytic bacterial isolates. Therefore, further exploration is necessary to understand their potential as antifungal agents capable of controlling plant diseases caused by pathogenic fungi. The results of this study are intended to contribute scientifically to the development of innovative, effective, environmentally friendly, healthier, and more sustainable solutions for humans and the environment in managing plant diseases. Additionally, this study supports efforts to reduce reliance on synthetic pesticides.

2. RESEARCH METHODS

Materials

Strawberry plant roots collected from the Malino area, Gowa regency, South Sulawesi province, Indonesia as a source of chitinolytic endophytic bacterial isolates, colloidal chitin as the enzyme substrate, distilled water, yeast extract, bacto agar, bacto peptone, peptone, Potato Dextrose Agar (PDA) (Merck Millipore), Aqua Pro Injection, NaOH (Merck Millipore), HCl (Merck Millipore), $(NH_4)_2SO_4$, NaH_2PO_4, Na_2CO_3, K_2HPO_4, NaCl, $CuSO_4.5H_2O$, $K_3[Fe(CN)_6]$, $MgSO_4.7H_2O$, N-acetyl-glucosamine (Sigma-Aldrich), Schales reagent, Crystal Violet solution (Himedia) as the primary stain, Gram's Iodine solution (Himedia) as the mordant, and 95% acetone alcohol decolorizer (Merck), Safranin solution (Himedia), Primer Forward 63f: 5'-CAG GCC TAA CAC ATG CAA GTC-3. Reverse 1387r: 5'-GGG CGG WGT GTA CAA GGC-3. PCR Enzyme (Go Taq Master Mix Green), RNAse Free water, Agarose (Sigma-Aldrich), Ethidium Bromide (Merck Millipore), Buffer TBE 0.5, DNA Leader / Marker (100 bp) (Geneaid).

Instruments

Laminar Air Flow Cabinet (LAFC, model JSCB-900SB), analytical balance (Ohaus), centrifuge (Hermle), autoclave (model 8000-DSE Napco), shaker water bath (Memmert), gel electrophoresis set (Bio-Rad), UV-Vis spectrophotometer (Genesys 20), vibrating platform shaker (Heidolph Titramax 1000), Polymerase Chain Reaction (PCR) machine (Bio-Rad), laboratory glassware (Pyrex), and refrigerator (Toshiba).

Isolation of Endophytic Bacteria from Strawberry Plant Roots

Endophytic bacteria were isolated from strawberry roots rinsed with distilled water. Surface sterilization was performed in a Laminar Air Flow Cabinet (LAFC) using 70% ethanol, 1% sodium hypochlorite, and injection-grade sterile water to eliminate external contaminants. Approximately 1 g of fresh strawberry root tissue was inoculated into 90 mL of Nutrient Broth (NB) and incubated at 180 rpm for 12 hours at 37 °C. The strawberry roots were then ground with the addition of 3 mL of sterile injection water. The homogenized root mixture was collected with a sterile pipette and transferred into sterile NB medium, then incubated with shaking for 2 hours. After mixing the homogenate, 1 mL of the bacterial suspension (supernatant) was collected using a sterile micropipette and transferred into a test tube containing 9 mL of NB. Serial dilution was performed up to a dilution factor of 10^{-8}. After dilution, 0.1 mL of the 10^{-4} to 10^{-8} dilutions was spread on Nutrient Agar (NA) media supplemented with 0.1% L-tryptophan (Raut et al., 2017). Incubation was then carried out for 24 hours at 30 °C.

Bacterial Clear Zone Testing

Screening for chitinase-producing bacteria can be performed on agar media containing colloidal chitin. The isolates are streaked on media containing 0.1% $(NH_4)_2SO_4$, 0.01% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 3% NaCl, 0.5 g yeast extract, 15 g agar, 1% colloidal chitin, and then incubated at pH 7 and 37°C for 72 hours. Colonies that show a clear zone around the bacteria indicate the presence of chitinase-producing bacteria [13].

Bacterial Identification

Selected bacterial isolate cultures were identified based on colony morphology (color, shape, margin elevation, and pigmentation), microscopic observations (cell arrangement and Gram staining). In addition, bacteria were also identified using 16S rRNA amplification [14].

DNA Extraction

Genomic DNA was extracted using a commercial extraction kit following the manufacturer's protocol. Bacterial cells were first pelleted by centrifugation at 14,000–16,000 rpm for 3 minutes. The supernatant was discarded, and the cell pellet was resuspended in GST buffer containing proteinase K, then vortexed and incubated at 60 °C to ensure effective cell lysis. Subsequently, the GSB buffer was added, and the mixture was incubated again at 60 °C to facilitate further breakdown of cellular components. For DNA binding, ethanol was added to the lysate, and the mixture was transferred into a GS column. After centrifugation, the bound DNA was washed sequentially with W1 buffer and Wash buffer to remove residual contaminants. The column was then centrifuged in a new collection tube to remove any remaining ethanol. Genomic DNA was finally eluted using pre-warmed elution buffer, yielding purified DNA suitable for downstream PCR amplification.

PCR Amplification of the 16S rRNA Gene

Amplification of the bacterial 16S rRNA gene was performed in a total reaction volume of 50 µL containing GoTaq Master Mix, the universal bacterial primers 63f(forward) and 1387r (reverse), nuclease-free water, and template DNA. The PCR cycling conditions consisted of an initial denaturation at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 90 s. A final extension was carried out at 72 °C for 20 minutes to ensure complete synthesis of the amplicons. In this study, molecular analysis was performed up to the verification of PCR products. The confirmed amplicons were then submitted for sequencing through a commercial sequencing service (PT. Genetika Science Indonesia).

Gel Electrophoresis

PCR products were analyzed using 2% agarose gel electrophoresis prepared in 0.5× TBE buffer. Agarose was dissolved by heating, slightly cooled, supplemented with ethidium bromide, poured into a casting tray, and allowed to solidify. PCR samples were mixed with loading dye and loaded into the gel wells alongside a 100 bp DNA ladder used as a molecular size marker. Electrophoresis was conducted at 100 V for approximately 60 minutes. The resulting DNA bands were visualized under UV illumination. Successful amplification was determined by the presence of a clear band of the expected size corresponding to the positive control.

