BIODIVERSITAS
Volume 24, Number 10, October 2023
Pages: 5831-5837

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241065

Glucanase activity produced by rhizospheric *Streptomyces tritolerans* ARJ 32 and *Streptomyces collinus* ARJ 38 and the analysis of their encoding genes

DEMMA ZILBA BUDIMAN[1], WIDYA ESTI PURWANINGTYAS[1], JEPRI AGUNG PRIYANTO[1], IVAN PERMANA PUTRA[1], ABDJAD ASIH NAWANGSIH[2], ARIS TRI WAHYUDI[1,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agathis, IPB Campus Dramaga, Bogor 16680, West Java, Indonesia. Tel./Fax.: 62-251-8622833, ♥email: ariswa@apps.ipb.ac.id

[2]Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, IPB Campus Dramaga, Bogor 16680, West Java, Indonesia

Manuscript received: 20 September 2023. Revision accepted: 31 October 2023.

Abstract. *Budiman DZ, Purwaningtyas WE, Priyanto JA, Putra IP, Nawangsih AA, Wahyudi AT. 2023. Glucanase activity produced by rhizospheric* Streptomyces tritolerans *ARJ 32 and* Streptomyces collinus *ARJ 38 and the analysis of their encoding genes. Biodiversitas 24: 5831-5837.* The enzyme β-1,3-glucanase is capable of breaking down the glucan components in the cell walls of phytopathogenic fungi. *Streptomyces* spp. isolated from the maize rhizosphere, are believed to produce enzymes with glucanolytic activity, making them promising candidates for use as a biological control agent against these fungi. This work aims to quantitatively analyze the enzymatic activity of glucanase in *Streptomyces tritolerans* ARJ 32 and *Streptomyces collinus* ARJ 38 and identify their encoding genes (*bglS*) and the three-dimensional modeling of their protein structures. The dinitrosalicylic acid (DNS) method quantitively assays the glucanase enzyme. The TA-Cloning of the *bglS* gene was performed using pGEM-T Easy Plasmid Vector, and the three-dimensional protein structure was constructed using the I-TASSER program. According to the findings, both *Streptomyces* isolates exhibited glucanolytic activity. The glucanase enzyme activities of *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38 peaked at eight days of incubation, with values of 31.116 U/mg and 41.599 U/mg, respectively. The partial *bglS* gene was present in both *Streptomyces* and identified as endo-β-1,3-glucanase from the glycoside hydrolase family 16 (GH 16). The deduced partial amino acid sequences were aligned and showed some highly conserved residue in the catalytic domain of GH 16. The three-dimensional structural model built from the partial bglS amino acid sequences displayed high-quality parameters and overlapped with the protein model of partial endo-β-1,3-glucanase from *Nocardiopsis* sp. F96 (2HYK). These preliminary results suggest that the two *Streptomyces* isolates had the potential to be used as glucanase enzyme producers.

Keywords: *bglS*, cloning, glucanase activity, protein modeling, *Streptomyces*

INTRODUCTION

Rhizosphere actinomycetes refer to Gram-positive filamentous bacteria belonging to the phylum Actinobacteria. Actinomycetes are commonly found in various environments and are renowned for producing numerous bioactive secondary metabolites, antibiotics, and growth-promoting substances (Elshafie and Camele 2022). These bacteria can inhabit the soil ecosystem surrounding the roots of plants. *Streptomyces* represent a prevalent genus of actinomycetes frequently found in the rhizosphere or soil environment. Several studies have indicated that this particular genus makes up about 95% of the whole actinobacteria population discovered in soil. This group of rhizosphere bacteria is known to be able to form mutualistic interactions with plants that contribute to growth-promoting agents, wherein they act as growth-promoting agents through both direct and indirect processes. These bacteria are called Plant Growth-Promoting Rhizobacteria (PGPR) (Yadav et al. 2018; Saeed et al. 2021). These bacteria play a direct role in PGPR through their ability to synthesize phytohormones, promote nitrogen fixation, and dissolve phosphates (Wahyudi et al. 2019). Through indirect mechanisms, organisms can engage in biological control by employing various strategies, such as the production of siderophores, antibiotics, and hydrolytic enzymes. Additionally, they can reduce the effects of diseases and minimize competition for nutrients and ecological niches (Goswami et al. 2016; Nozari et al. 2021). Hence, it is important to investigate the utilization of rhizosphere actinomycetes as biofertilizers and agents for controlling phytopathogens. Actinomycetes provide several advantages as biocontrol agents, including their non-toxicity to both humans and plants, their ability to enhance plant productivity, and their potential to reduce reliance on synthetic fungicides (Torres-Rodriguez et al. 2022).

Actinomycetes have been widely acknowledged as valuable sources of enzymes capable of degrading β-glucan. Among the genera, *Streptomyces* stands out as the predominant producer of enzymes, including *S. sioyaensis*, *S. albogriseolus*, *S. rimosus*, *S. murinus*, and *S. angustmyceticus* NR8-2 (Edison and Pradeep 2020). Extensive research has been conducted on the capacity of rhizospheric actinomycetes to regulate the growth and spread of phytopathogenic fungi. Producing the hydrolytic

enzyme β-1,3-glucanase by Streptomyces is considered one of the antagonistic mechanisms against phytopathogenic fungi (Torres-Rodriguez et al. 2022). The enzyme can catalyze the hydrolysis of β-1,3-glycosidic linkages in glucan (Kim et al. 2013). The cell wall of fungi is typically comprised of β-glucan, which accounts for approximately 50-60% of its total weight. Beta-1,3-glucanases may be involved in the expansion of cells, the fusion of cells, and the release of spores. The extensive breakdown of glucan polymers by endo-1,3-β-D-glucanases may cause fungal cell lysis (Shi et al. 2009). Consequently, the existence of these polymers within the fungal cell wall presents a promising focus for glucan-degrading enzymes (Free 2013). Based on the hydrolysis mechanism of the β-1,3-glucan substrate, these enzymes can be divided into endo-β-1,3-glucanase and exo-β-1,3-glucanase. The enzyme endo-β-1,3-glucanase (EC 3.2.1.39) catalyzes the hydrolysis of the β-1,3 bond located on the non-specific side of the polysaccharide chain, leading to the formation of oligosaccharides (Mouyna et al. 2013). The β-1,3-glucanase can remarkably break down β-1,3-glucans as a defense mechanism against fungal pathogens in plants and inhibit fungal growth (Wu et al. 2018).