Determination of the Growth Curve of Chitinolytic Bacteria

The growth curve of chitinolytic bacteria was conducted by measuring bacterial growth every 12 h. The selected isolate was inoculated into 20 mL of inoculum medium. Luria Broth medium was prepared by mixing 10 g of tryptone, 10 g of yeast extract, 0.1 g of NaCl, and 1000 mL of distilled water. The medium was then incubated at 30°C for 24 hours while being shaken at 150 rpm. A total of 2 mL of the inoculum solution was added to 20 mL of the same medium in a 100 mL Erlenmeyer flask, and the flask was incubated under the same conditions. Then, 2 mL samples were taken every 2 hours to measure the bacterial optical density (OD) until the early death phase. Bacterial OD measurement was conducted using a spectrophotometer at 600 nm. The growth curve was determined by plotting incubation time against optical density [15].

Chitinase Production

Inoculum Preparation

One loop of the selected isolate was transferred into the inoculum medium and agitated on a shaker at 180 rpm for 24 hours at 24 °C to increase aeration.[16].

Chitinase Production

A total of 15 mL of the inoculum medium was pipetted into the production medium, then homogenized on a shaker with aeration at 180 rpm for 72 hours. The culture was then centrifuged in a refrigerated centrifuge at 4500 rpm for 15 minutes to separate the pellet from the supernatant. The resulting supernatant, collected as the crude chitinase extract, was transferred into a storage bottle and refrigerated for subsequent analysis. The pellet (residue) was discarded. The crude enzyme extract was analyzed to determine chitinase activity using the Schales method

and total protein concentration using the Lowry method [17].

Measurement of Chitinase Activity

The principle of measuring chitinase activity is based on the amount of reducing sugar produced from the hydrolysis of colloidal chitin by mixing 50 µL of enzyme, 100 µL of colloidal chitin, and 150 µL of phosphate buffer solution in a test tube, then incubating at 55 °C for 30 minutes and heating at 100 °C for 5 minutes. From the resulting mixture, 400 µL is taken and placed in a test tube, followed by the addition of 1600 µL of distilled water and 2 mL of Schales reagent. The mixture is then covered with aluminum foil and incubated in a boiling water bath for 15 minutes. Subsequently, the reaction mixture is centrifuged at 3000 rpm for 5 minutes at 4 °C. The absorbance is then measured at 420 nm. This measurement uses GlcNAc (N-acetyl-glucosamine) as the standard solution. One unit of chitinase activity is defined as the amount of enzyme that produces 1 µmol of N-acetyl-glucosamine (reducing sugar) per minute under the enzyme's optimum conditions [17]. The activity of the chitinase can be determined using the following formula (1).

$$\text{Enzyme activity} = [\text{Xs-Xc}] = \frac{1000}{200} \times \frac{600}{200} \times \frac{1}{30} \times \frac{1}{MW} \quad (1)$$

Where:

Xs = Glucosamine levels in the sample (µg/mL)
Xc = Glucosamine levels in the control (µg/mL)
1000 = Correction factor (1 = 1000 µL (p))
200 = Enzyme volume (µL) (w)
600 = Total volume of enzymatic reaction (µL) (x)
400 = The volume of sample mixture used for staining (µL) (y)
30 = Incubation time
BM = Molecular weight of N-acetylglucosamine (221.2 g/mol)

3. RESULTS AND DISCUSSION

Endophytic Bacteria from Strawberry Plant Roots (*Fragaria* sp.) with Chitinolytic Activity

Samples were taken from 2–3-month-old strawberry plants (*Fragaria* sp.) growing in Malino, South Sulawesi. This study successfully isolated four endophytic bacterial isolates from the root tissues of strawberry plants (*Fragaria* sp.). The endophytic bacterial isolates that grew were given the codes RS-A1, RS-A2, RS-A3, and RS-A4. The isolates obtained were then selected for their chitinolytic activity using an initial test on colloidal chitin as the substrate. Chitinolytic activity was assessed by observing the formation of clear zones around the colonies of the four bacterial isolates tested. The clear zones that formed were caused by the activity of extracellular chitinase produced by the bacteria, which hydrolyze chitin macromolecules into smaller molecules or N-acetyl-D-glucosamine monomers [18]. The results of the chitinolytic index screening are shown in **Table 1** and **Figure 1**.

Table 1. Results of chitinolytic index screening (cm)

Isolate Name	Day-1	Day-2	Day-3	Day-4
RS-A1	1.1	1.4	1.45	1.65
RS-A2	0.9	1.3	1.4	1.55
RS-A3	0.85	1.15	1.25	1.4
RS-A4	0.9	1.25	1.25	1.35

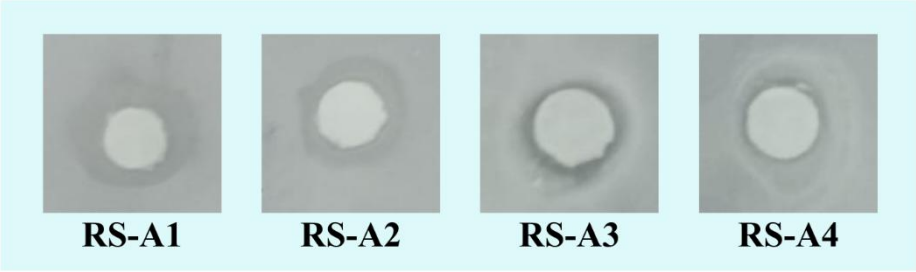

Figure 1. Activity of chitinolytic bacterial isolates

The chitinolytic index is a quantitative measure of a microorganism's ability to degrade chitin. The chitinolytic index value is obtained from the ratio between the diameter of the clear zone formed around bacterial colonies on chitin-containing media and the diameter of the bacterial colony itself. The chitinolytic index of each bacterial isolate varies, with the highest from isolate RS-A1 (1.65) and the lowest from isolate RS-A4 (1.35). The variation in chitinolytic index values obtained falls within the range generally reported in previous studies, such as research by 19, which reported that chitinolytic index values of isolates from pepper plant roots ranged from 0.3 to 7.5. Previous studies reported that the range of chitinolytic index values from isolates obtained from the roots of Ipomoea pes-caprae was 0.09 to 0.7 [20]. These results

are even lower than those obtained in this study. Each isolate produces a different chitinolytic index, influenced by the amount of N-acetyl-D-glucosamine monomers produced, so variation in monomer number plays a role in determining the size of the clear zone formed around the colony [21].

The two isolates with the highest chitinolytic indices, namely isolates RS-A1 and RS-A2, were proceeded to the chitinase production stage. The selection of these two isolates was based on their chitinolytic index values, which indicated they had the most effective chitin-degrading ability compared to other isolates. A higher chitinolytic index reflects stronger enzymatic activity, thus these isolates have a greater potential to inhibit pathogens that have chitin-containing cell walls. Differences in chitinolytic index values can also be influenced by several factors that affect chitinase activity, such as temperature, pH, substrate type, substrate concentration, and incubation time [22]. Previous research stated that chitinase activity can also be affected by inappropriate chitin colloid pH and the presence of contaminants, which can result in suboptimal chitinase activity [23].

Morphological and Molecular Characterization of 16S rRNA Isolates of Bacteria RS-A1 and RS-A2

Morphological and molecular characterization is an important step toward a comprehensive understanding of the features and identity of the bacterial isolates RS-A1 and RS-A2. Morphological characterization is conducted to observe the characteristics of bacterial colonies and cells, such as shape, size, color, and Gram staining reactions, which serve as initial indicators in grouping the isolates. Molecular analysis of the 16S rRNA gene is performed to obtain more accurate genetic information and to determine phylogenetic relationships with other bacteria. This combined approach provides a comprehensive picture, making the identification results of the bacterial isolates more valid.

Morphological Characterization

Microscopic and physiological observations revealed variations in morphology, including the shape and characteristics of the bacterial isolates. The results of the morphological observations are shown in **Table 2** and **Figure 2** below.

Macroscopic morphological observations showed that the RS-A1 isolate has smooth, round, yellowish-white colonies with flat edges and convex elevations. The RS-A2 isolate, macroscopically, has irregularly round, white colonies with wavy edges and a convex elevation. Differences in colony morphology of bacterial isolates can be influenced by environmental factors or, in this case, the ability of different bacterial species to metabolize different culture media components [24].

Table 2. Morphological characterization of bacterial isolates from strawberry plant roots (*Fragaria sp.*)

Morphological Observation of Bacteria	RS-A1 Isolate	RS-A2 Isolate
Colony shape	Smooth round	Irregular sphere
Color	Yellowish white	White
Edge	Flat	Wavy
Elevation	Convex	Convex
Cell shape	Coccobacillus	Bacillus
Gram staining	Negative	Positive

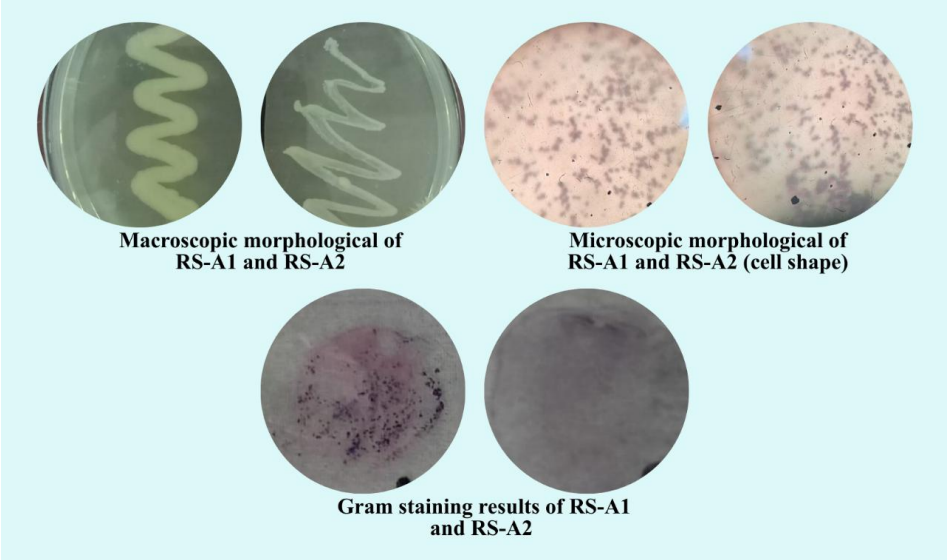

Figure 2. Morphological characterization of bacterial isolates from strawberry plant roots (*Fragaria sp.*)

Microscopic observations were also conducted to determine the cell morphology of the bacterial isolates, and the results showed that isolate RS-A1 has a coccobacill shape, whereas RS-A2 is rod-shaped. This morphological difference is influenced by the genetic traits of each isolate. Gram staining was also performed to complement the characterization results of the test isolates. Gram staining is a classical method that is still widely used in bacteriology because its procedure is considered relatively simple and quick for distinguishing between Gram-positive and Gram-negative bacteria using different staining solutions [25]. The Gram staining results indicated that the bacterial isolate RS-A1 is Gram-negative, while isolate RS-A2 is Gram-positive. This is marked by a pinkish-purple color in isolate RS-A1 and a purple color in isolate RS-A2 after staining. Previous studies indicate that color variations observed in bacterial cells after staining result from differences in the structure of bacterial cell walls [26].

Gram-negative bacteria have cell walls dominated by lipid layers. This causes the bacteria to fail to retain the primary stain during staining, especially after the alcohol wash, which can damage the lipid layer. As a result, in the Gram staining process, these bacteria will appear red or pink [27]. Gram-positive bacteria will appear purple after the Gram staining process because they can retain the primary stain, which is crystal violet, even after washing with alcohol. This is due to the cell wall structure having a thick peptidoglycan layer, so the crystal violet remains strongly bound and is not replaced by secondary stains like safranin [23].

The results of macroscopic and microscopic observations of the bacterial isolate RS-A1 are consistent with previous observations of *Acinetobacter baumannii* morphology, which has creamy-colored colonies, smooth or flat edges, bacillococci-shaped cells, and is a gram-negative bacterium indicated by its pink Gram stain [28]. When compared to previous studies, isolate RS-A2 also showed consistent results with *Bacillus subtilis*, which have white colonies, irregular colony shapes, and are Gram-positive bacteria, indicated by its purple Gram stain [29].

Molecular Characterization of 16S rRNA

Molecular characterization using the 16S rRNA gene was performed to accurately identify and classify bacteria based on their 16S rRNA gene sequences. The 16S rRNA gene is widely used because it is highly conserved, making it a universal marker, yet it also contains variable regions that enable species differentiation, providing high sensitivity and specificity in bacterial identification. The 16S rRNA gene has proven highly effective for bacterial identification due to its high accuracy and relatively fast analysis compared to conventional methods. This makes it one of the most preferred methods for molecular classification. The gel electrophoresis results of the 16S rRNA gene amplification are presented in **Figure 3**. Molecular analysis based on the 16S rRNA gene, with a base sequence similarity of 97%, generally indicates similarity at the genus level, whereas a similarity of around 99% or higher may indicate similarity at the species level [30].