Various recombinant and wild-type enzymes from different sources have been actively studied in bacteria. Bacteria-derived β-1,3-Glucanase enzymes are primarily classified into the glycoside hydrolase family 16 (GH 16) based on their hypothetical amino acid sequences and three-dimensional structures. While those from fungi are grouped into the glycoside hydrolase family 17 (GH 17). In *Streptomyces*, the enzyme endo-β-1,3-glucanase is encoded by the *bglS* gene. This gene has been successfully cloned and expressed in *Escherichia coli* E (Wu et al. 2018; Shi et al. 2010). A prior study has successfully isolated rhizospheric actinomycetes that can promote the sprouting of maize. In addition, these actinomycetes are also capable of producing chitinase enzymes (Wahyudi et al. 2019). Therefore, using β-glucanase enzymes produced by actinomycetes for cell wall degradation in phytopathogenic fungi represents a significant environmentally sustainable option. Hence, undertaking research endeavors to achieve sustainable agriculture by utilizing biological agents, specifically actinomycetes that produce β-glucanase, is imperative. In this research, we analyze the glucanolytic ability of two *Streptomyces* isolates as well as the identification of its encoding gene.

MATERIALS AND METHODS

Bacterial strain

This study used two *Streptomyces* isolates, *Streptomyces tritolerans* ARJ 32 and *Streptomyces collinus* ARJ 38, which were isolated from the maize rhizosphere in East Nusa Tenggara, Indonesia, and displayed plant growth-promoting activity (Wahyudi et al. 2019; Deviani 2021).

Extraction of β-glucan from oat

First, 6 g of oat powder was dissolved in 90 mL of distilled water at 55°C. Subsequently, 0.25 N NaOH solution was added to adjust the pH to 8 and stirred for 30 minutes. The solution was centrifuged at 4,000 rpm for 20 minutes at 4°C. The filtrate was added to 1 M HCl to reach pH 6.5 and incubated at 80°C for 1 hour. The filtrate was cooled to ± 28°C before adding 1 M HCl adjusted to pH 4.5 and then centrifuged at 4,000 rpm for 25 minutes at 4°C. The filtrate was added to absolute ethanol (2:1, v/v), stirred at 10°C, and precipitated overnight. The filtrate was centrifuged again at 4000 rpm for 25 min at 4°C. The pellet was dissolved in 50 mM phosphate buffer with pH 7 at 10°C (Putri et al. 2021).

Quantitative determination of β-1,3-glucanase activity and protein content

The β-1,3-glucanase activity of *Streptomyces* spp. was measured by the dinitrosalicylic acid (DNS) method with slight modifications (Wang et al. 2023). *Streptomyces* isolates were inoculated on liquid International Streptomyces Project (ISP4) medium (10 g soluble starch; 2 g $(NH_4)_2SO_4$; 2 g $CaCO_3$; 1 g $K2HPO_4$; 1 g $MgSO_4 \bullet 7H2O$; 1 g NaCl; 0,001 $FeSO_4 \bullet 7H_2O$; 0,001 g $MnCl_2$; 0,001 g $ZnSO_4 \bullet 7H_2O$) supplemented with 1% of β-glucan (b/v) and incubated on a shaker at 120 rpm at room temperature for 14 days. Cell-free supernatants were collected at every day by centrifugation at 8000 rpm at 4°C for 20 min. A total of 0.5 mL of cell-free supernatant was reacted with 0.5 mL of 3% (w/v) β-glucan as a substrate in 50 mM phosphate buffer pH 7, then incubated at 40°C for 30 min. The mixture of supernatant and substrate was added with 1 mL of DNS reagent and then boiled for 15 minutes. The enzyme unit activity was measured by determining the absorbance value at 540 nm, and the protein concentration was determined using Bradford (1976) with a bovine serum albumin (BSA) standard.

PCR amplification of *bglS*

The PCR amplification of *bglS* gene was conducted based on Shi et al. (2010). The genomic DNA of *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38 were extracted using Zymo Research Quick-DNA™ Fungal/Bacterial Miniprep Kit (CA, US), following the manufacturer's protocols. The gene encoding endo-β-1,3-glucanase (*bglS*) was amplified by PCR using the degenerative primers Bgl16F (5′-GGG ATC TGG CCS GCN TTY TGG ATG-3′) and Bgl16R (5′-CCG CCG GAG TAN CCS GGS CCR TG-3′). The total PCR reaction components in a volume of 50 µL consist of 25 µL of MyTaq™ HS Red Mix 2x (Bioline, UK); 5 µL of each forward and reverse primer (10 pmol); 5 µL of template DNA (10 ng); and 10 µL of nuclease-free water. The gene was amplified in 35 cycles with the condition: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 63°C for 30 s, elongation at 72°C for 30 s, and final elongation at 72°C for 10 min. The amplicons were visualized using 1% (b/v) gel agarose electrophoresis. The targeted DNA fragment (±160 bp) was purified following the GenepHlow™ Gel/PCR Kit (Geneaid, Taiwan).