The sequencing data were then analyzed using the BLAST-N program in the NCBI nucleotide database to assess sequence similarity and predict the possible genus of each isolate. The sequence alignment results for isolates RS-A1 and RS-A2 are presented in **Table 3** and were subsequently used to construct the phylogenetic tree. The phylogenetic trees shown in Figures 1 and 2, based on BLAST analysis results obtained from RNA sequences, indicate that isolate RS-A1 has 98.06% similarity with *Acinetobacter baumannii*, and isolate RS-A2 has 97.21% similarity with *Bacillus aerius*.

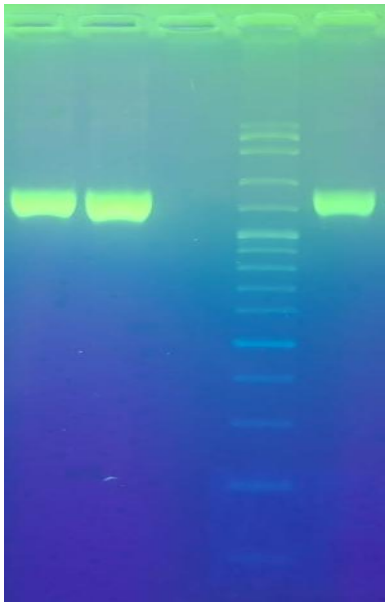

Figure 3. Gel electrophoresis results of 16S rRNA gene amplification

Several previous studies have reported various microorganisms successfully identified from strawberry plants, including *Bacillus safensis* from the fruit[31], *Bacillus cereus* from the leaves [32], *Streptomyces thermocarboxydus* from the leaves and leaf petioles [33], *Staphylococcus sciuri* from the stolon [34], and *Sphingopyxis* sp. from the meristem tissue [35]. These endophytic bacteria are also known to produce various bioactive compounds that contribute to plant growth and protection against pathogens. The isolates found in this study belong to the genus *Acinetobacter*, which previous studies have reported that the species *Acinetobacter* indicus possesses antifungal properties and can produce chitinase, making it effective for

development as a biocontrol agent [36]. Bacterial isolates from the genus *Bacillus*, according to research [37]have shown that Bacillus safensis isolated from oil palm plants can produce chitinase that antagonistically acts against the pathogenic fungus *Fusarium oxysporum*.

Table 3. BLAST alignment results of 16S rRNA sequences from endophytic bacterial isolates

Isolate Code	Closest sequence match	Similarity (%)	Accession number	Query coverage (%)	E-value
RS-A1	*Acinetobacter baumannii* strain 5689	98.06	CP096731.1	97	0.0
	Acinetobacter baumannii strain ATCC BAA1605	98.06	CP058625.1	97	0.0
	Acinetobacter baumannii strain 2018HLJAB1	98.06	CP059358.1	97	0.0
	Acinetobacter baumannii strain VB958	98.06	CP040041.1	97	0.0
	Acinetobacter baumannii strain Aci44	97.98	CP101653.1	97	0.0
RS-A2	*Bacillus sonorensis* strain ONF1P	97.21	KT362744.1	97	0.0
	Bacillus licheniformis strain S1	97.57	KP342533.1	95	0.0
	Bacillus sp. (in: Bacteria) strain VPS45	97.06	MH819973.1	96	0.0
	Bacillus licheniformis strain S3T1411	97.06	MH017407.1	96	0.0

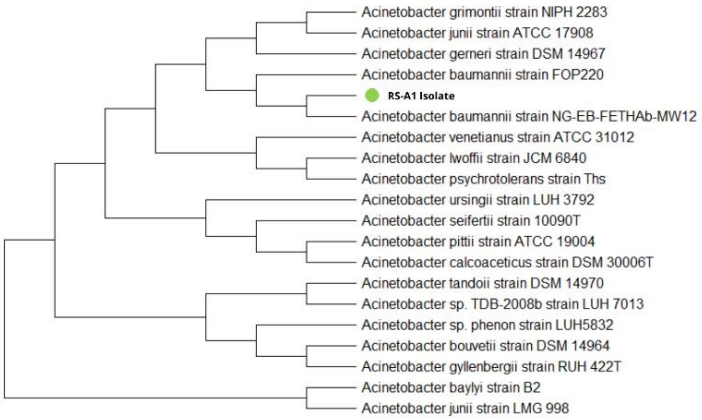

Figure 4. Phylogenetic analysis of the RS-A1 isolate derived from 16S rRNA gene sequence alignment with homologous sequences sourced from the NCBI database

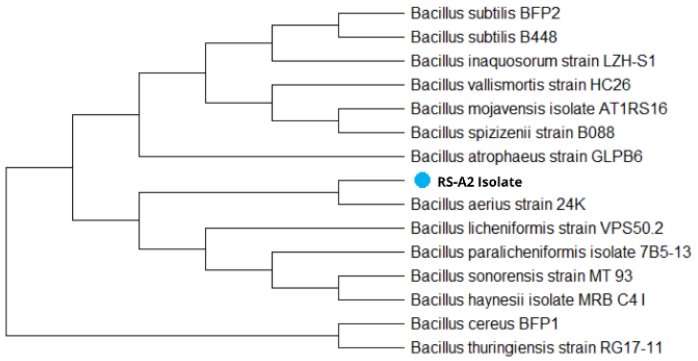

Figure 5. Phylogenetic analysis of the RS-A2 isolate derived from 16S rRNA gene sequence alignment with homologous sequences sourced from the NCBI database

Growth Curves of *Acinetobacter baumannii* and *Bacillus aerius* Bacteria

The bacterial isolates *Acinetobacter baumanii* RS-A1 and *Bacillus aerius* RS-A2 were then cultured in production medium at 37°C and 180 rpm for 3 days. Samples were collected every 6 hours to monitor microbial growth using Optical Density (OD_{660nm}) measurements, enzyme activity assays, and protein content analysis. OD measurements were conducted at a wavelength of 660 nm. The OD measurement data are presented in Figure 3.