TA-cloning of endo-β-1,3-glucanase encoding gene

The TA-Cloning of the *bglS* gene was performed using pGEM®-T Easy Plasmid Vector (Promega, USA). The recombinant plasmid was transformed into *Escherichia coli* DH5α using the TransformAid Bacterial Transformation Kit (Thermo Scientific, USA). Transformed *E. coli* DH5α bacteria were grown on LA (Luria Bertani Agar) medium supplemented with 100 µg/mL ampicillin and 20 µg/mL X-Gal. Furthermore, PCR colony using SP6/T7 primers and *Eco*RI (Thermo Scientific, USA) digestion was used to verify the cloned gene. The recombinant plasmids were extracted using the GeneJET™ Plasmid Miniprep Kit protocol (Thermo Scientific, USA). The target genes were sequenced at First Base (Selangor, Malaysia).

Amino acid sequences alignment and phenetic tree construction

The nucleotide sequences were then aligned with the BlastX program at the National Centre for Biotechnology Information (NCBI). The amino acid sequence inferred from the partial *bglS* nucleotide sequences was deduced using ExPasy and aligned using the ClustalW method. The reference sequences used in this analysis were partial endo-β-1,3-Glucanase (Table 1). The phenetic tree of the actinomycetes was constructed in the MEGA 11 program according to the Best Fit Model results in Whelan and Goldman (WAG+I) with 1000x bootstraps (Whelan and Goldman 2001).

Domain prediction and three-dimensional structure modeling

The *Streptomyces* isolate with the higher glucanase activity was subjected to domain prediction and the construction of a 3D protein structure model. Domain prediction of *bglS* of the *Streptomyces* isolates and the position of deducted amino acid is within complete *Streptomyces* endo-β-1,3-glucanase from *Streptomyces* sp. S27 (Shi et al. 2010) was predicted and carried out using the InterPro classification of protein (Paysan-Lafosse et al. 2022). A three-dimensional protein structural model was constructed using the I-TASSER website, as described by Zhou et al. (2022). The I-TASSER algorithm was employed to generate a structural model of the bglS protein by comparing it to the most closely matching protein structure available in the Protein Data Bank (PDB) Library. The visualization and analysis of a generated three-dimensional model were conducted using UCSF ChimeraX to observe and evaluate the overlap region and the positioning of important residues to the reference structure of partial endo-β-1,3-glucanase from *Nocardiopsis* sp. F96 (Pettersen et al. 2021).

RESULTS AND DISCUSSION

Glucanase activity of two *Streptomyces* isolates

Both *Streptomyces* isolates could grow in ISP 4 medium supplemented with 1% of oat glucan. The glucanase production started within 5 days and gradually increased until reaching its maximum on the 7th and 8th-day incubation for *S. tritolerans* ARJ 32 (31.116 U/mg) and *S. collinus* ARJ 38 (41.599 U/mg), respectively (Figure 1). The glucanase production generally decreased after 10 days of incubation. The enzyme-specific activity of *S. collinus* ARJ 38 was higher than *S. tritolerans* ARJ 32.

Cloning of *bgl*S gene and DNA sequence analysis

The *bglS* gene was observed in both *Streptomyces* as a specific band of approximately 160 bp after the recombinant plasmids pGEM-T carrying the *bgl*S gene were digested with *Eco*RI (Figure 2). The partial nucleotide sequences of *bgl*S genes were subsequently deduced into 45 amino acids. Following the alignment to the NCBI database, the *bgl*S gene of the *Streptomyces* isolates belonged to the glycoside hydrolase family 16 protein (Table 2). The amino acid sequences were aligned with reference sequences of *bglS* from *Streptomyces* spp., *Nocardiopsis* sp. (2HYK), and *Cellulosimicrobium cellulans* (3ATG) (Figure 3). Fifteen amino acid residues were conserved in *Streptomyces* spp., *Nocardiopsis* sp., and *C. cellulans*. The conserved amino acid residue is dominated by glycine (G), tryptophan (W), proline (P), and glutamic acid (E); the domain prediction of the complete *bglS* gene of *Streptomyces* sp. S27 consisted of three domains: TAT-signal, glycoside hydrolase family 16 (GH16), and carbohydrate-binding molecule family 13 (CBM13) (Figure 4). While the partial amino acid sequences of *S. collinus* ARJ 38 are located in the GH16 domain.

Table 1. The reference amino acid sequences used in this study

Strain name	Accession number / pdb code
Nocardiopsis sp. F96	2HYK
Streptomyces sp. S27	ACO94508.1
Streptomyces plumbidurans	WP_222812332.1
Streptomyces murinus	WP_085568052.1
Streptomyces malaysiense	WP_046426660.1
Streptomyces musisoli	WP 201814453.1
Streptomyces hygroscopicus	WP 058083161.1
Streptomyces olivaceoviridis	WP_189816684.1
Streptomyces collinus	WP_020943690.1
Streptomyces diastatochromogenes	WP 094218957.1
Cellulosimicrobium cellulans	3ATG

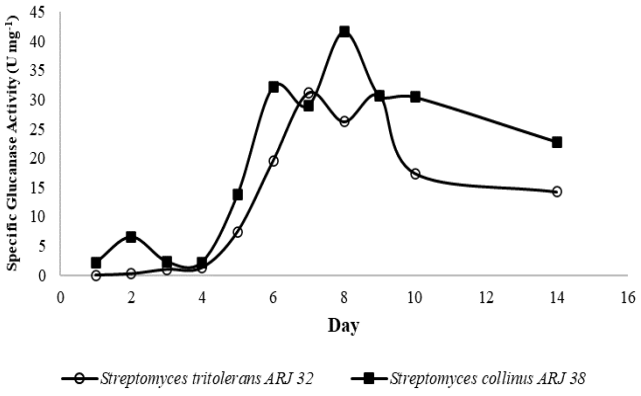

Figure 1. Activity of β-1,3-glucanase produced by *S. tritolerans* ARJ 32 and *S. collinus* AR 38