The bacterial growth curve consists of four phases: the adaptation (lag) phase, the exponential (log) phase, the stationary phase, and the death phase [38]. The research results shown in **Figure 6** indicate that the growth of the bacterial isolate RS-A1 experienced a lag or adaptation phase at the beginning of the conditioning time, from hour 0 to hour 6. The bacteria were not yet actively growing because they were still adapting to the media environment. This lag phase occurs when the OD starts to rise slightly but is not yet significant. The log or exponential phase occurred between hours 6 and 42. The exponential phase is characterized by rapid bacterial growth and division, causing the OD to increase quickly and sharply. The stationary phase in this graph is indicated by the OD reaching a peak and starting to stabilize or slightly decline. This phase occurred at hour 42 as the critical point marking the beginning of the stationary phase. Based on the presented data, the stationary phase was very brief, with maximum OD reached at 42 hours. The death phase is marked by a continuous decrease in OD as many bacterial cells die. This death phase begins around 42-72 hours and occurs because the levels of substrate and nutrients are almost depleted, so the cells can no longer grow.

The growth curve results for the RS-A2 bacterial isolate showed an unusually short lag phase. Ideally, the lag phase is a period of adaptation at the beginning of conditioning, with no significant biomass increase. However, the high initial OD on the curve, which was 0.87 at 0 hours and immediately increased to 1.14 at 6 hours, indicates that the inoculum used was already in an active metabolic state. This was also reported by [39], who found that the lag phase was extremely short in fresh media where cells had already expressed genes, making them ready to grow. The lag phase was brief, occurring from hour 0 to hour 6. Although the OD value increased from 0.87 to 1.14, the increase was still slower than the subsequent growth phase.

The log or exponential phase occurs between 6 and 42 hours. The exponential phase is characterized by bacteria growing and dividing rapidly, causing the OD to increase quickly and sharply. *Bacillus aerius* RS-A2 reaches its highest OD peak at 42 hours, followed by the stationary phase, marked by the OD reaching its peak and beginning to stabilize or slightly decrease. This phase occurs between 42 and 72 hours. The initial gradual decrease cannot yet be classified as the death phase, which is characterized by a sharp decline in OD due to the massive death of bacterial cells. The 72nd hour is more accurately defined as the late stationary phase leading toward cell death. Longer data sampling is required to observe a clear decline in cell death.

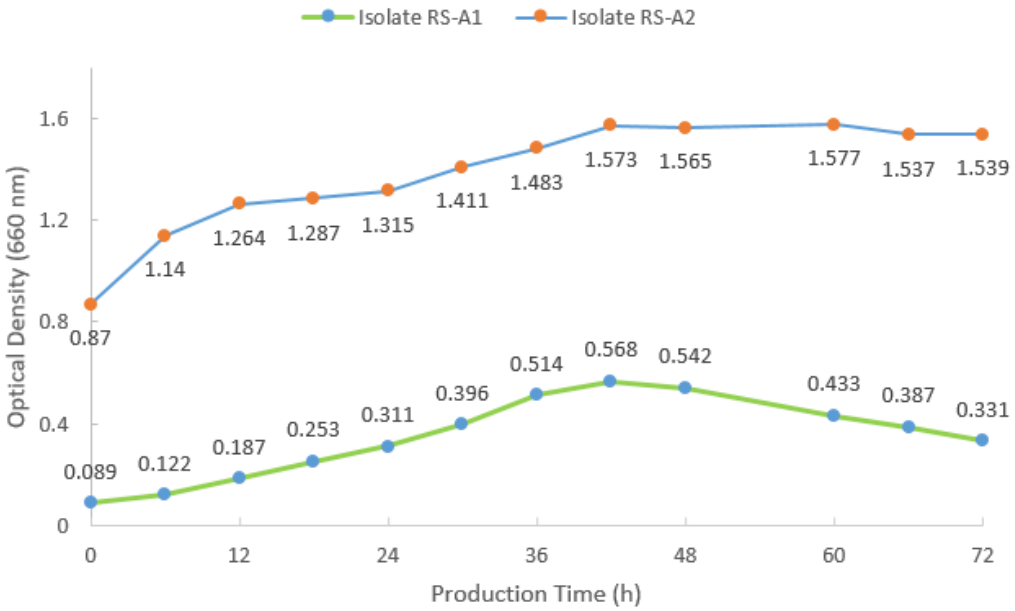

Figure 6. The effect of production time on chitinase by *Acinetobacter baumanii* RS-A1 and *Bacillus aerius* over 72 hours

Chitinase Activity and the Growth of *Acinetobacter baumannii* and *Bacillus aerius*

The results of measuring the specific activity of chitinase, compared with the growth curves of two endophytic bacterial isolates, namely RS-A1 and RS-A2, are shown in **Figures 7 and 8**. Based on the comparison of the growth curves and the specific activity of chitinase from both bacterial isolates, there appears to be a pattern linking the cell growth phase to enzyme production. In isolated RS-A1, the specific activity of chitinase reached its maximum value at 36 hours, while bacterial growth reached the stationary phase at 42 hours. In isolated RS-A2, chitinase activity increased with incubation time and peaked during the early logarithmic phase at 24 hours before eventually declining upon entering the stationary phase.

Both results indicate that chitinase production by endophytic bacteria from strawberry plant roots is growth-associated, meaning that enzyme activity increases alongside the logarithmic growth phase. During this phase, the bacteria undergo active division and require high energy and carbon sources. To meet these needs, the bacteria secrete chitinase extracellularly to hydrolyze chitin in the medium into simpler compounds such as N-acetylglucosamine, which is then used as a carbon source for cellular metabolism.

The decline in enzyme activity after the logarithmic phase in both isolates is likely caused by several factors, such as the decreased availability of chitin substrate, the accumulation of metabolites that inhibit enzyme activity, and changes in the physiological conditions of cells during the stationary phase, which no longer support enzymatic protein biosynthesis [40]. This phenomenon also indicates that enzyme production does not occur continuously but depends on the bacterial physiological conditions and metabolic needs. These findings are consistent with the report, which stated that the specific activity of chitinase in *Pseudomonas aeruginosa* DSM 50071 reaches its maximum in the logarithmic phase and decreases upon entering the stationary phase [41].