Phenetic tree analysis

Based on the bglS amino acid sequences of *S. tritolerans* ARJ 32, *S. collinus* ARJ 38, and reference strains, a phenetic tree was constructed (Figure 5). The two Streptomyces isolates found to be homologous to glycoside hydrolase family 16 were grouped within glycoside hydrolase family cluster 16 and exhibited a close relationship with other Streptomyces spp. This correlation is further supported by the Whalen and Goldman model (WAG+I) bootstrap value of the tree, which had more than 50 bootstrap values in 1000 replicates. The bootstrap values of between *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38 were 94, while both isolates had 66 bootstrap values to the GH 16 protein from the reference isolate. Bootstrap values are shown at the nodes to assess the robustness of the tree topology.

Prediction of 3D protein model and superposition analysis

I-Tasser successfully generated the three-dimensional structural protein model of the partial bglS amino acid sequences with quality parameters including C-score 0.30, TM-score 0.75±0.10, and RMSD 1.9±1.6 Å (Figure 6). The value demonstrated a high similarity structure with the original protein. The protein model of *S. collinus* ARJ 38 exhibited a notable topological similarity with the structure of the endo-β-1,3-glucanase protein (EC 3.2.1.39) from *Nocardiopsis* sp. F96 (2HYK) with TM-score 0.91 and RMSD 0.77 Å. Superposition analysis demonstrated the presence of overlapping structural parts between the model and the endo-β-1,3- glucanase protein from *Nocardiopsis* sp. F96.

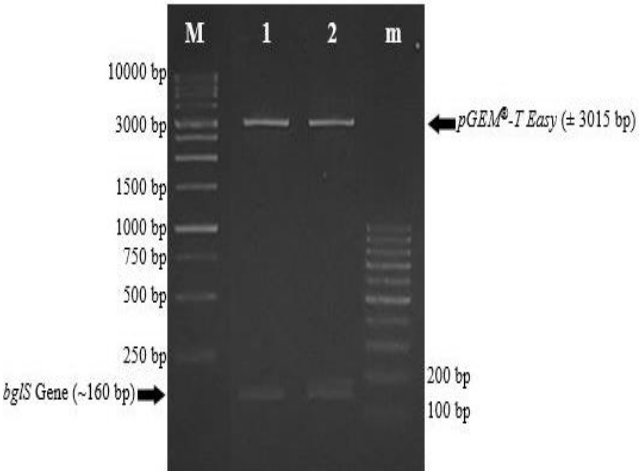

Figure 2. Agarose gel electrophoresis of the recombinant plasmid pGEMT-Easy carrying *bglS* gene from *S. tritolerans* ARJ 32 (1) dan *S. collinus* ARJ 38 (2), digested with *Eco*RI. M : 1kb DNA ladder, and m : 100 bp DNA ladder

Table 2. Homology analysis of partial endo-β-1,3-glucanase (*bglS*) encoding genes from two *Streptomyces*

Isolate code	Homologous sequence	Query cover (%)	Similarity (%)	E-value	Accession number
S. tritolerans ARJ 32	Glycoside hydrolase family 16 protein (*Streptomyces plumbidurans*)	95	95	6e-23	WP_222812332.1
S. collinus ARJ 38	Glycoside hydrolase family 16 protein (*Streptomyces plumbidurans*)	95	97	1e-23	WP_222812332.1

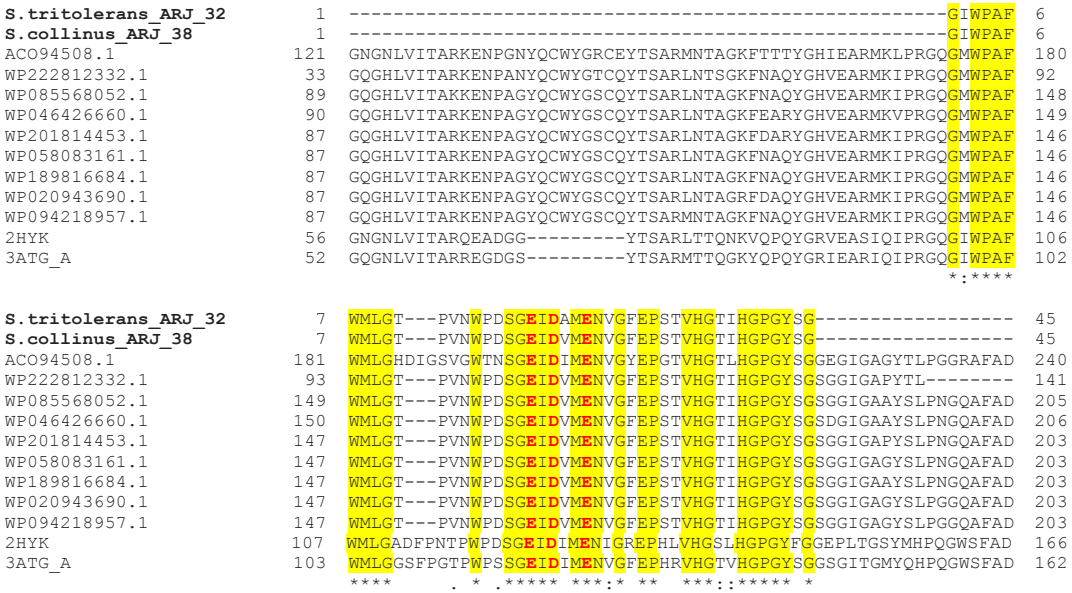

Figure 3. Deduced amino acid alignment of partial *bglS* gene of the *S. tritolerans* ARJ 32, *S. collinus* ARJ 38, the references *Streptomyces* spp., *Nocardiopsis* sp. (2HYK), and *C. cellulans* (3ATG). Red: conserved residues in the catalytic domain of the *bglS* gene. Yellow highlight: conserved residues among the isolates, references *Streptomyces* spp., *Nocardiopsis* sp. (2HYK), and *C. cellulans* (3ATG)