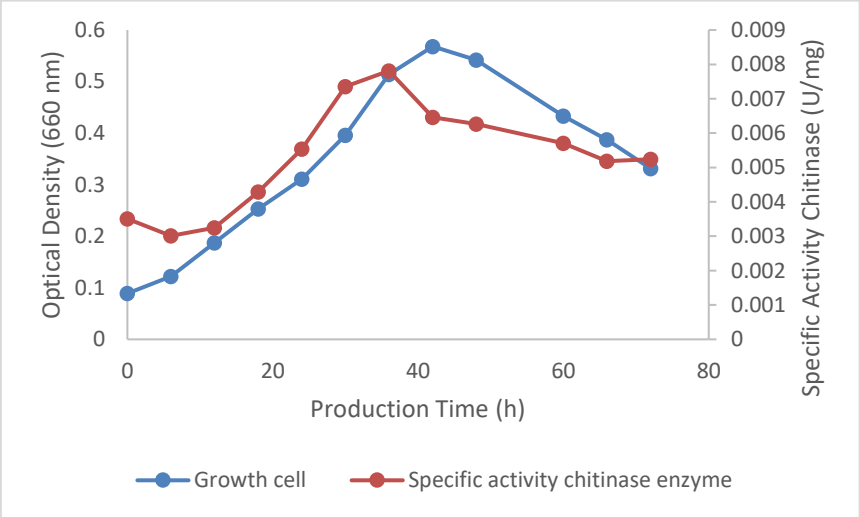

Figure 7. Comparison chart of the growth curve and chitinase activity of *Acinetobacter baumannii* RS-A1 isolate

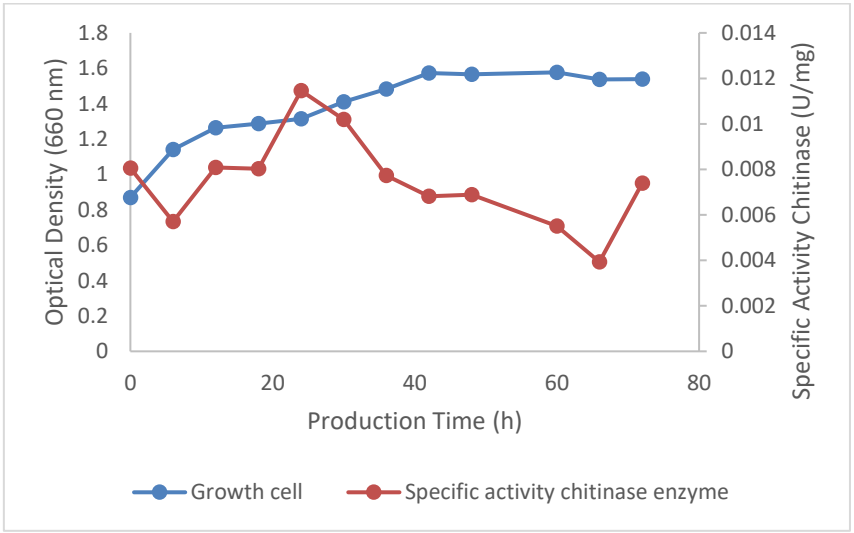

Figure 8. Comparison chart of the growth curve and chitinase activity of *Bacillus aerius* RS-A2 isolate

The specific activity of chitinase produced by the endophytic bacterial isolates in this study was 0.00781 U/mg for RS-A1 and 0.01146 U/mg for RS-A2. These values indicate the enzyme's ability to hydrolyze chitin substrate per unit of protein mass. The specific activity obtained here, when compared with previous research, shows that the chitinase produced by the endophytic bacterium *Bacillus safensis* from strawberries was 0.00966 U/mg [31]. This value is higher than that of isolated RS-A1 and lower than that of RS-A2. Endophytic *Pseudomonas aeruginosa* bacteria from onion roots have also been reported to exhibit a specific chitinase activity of 0.00566 U/mg [41]. This value is also lower than the specific activity obtained from isolates RS-A1 and RS-A2.

The differences in specific chitinase activity among each isolate can be influenced by several factors, such as pH and temperature conditions during bacterial growth, which play an important role in maintaining enzyme stability and performance, as reported in previous studies, indicating that chitinase works optimally under certain environmental conditions [42]. This phenomenon is not restricted to chitinase enzymes but broadly influences enzymatic activity in general [43]. The concentration of chitin substrate in the medium can also enhance or inhibit enzyme production. In addition to substrate concentration, the type of substrate used is also a factor that causes differences in specific chitinase activity, in line with previous research findings, which reported that among the three types of substrates used, namely colloidal chitin, chitin powder, and shell powder, colloidal chitin exhibited the highest chitinase activity [44]. Differences in the genetic ability of each bacterial isolate to express chitinase also contribute to variations in specific activity.

In general, both RS-A1 and RS-A2 isolates show potential as chitinase producers. However, the RS-A2 isolate demonstrates slightly greater stability of enzyme activity during the later growth phase than RS-A1. This is due to differences in the isolates' metabolic adaptation to the culture environment. Therefore, both isolates have the potential to be developed as biocontrol agents producing chitinase, but the RS-A2 isolate could be prioritized for enzyme production on a biotechnological scale due to its better activity stability.

4. CONCLUSIONS

This study successfully isolated and identified endophytic bacteria from strawberry plant roots. Molecular identification using 16S rRNA gene analysis showed that isolate RS-A1 had 98.06% similarity to *Acinetobacter baumannii*, while isolate RS-A2 had 97.21% similarity to *Bacillus aerius*. Quantitative measurements of chitinase production indicated that isolate RS-A2 exhibited the highest specific activity at 0.01146 U/mg. The observed chitinase activity suggests that *Bacillus aerius* RS-A2 could be explored in future research as a candidate for biocontrol or biotechnological applications.