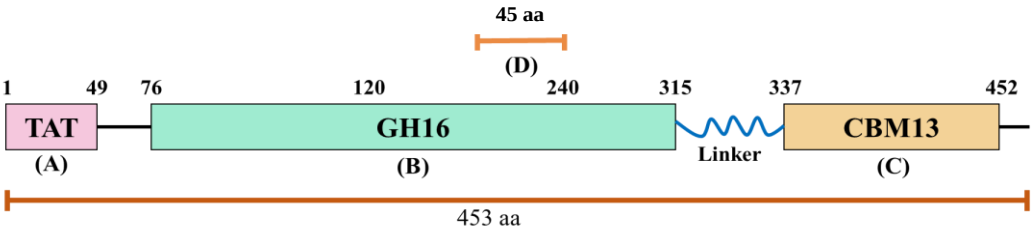

Figure 4. The complete *Streptomyces bglS* gene domain prediction (453 residues) (ACO94508) and the position of deduced amino acid of *S. collinus* ARJ 38. A. TAT-signal peptide, B. GH16 domain (IPR000757/cd08023), C. CBM13 domain (IPR000772/cd00161), and D. partial 45 deduced amino acid of *S. collinus* ARJ 38

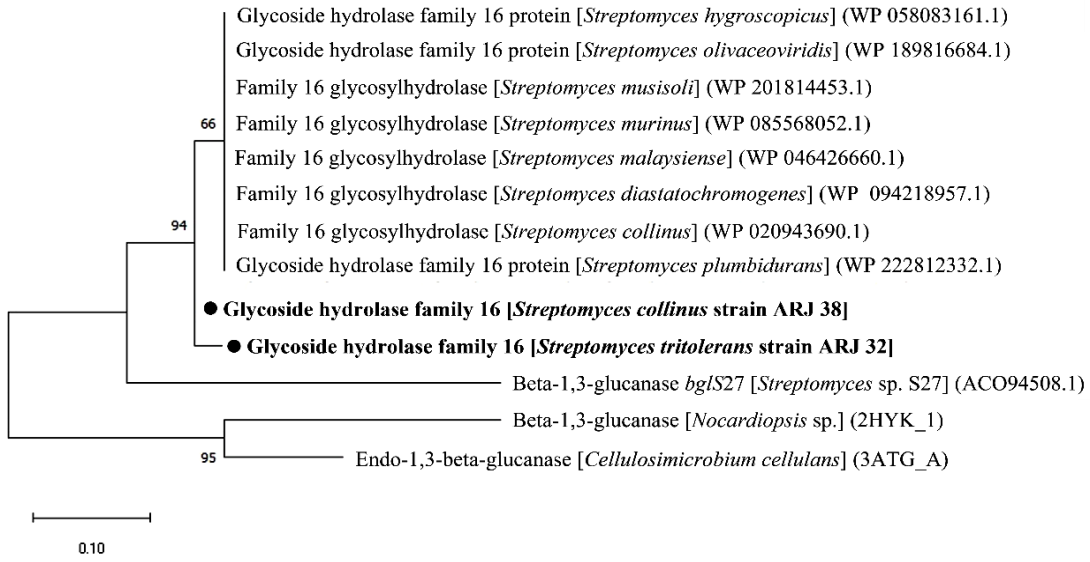

Figure 5. Phenetic tree based on partial bglS amino acid sequences of *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38

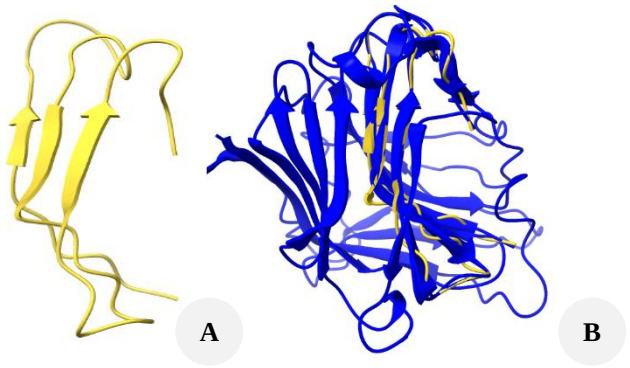

Figure 6. Three-dimensional structure prediction of the *S. collinus* ARJ 38 and the superposition to bglS *Nocardiopsis* sp. F96 (2HYK). A. Partial bglS of *S. collinus* ARJ 38 and B. Superposition of the partial bglS of *S. collinus* ARJ 38 (yellow) and bglS of *Nocardiopsis* sp. F96 (2HYK) (blue)