REFERENCES

1. Shekhar C, Khosya R, Thakur K, et al. A Systematic Review of Pesticide Exposure, Associated Risks, and Long-term Human Health Impacts. *Toxicol Reports*. 2024;13:1-23. doi:10.1016/j.toxrep.2024.101840
2. Kashyap U, Garg S, Arora P. Pesticide Pollution in India: Environmental and Health Risks, and Policy Challenges. *Toxicol Reports*. 2024;13:1-17. doi:10.1016/j.toxrep.2024.101801
3. Tu CK, Huang W Di, Wang PH, et al. The Rice Endophytic Bacterium Bacillus velezensis LS123N Provides Protection Against Multiple Pathogens and Enhances Rice Resistance to Wind with Increase in Yield. *Biol Control*. 2024;192:1-14. doi:10.1016/j.biocontrol.2024.105507
4. Sriwati R, Maulidia V, Intan N, et al. Endophytic Bacteria as Biological Agents to Control Fusarium wilt Disease and Promote Tomato Plant Growth. *Physiol Mol Plant Pathol*. 2023;125. doi:10.1016/j.pmpp.2023.101994
5. Rostami A, Hinc K, Goshadrou F, et al. Display of B. pumilus Chitinase on The Surface of B. subtilis Spore As a Potential Biopesticide. *Pestic Biochem Physiol*. 2017;140:17-23. doi:10.1016/j.pestbp.2017.05.008
6. Schiphof K, Kawauchi M, Yoshimi A, Tanaka C. Functional Analysis of Basidiomycete Specific Chitin Synthase Genes in The Agaricomycete Fungus Pleurotus ostreatus. *Fungal Genet Biol*. 2024;172:1-12. doi:10.1016/j.fgb.2024.103893
7. Ferreira JM, Elias F, Soares DF. Entomopathogenic Fungi Hydrolytic Enzymes: A New Approach to Biocontrol? *J Nat Pestic Res*. 2023;3:1-6. doi:10.1016/j.napere.2023.100020
8. Rathod K, Rana S, Dhandhukia P, Thakker JN. Plant Stress Marine Bacillus as a Potent Biocontrol Agent Against Fusarium oxysporum f. sp. ciceris. *Plant Stress*. 2023;10:1-8. doi:10.1016/j.stress.2023.100289
9. Yunita VA, Natsir H, Ahmad A, Baharuddin M. Chitinase Enzyme-Producing Endophytic Bacterias From the Roots of the Plant Gembolo (Dioscorea bulbifera): Isolation, Characterization and its Potential as an Antifungal Agent. *Molekul*. 2024;19(1):117-

127. doi:https://doi.org/10.20884/1.jm.202 4 .1 9 . 1 . 9422
10. Manda-Hakki K, Hassanpour H. Effect of L-glutathione treatment on biochemical properties, antioxidant capacity and antioxidant enzymes activity in strawberry fruits during storage. *Heliyon*. 2024;10(18):e38046. doi:10.1016/j.heliyon.2024.e38046
11. Xu L, Liu W, Pan Z, et al. Characterization and Comparative Analysis of Volatile Organic Compounds in Four Aromatic Wild Strawberry Species Using HS-SPME-GC-MS. *Food Chem X*. 2025;25:1-9. doi:10.1016/j.fochx.2024.102092
12. Abdelhadi H, Qryefy M El, Ounine K. Identification of Four Antifungal Bacteria from Potato (Solanum tuberosum) and Strawberry (Fragaria x ananassa) Plants and their Rhizosphere Soils. *Trop J Nat Prod Res*. 2024;8(7):7693-7697. doi:10.26538/tjnpr/v8i7.10
13. Kurniawan E, Panphon S, Leelakriangsak M. Potential of Marine Chitinolytic Bacillus Isolates as Biocontrol Agents of Phytopathogenic Fungi. *IOP Conf Ser Earth Environ Sci*. 2019;217:1-5. doi:10.1088/1755-1315/217/1/012044
14. Bahmani K, Hasanzadeh N, Harighi B, Marefat A. Isolation and Identification of Endophytic Bacteria from Potato Tissues and Their Effects as Biological Control Agents Against Bacterial Wilt. *Physiol Mol Plant Pathol*. 2021;116:1-9. doi:10.1016/j.pmpp.2021.101692
15. Sudin S, Sulistijowati R, Hermain RM. Penapisan dan Pola Pertumbuhan Bakteri Kitinolitik dari Cangkang Rajungan (Portunus pelagicus). *Jambura Fish Process J*. 2020;2(1):36-45. doi:10.37905/jfpj.v2i1.5961
16. Madonna S. Produksi Enzim Amilolitik dari Bacillus Megaterium Menggunakan Variasi Kadar Pati Sagu (Metroxylon sp.). *Al-Kauniyah J Biol*. 2014;7(1):22-27. doi:https://doi.org/10.15408/kauniyah.v7i1.270 9
17. Natsir H, Patong AR, Suhartono MT, Ahmad A. Production and Characterization of Chitinase Enzymes from Sulili Hot Spring in South Sulawesi, Bacillus sp. HSA,3-1a. *Indones J Chem*. 2010;10(2):263-267. doi:10.22146/ijc.21470
18. Nafiah H, Pujiyanto S, Raharjo B. Isolasi Dan Uji Aktivitas Kitinase Isolat Bakteri Dari Kawasan Geotermal Dieng. *Bioma*. 2017;19(1):22-29. doi:10.14710/bioma.19.1.22-29
19. Tola SD, Muleta D, Assefa F, Ghadamgahi F, Vetukuri RR. Characterization and Identification of Hot Pepper-Associated Endospore-Forming Bacteria with Potential Applications as Biofertilizers and in Biocontrol of Pepper Wilt Pathogens. *BMC Microbiol*. 2025;25(198):1-14. doi:10.1186/s12866-025-03896-7
20. Bahar AK., Patandjengi B, Hardiansyah M., Membalik V. Characterization of Chitinolytic Bacteria Isolated from Ipomea pes caprae Characterization of Chitinolytic Bacteria Isolated from Ipomea pes caprae. In: *IOP Conference Series: Earth and Environmental Science Paper*. Purpose-Led Publishing; 2023:1-9. doi:10.1088/1755-1315/1230/1/012105
21. Giyanto AN, Mutaqin KH, Damayanti TA. Exploration of Chitinolytic Bacteria as Biocontrol Agents Against Ganoderma boninense , The Causal Agent of Basal Stem Rot in Oil Palm. In: *IOP Conference Series: Earth and Environmental Science*. IOP Publishing; 2025:1-7. doi:10.1088/1755-1315/1494/1/012025
22. Wahyudi AT, Fithriansyah NG, Amri MF, Priyanto JA, Nawangsih AA. Screening of Chitinase-producing Rhizosphere Actinomycetes and Their Genetic Diversity. *Biodiversitas*. 2021;22(10):4186-4192. doi:10.13057/biodiv/d221008
23. Chanifah N, Nurhidayat SW, Alifianto LG, Pungky D. Aktivitas Enzimatik Dan Potensi Bakteri Asal Produk LifeGrow Endofit Terhadap Jamur Fusarium s p . Di PT Biotek Cipta Kreasi Enzimatic Activity And Bacterial Potential Of Endofit LifeGrow Products Against Fusarium sp . At PT Biotek Cipta Kreasi. In: *Prosiding Seminar Nasional Penelitian Dan Pengabdian Kepada Masyarakat*. Vol 3. ; 2025:1308-1324.
24. Alifianto LG, Nugraheni IA, Rohmawati R. Aktivitas Antagonis Isolat Bakteri Endofit Cabai dan Ciplukan terhadap Patogen Fusarium sp . dan Colletotrichum sp . Antagonistic Activity of Endophytic Bacterial Isolates from Chili and Ciplukan Plants Against the Pathogens Fusarium sp and Colletotrichum. *Agrium*. 2025;28(1):1-10.
25. Paray AA, Singh M, Amin Mir M. Gram Staining: A Brief Review. *Int J Res Rev*. 2023;10(9):336-341. doi:10.52403/ijrr.20230934
26. Emitaro WO, Kawaka F, Musyimi DM, Adienge A. Diversity of Endophytic Bacteria Isolated from Leguminous agroforestry Trees in Western Kenya. *AMB Express*. 2024;14(18):1-9. doi:10.1186/s13568-024-01676-6
27. Dwiyanti AM, Lawalata HJ, Setyawati I,