Discussion

The maize rhizospheric *S. collinus* ARJ 38 and *S. tritolerans* ARJ 32 isolates were tested for β-1,3-glucanase enzyme activity. In this research, the medium was supplemented with oat β-glucan to stimulate the production of β-1,3-glucanase by the *Streptomyces* isolates. The β-1,3-glucanase, produced by *Streptomyces* isolates, exhibited peak production at 7 and 8 days post-inoculation, followed by a decline at 10-day incubation. The results of the enzyme quantitative test showed that the specific activity of the enzyme in the *S. collinus* ARJ 38 and *S. tritolerans* ARJ 32 isolates were higher when compared with the specific activity of the enzyme from the isolate of *S. hygroscopicus* of 0.67 U/mg on the second day of incubation (Prapagdee et al. 2008) and *S. torulosus* PCPOK 0324 at 1.71 U/mg on the third day incubation time (Park et al. 2012). However, the time required to achieve optimum enzyme-specific activity in *S. collinus* ARJ 32 and *S. tritolerans* ARJ 38 isolates was longer when compared to *S. hygroscopicus* and *S. torulosus* PCPOK 0324. This difference in growth time between the isolates may be due to *S. collinus* ARJ 32 and *S. tritolerans* ARJ 38 requiring a longer duration for growth. Actinomycetes can exhibit antagonistic behavior by producing β-1,3-glucanase, which hydrolyzes the cell wall. The production of hydrolytic enzymes can also significantly impact the control of phytopathogenic fungi. Glucanase, a type of cell wall lytic enzyme, contributes to the degradation of fungal cell wall components by being embedded inside its protein matrix (AbdElgawad et al. 2020; Wang et al. 2021).

The *bglS* gene encodes an endo-β-1,3-glucanase enzyme that belongs to the protein glycoside hydrolase family 16 (GH16) group previously identified as belonging to *Streptomyces* sp. S27 by Shi et al. (2010). The partial sequences of the *bglS* gene from *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38 were identified as highly similar to glycoside hydrolase family 16 from *Streptomyces plumbidurans* (≥95%). Both isolates also showed a closer relation to the *Streptomyces* spp. family 16 glycosylhydrolase (Figure 5). The enzyme β-1,3-glucanase, especially endo-β-1,3-glucanase, is part of the GH 16 family protein (Mouyna et al. 2013; Li et al. 2023). Several studies have reported that this enzyme can be produced by several bacteria from the *Streptomyces* group, such as *S. corchorusii* UCR3-16 (Tamreihao et al. 2016), *S. philanthi* RM-1-1-38 (Boukaew et al. 2016), *S. goshikiensis* YCXU (Faheem et al. 2015), *S. violaceusniger* MTCC 3959 (Nagpure et al. 2014), and *S. hygroscopicus* subs. *hygroscopicus* 5-4 (Yun et al. 2022). Moreover, Based on the alignment results, 15 essential amino acid residues are conserved. Some conserved residues are dominated by glycine (G), tryptophan (W), proline (P), and glutamic acid (E). Three essential residues in the partial amino acid sequences of *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38, which are Glu^{20}, Asp^{22}, and Glu^{25}, are thought to be catalytic residues involved in the hydrolysis of β-1,3-glycosidic bonds (Fibriansah 2007; Nan-Hill et al. 2023). The three residues were also found to be in *Streptomyces* spp. *Nocardiopsis* sp. and *Cellulosimicrobium cellulans*.

The complete structure of the *Streptomyces* bglS protein on the reference isolates *Streptomyces* sp. S27, based on research by Shi et al. (2010) is predicted to consist of 3 domains that are twin-arginine translocation (TAT) signal, glycoside hydrolase family 16 (GH16), and carbohydrate-binding molecule family 13 (CBM13). The GH16 is a domain that plays a role in the process of hydrolyzing glycosidic bonds in complex sugars, one of which is glucan, while the CBM13 domain plays a role in substrate attachment during the catalysis process by glucanase (Lombard et al. 2014; Edison et al. 2020). A three-dimensional protein structure model of the amino acid sequence of *S. collinus* ARJ 38 isolate has been successfully constructed. The 3D structural model of *S. collinus* ARJ 38 endo-β-1,3-glucanase protein constructed on the I-TASSER has high-quality parameter values: C-score 0.30, TM-score 0.75 ± 0.10, and RMSD 1.9 ± 1.6. The quality parameters evaluated are TM-score, C-score, and RMSD. The TM-score values lie within the interval of 0 to 1, indicating the structural fit of the constructed model. The C-score, on a scale from -5 to 2, evaluates the accuracy of the protein structure, with higher values suggesting a higher-quality model. Furthermore, a C-score greater than or equal to -1.5 indicates that the protein has a correct fold and structure. Higher TM-score and C-score values indicate a high structural fit (Saepuloh et al. 2020). RMSD values below 2 Å also indicate a high topological structure similarity between the predicted model and the original protein (Mafakher et al. 2022). Superposition analysis of the 3D model of the endo-β-1,3- glucanase protein of *S. collinus* ARJ 38 has a high similarity with the structure of the endo-β-1,3-glucanase protein (EC 3.2.1.39) from *Nocardiopsis* sp. F96 (2HYK) indicated from the TM-score value of 0.91 and RMSD of 0.77 Å. Therefore, the partial amino acid sequence of the endo-β-1,3- glucanase of S. *collinus* ARJ 38 is predicted to have similar properties and functions to the experimental protein endo-β-1,3-glucanase from *Nocardiopsis* sp. F96.

In conclusion, maize rhizosphere *S. tritolerans* ARJ 32 and *S. collinus* ARJ 38 exhibited β-1,3-glucanase enzyme activity, which can degrade β-glucan substrate extracted from oats. The highest specific enzyme activity was obtained on the 7^{th} and 8^{th} day of incubation. The glucanolytic enzyme activity was supported by the presence of an endo-β-1,3-glucanase encoding gene (*bglS*) in both isolates identified as glycoside hydrolase family 16, and the deduced partial amino acid sequences showed some conserved residue in the catalytic domain of GH16. The three-dimensional model of the deduced amino acid sequence of the partial endo-β-1,3-glucanase was highly similar to the endo-β-1,3-glucanase protein model of the GH16 domain in *Nocardiopsis* sp. F96 (2HYK) and is supported by overlapping superposition analysis. Further investigation into the comprehensive characterization of the complete *Streptomyces bglS* gene and structure is necessary as there is currently a lack of recorded data related to this particular enzyme in the protein data bank.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through Regular Fundamental Research, fiscal year 2023 (Contract Number: 102/E5/PG.02.00.PL/2023) to ATW. Therefore, the authors thank and appreciate all the support for this research.