Constanci A, Tengker C, Manampiring N. Identification of Bacteria Isolated from Sea Urchin (Diadema setosum) Gonads Inhibiting Pseudomonas fluorescens and Staphylococcus aureus Growth. *Adv Trop Biodivers Environ Sci*. 2025;9(2):100-106. doi:10.24843/ATBES.2025.v09.i02.p05

28. Yadzir ZHM, Shukor MY, Nazir MS, Abdullah MA. Characterization and Identification of Newly Isolated Acinetobacter baumannii Strain Serdang 1 for Phenol Removal. In: *International Conference on Fundamental and Applied Sciences*. Vol 223. ; 2014. doi:10.1063/1.4757470
29. Gireesha D, Patil P V, Gowda GRV, Vijaykumar KN, Doggalli G. Morphological and Biochemical Characterization of Bacillus subtilis Isolated from Rhizosphere of Sugarbeet. *Biochem Cell Arch*. 2024;24(1):1077-1082. doi:10.51470/bca.2024.24.1.1077
30. Akihary CV, Kolondam BJ. Utilization of the 16S rRNA Gene as a Bacterial Identification Device for Research in Indonesia. *Pharmacon*. 2020;9(1):16-22. doi:10.35799/pha.9.2020.27405
31. Hassan EA, Mostafa YS, Alamri S, Hashem M, Nafady NA. Biosafe Management of Botrytis Grey Mold of Strawberry Fruit by Novel Bioagents. *Plants*. 2021;10(12):1-20. doi:10.3390/plants10122737
32. Martins J, Ares A, Casais V, Costa J, Canhoto J. Identification and Characterization of Arbutus unedo l. Endophytic Bacteria Isolated from Wild and Cultivated Trees for The Biological Control of Phytophthora cinnamomi. *Plants*. 2021;10(8):1-17. doi:10.3390/plants10081569
33. Marian M, Ohno T, Suzuki H, Kitamura H, Kuroda K, Shimizu M. A Novel Strain of Endophytic Streptomyces for The Biocontrol of Strawberry Anthracnose Caused by Glomerella cingulata. *Microbiol Res*. 2020;234:1-9. doi:10.1016/j.micres.2020.126428
34. Alijani Z, Amini J, Ashengroph M, Bahramnejad B. Antifungal Activity of Volatile Compounds Produced by Staphylococcus sciuri Strain MarR44 and Its Potential for the Biocontrol of Colletotrichum nymphaeae, Causal Agent Strawberry Anthracnose. *Int J Food Microbiol*. 2019;307:1-9. doi:10.1016/j.ijfoodmicro.2019.108276
35. Dias ACF, Costa FEC, Andreote FD, et al. Isolation of Micropropagated Strawberry Endophytic Bacteria and Assessment of Their Potential for Plant Growth Promotion. *World J Microbiol Biotechnol*. 2009;25(2):189-195. doi:10.1007/s11274-008-9878-0
36. Akram F, Haq I, Roohi A, Akram R. Acinetobacter indicus CCS-12: A New Bacterial Source for the Production and Biochemical Characterization of Thermostable Chitinase with Promising Antifungal Activity. *Waste and Biomass Valorization*. 2022;13. doi:10.1007/s12649-022-01753-6
37. Okunwaye T, Okogbenin EA, Ikhajiagbe B. Isolation and Optimization of Chitinase Production from Bacillus safensis from the Oil Palm Rhizosphere. *Asian J Biotechnol Bioresour Technol*. 2025;11(3):86-97. doi:10.9734/ajb2t/2025/v11i3245
38. Fernández-Martínez LT, Javelle A, Hoskisson PA. Microbial Primer: Bacterial Growth Kinetics. *Microbiology*. 2024;170:1-6. doi:10.1099/mic.0.001428
39. Madar D, Dekel E, Bren A, Zimmer A, Porat Z, Alon U. Promoter Activity Dynamics in The Lag Phase of Escherichia coli. *Bio Med Cent Syst Biol*. 2013;7(136):1-13. doi:10.1186/1752-0509-7-136
40. Elawati N., Pujiyanto S, Kusdiyantini E. Production of Extracellular Chitinase Beauveria bassiana Under Submerged Fermentation Conditions. In: *Journal of Physics: Conference Series PAPER*. ; 2018:1-6. doi:10.1088/1742-6596/1025/1/012074
41. Khairah M, Mubarik NR, Manaf LA. Bacterial Selection and Characterization of Chitinase Enzyme from Bacteria Controlling Fusarium proliferatum. *Biodiversitas*. 2023;24(3):1926-1933. doi:10.13057/biodiv/d240370
42. Harvinda Y, Ustadi, Putra MMP. Production, Purification and Characterization of Chitinase from Mi-cromonospora sp. AR17. *Indones J Biotechnol*. 2023;28(1):46-55. doi:10.22146/ijbiotech.77137
43. Sarjono PR, Choirunnisa NF, Triwijayanti Y, et al. Isolation of Endophytic Pseudomonas Strains from Papaya Leaves and Their Extracellular Enzyme Production and Antioxidant Profile. *J Kim Val*. 2025;11(1):69-80. doi:10.15408/jkv.v11i1.40921
44. Pamungkas SA, Puspita ID, Ustadi. Pengaruh pH , Suhu dan Jenis Substrat terhadap Aktivitas Kitinase Bacillus sp . RNT9. *Indones J Fish Sci Technol*. 2023;19(1):29-39. doi:10.14710/ijfst.19.1.29-39