REFERENCES

AbdElgawad H, Abuelsoud W, Madany MM, Selim S, Zinta G, Mousa AS, Hozzein WN. 2020. Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomolecules 10 (12): 1675. DOI: 10.3390/biom10121675.

Boukaew S, Petlamul W, Suyotha W, Prasertsan P. 2016. Simultaneous fermentative chitinase and beta-1, 3-glucanase production from *Streptomyces philanthi* RM-1- 1-38 and their antifungal activity against rice sheath blight disease. BioTechnologia 97 (4): 271-284. DOI: 10.5114/bta.2016.64544.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding. Anal Biochem 72 (1-2): 248-254. DOI: 10.1016/0003-2697(76)90527-3.

Deviani C. 2021. Formulasi Aktinomiset Rizosfer Pemacu Tumbuh Dengan Menggunakan Bahan Pembawa Gambut [Thesis]. Institut Pertanian Bogor, Bogor. [Indonesian]

Edison LK, Pradeep NS. 2020. Beta-Glucanolytic soil Actinomycetes: diversity and applications. Rhizosphere Microbes: Soil Plant Funct 23: 471-482. DOI: 10.1007/978-981-15-9154-9_19.

Edison LK, Reji SR, Pradeep NS. 2020. In silico perceptions in structural elucidation of exo-ß-1, 4-glucanase and endo-ß-1, 3-glucanase from *Streptomyces* spp. J Appl Biol Biotechnol 8 (6): 67-81. DOI: 10.7324/JABB.2020.80612.

Elshafie HS, Camele I. 2022. Rhizospheric actinomycetes revealed antifungal and plant-growth-promoting activities under controlled environment. Plants 11 (14): 1872. DOI: 10.3390/plants11141872.

Faheem M, Raza W, Zhong W, Nan Z, Shen Q, Xu Y. 2015. Evaluation of the biocontrol potential of *Streptomyces goshikiensis* YCXU against *Fusarium oxysporum* f. sp. niveum. Biol Control 81: 101-110. DOI: 10.1016/j.biocontrol.2014.11.012.

Fibriansah G, Masuda S, Koizumi N, Nakamura S, Kumasaka T. 2007. The 1.3 Å crystal structure of a novel endo-b-1, 3-glucanase of glycoside hydrolase family 16 from alkaliphilic *Nocardiopsis* sp. strain F96. Proteins 69 (3): 683. DOI: 10.1002/prot.21589.

Free SJ. 2013. Fungal cell wall organization and biosynthesis. Adv Genet 81: 33-82. DOI: 10.1016/B978-0-12-407677-8.00002-6.

Goswami D, Thakker JN, Dhandhukia PC. 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food Agric 2 (1): 1127500. DOI: 10.1080/23311932.2015.1127500.

Kim YR, Kim EY, Lee JM, Kim JK, Kong IS. 2013. Characterisation of a novel Bacillus sp. SJ-10 β-1, 3–1, 4-glucanase isolated from jeotgal, a traditional Korean fermented fish. Bioprocess Biosyst Eng 36: 721-727. DOI: 10.1007/s00449-013-0896-4.

Li J, Liang Y, He Z, Zhong M, Hu Z. 2023. Mutation of conserved residues in the laminarinase Lam1092 increases the antioxidant activity of the laminarin product hydrolysates. Enzyme Microb Technol 162: 110135. DOI: 10.1016/j.enzmictec.2022.110135.

Lombard V, Golaconda RH, Drula E, Coutinho PM, Henrissat B. 2014. Thecarbohydrate-active enzymes database (CAZy). Nucleic Acids Res 42 (D1): D490-D495. DOI: 10.1093/nar/gkt1178.

Mafakher L, Rismani E, Rahimi H, Enayatkhani M, Azadmanesh K, Teimoori-Toolabi L. 2022. Computational design of antagonist peptides based on the structure of secreted frizzled-related protein-1 (SFRP1) aiming to inhibit Wnt signaling pathway. J Biomol Struct Dyn 40 (5): 2169-2188. DOI: 10.1080/07391102.2020.1835718.

Mouyna I, Hartl L, Latge JP. 2013. β-1,3-glucan modifying enzymes in *Aspergillus fumigatus*. Front Microbiol 4 (81): 1-9. DOI: 10.3389/fmicb.2013.00081.

Nagpure A, Choudhary B, Gupta RK. 2014. Mycolytic enzymes produced by *Streptomyces violaceusniger* and their role in antagonism towards wood-rotting fungi. J Basic Microbiol 54 (5): 397-407. DOI: 10.1002/jobm.201200474.

Nin-Hill A, Ardevol A, Biarnés X, Planas A, Rovira C. 2023. Control of substrate conformation by hydrogen bonding in a retaining β-endoglycosidase. Chem Eur J 7: e202302555. DOI: 10.1002/chem.2023025.

Nozari RM, Ortolan F, Astarita LV, Santarém ER. 2021. *Streptomyces* spp. enhance vegetative growth of maize plants under saline stress. Braz J Microbiol 52: 1371-83. DOI: 10.1007/s42770-021-00480-9.

Park JK, Kim JD, Park YI, Kim SK. 2012. Purification and characterization of a 1, 3-β-d-glucanase from *Streptomyces torulosus* PCPOK-0324. Carbohydr Polym 87 (2): 1641-1648. DOI: 10.1016/j.carbpol.2011.09.058.

Paysan-Lafosse T, Blum M, Chuguransky S, Grego T, Pinto BL, Salazar GA, Bileschi ML, Bork P, Bridge A, Colwell L, Gough J, Haft DH, Letunić I, Marchler-Bauer A, Mi H, Natale DA, Orengo CA, Pandurangan AP, Rivoire C, Sigrist CJA, Sillitoe I, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Bateman A. 2022. InterPro in 2022. Nucleic Acids Res 51 (D1): D418-D427. DOI: 10.1093/nar/gkac993.

Pettersen EF, Goddard TD, Huang CC, Meng EC, Couch GS, Croll TI, Morris JH, Ferrin TE. 2021. UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Sci 30 (1): 70-82. DOI: 10.1002/pro.3943.

Prapagdee B, Kuekulvong C, Mongkolsuk S. 2008. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. Intl J Biol Sci 4 (5): 330-337.DOI: 10.7150/ijbs.4.330.

Putri RE, Mubarik NR, Ambarsari L, Wahyudi AT. 2021. Antagonistic activity of glucanolytic bacteria *Bacillus subtilis* W3. 15 against *Fusarium oxysporum* and its enzyme characterization. Biodiversitas 22 (9): 4067-4077. DOI: 10.13057/biodiv/d220956.

Saeed Q, Xiukang W, Haider FU, Kučerik J, Mumtaz MZ, Holatko J, Naseem M, Kintl A, Ejaz M, Naveed M, Brtnicky M. 2021. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. Intl J Mol Sci 22 (19): 10529. DOI: 10.3390/ijms221910529.

Saepuloh U, Iskandriati D, Pamungkas J, Solihin DD, Mariya SS, Sajuthi D. 2020. Construction of a preliminary three-dimensional structure simian betaretrovirus serotype-2 (SRV-2) reverse transcriptase isolated from Indonesian cynomolgus monkey. Trop Life Sci Res 31 (3): 47-61. DOI: 10.21315/tlsr2020.31.3.4.

Shi P, Yao G, Yang P, Li N, Luo H, Bai Y, Wang Y, Yao B. 2010. Cloning, characterization, and antifungal activity of an endo-1,3-β-D-glucanase from *Streptomyces* sp. S27. Appl Microbiol Biotechnol 85: 1483-1490. DOI: 10.1007/s00253-009-2187-1.

Tamreihao K, Ningthoujam DS, Nimaichand S, Singh ES, Reena P, Singh SH, Nongthomba U. 2016. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiol Res 192: 260-270. DOI: 10.1016/j.micres.2016.08.005.

Torres-Rodriguez JA, Reyes-Pérez JJ, Quiñones-Aguilar EE, Hernandez-Montiel LG. 2022. Actinomycete potential as biocontrol agent of phytopathogenic fungi: mechanisms, source, and applications. Plants 11 (23): 3201. DOI: 10.3390/plants11233201.

Wahyudi AT, Priyanto JA, Fijrina HN, Mariastuti HD, Nawangsih AA. 2019. *Streptomyces* spp. from rhizosphere soil of maize with potential as plant growth promoter. Biodiversitas 20 (9): 2547-2553. DOI: 10.13057/biodiv/d200916.

Wang K, Huai S, Tan Z, Ngea GLN, Godana EA, Shi J, Yang Q, Zhang X, Zhao L, Zhang H. 2023. A First Expression, Purification and Characterization of Endo-β-1, 3-Glucanase from *Penicillium expansum*. J Fungi 9 (10): 961. DOI: 10.3390/jof9100961.

Wang Z, Solanki MK, Yu ZX, Anas M, Dong DF, Xing YX, Malviya MK, Pang F, Li YR. 2021. Genome characteristics reveal the biocontrol potential of actinobacteria isolated from sugarcane rhizosphere. Front Microbiol 12: 797889. DOI: 10.3389/fmicb.2021.797889.

Whelan S, Goldman N. 2001. A General empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol Biol Evol 18 (5): 691-699. DOI: 10.1093/oxfordjournals.molbev.a003851.

Wu Q, Dou X, Wang Q, Guan Z, Cai Y, Liao X. 2018. Isolation of β-1, 3-glucanase-producing microorganisms from *Poria cocos* cultivation soil via molecular biology. Molecules 23 (7): 1555. DOI: 10.3390/molecules23071555.

Yadav AN, Verma P, Kumar S, Kumar V, Kumar M, Sugitha TCK, Singh BP, Saxena AK, Dhaliwal HS. 2018. Actinobacteria from rhizosphere: Molecular diversity, distributions, and potential biotechnological applications. In: Singh BP, Gupta VK, Passari AK (eds.) New and future Developments in Microbial Biotechnology and Bioengineering. Elsevier, New York. DOI: 10.1016/B978-0-444-63994-3.00002-3.

Yun T, Jing T, Zhou D, Zhang M, Zhao Y, Li K, Zang X, Zhang L, Xie J, Wang W. 2022. Potential biological control of endophytic *Streptomyces* sp. 5-4 against *Fusarium* wilt of banana caused by *Fusarium oxysporum* f. sp. cubense tropical race 4. Phytopathology 112 (9): 1877-1885. DOI: 10.1094/PHYTO-11-21-0464-R.

Zhou X, Zheng W, Li Y, Pearce R, Zhang C, Bell EW, Zhang G, Zhang Y. 2022. I- TASSER-MTD: a deep-learning-based platform for multi-domain protein structure and function prediction. Nat Protoc 17: 2326-2353. DOI: 10.1038/s41596-022-00728-0.