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Bioprospecting of Pectinase-Producing Bacteria from Marine Actinomycetes

Neneng Hasanah[1,2], Hana Nadhifah[3], Mulyorini Rahayuningsih[1], Akhirta Atikana[3], Shanti Ratnakomala[4], Puspita Lisdiyanti[4], Nanik Rahmani[3]*

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ABSTRACT

Pectinase is used in a variety of industries, including fruit juice production, textile processing, papermaking, biodegumming, coffee and tea manufacturing, medicines, feed, and nutraceuticals. The demand for pectinase enzymes grows year after year. Industrial applications require high-activity enzymes that can increase considerable product yields while also improving based on needs and byproduct use. Indonesia's industry continues to rely on imported pectinase enzymes. Indonesia, on the other hand, possesses megabiodiversity, particularly marine microorganisms, which have the potential to provide new enzymes with high activity for industrial applications. This work aims to undertake bioprospecting of marine actinomycetes producing pectinase enzymes that have the capacity to hydrolyze pectin polymer, both from commercial and biomass sources. A total of 20 marine actinomycetes isolates from sediment, seawater, and sponges were bioprospected, and one isolate was selected with high pectinase activity (BLH 1.20), which was then used to characterize pH, temperature, hydrolysis analysis on pectin polymers, and isolate identification using 16s rRNA analysis. The selected isolate (BLH 1.20) performed best in a sodium phosphate buffer with a pH of 6.0 and a temperature of 30°C, achieving an activity of 5.4 U/mL. The 16S rRNA analysis revealed that the isolate is from the genus Streptomyces and the species *Streptomyces sampsonii*.

Keywords: bioprospecting, marine Actinomycetes, pectinase, *Streptomyces sampsonii*

INTRODUCTION

Indonesian marine ecosystems are home to a varied range of hitherto unknown microorganisms. Researchers are studying marine bacteria that can withstand extreme climatic conditions to collect and find new products, such as possible enzymes (*Amador et al.* 2003). Marine actinomycetes have been reported to play an important role in a variety of applications, particularly the production of secondary metabolites and commercial/industrial enzymes (Boovaragamoorthy *et al.* 2019). The vast majority of actinomycetes have been isolated from soil and compost. However, information on actinomycetes from saline settings is scarce. The search and discovery of new enzymes derived from actinomycetes has received a lot of attention recently due to the growing need for applications in a variety of industries, including the food industry. Streptomyces are particularly useful in a variety of industrial applications due to their capacity to produce a wide range of potential enzymes such as protease, amylase, lipase, pectinase, cellulase, xylanase, glutaminase, and asparaginase (Al-Dhabi *et al.* 2019, 2020).

Our earlier search looked at marine Streptomyces from Indonesian water as prospective enzyme makers, including lipase (Agustriana *et al.* 2023), xylanase (Nadhifah *et al.* 2023), mannanase (Putri *et al.* 2024), and alginate lyase (Jimat *et al.* 2024). Pectinase has been shown to have potential in a variety of industrial applications, including food, animal feed, textiles, pulp and paper, and energy (Abdollahzadeh *et al.* 2020). Pectinolytic enzymes convert pectin-containing substrates into monomeric sugars and pectioligosaccharides. Continuing our previous research, the current study aims to bioprospect marine actinomycetes from Indonesian water with the goal of selecting and identifying the most promising pectinase producers among the collection of marine actinomycetes, particularly those with the potential to develop for biotechnology, industrial applications, and functional foods. This research adds to marine bioprospecting in Indonesia, particularly in biotechnology and industry.

[1] Study Program of Biotechnology, Graduate School, IPB University, IPB Darmaga Campus, Bogor 16680
[2] Directorate of Scientific Collections Management, National Research and Innovation Agency (BRIN), Bogor 16911
[3] Center for Applied Microbiology Research, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Bogor 16911
[4] Biotechnology, IPB University, IPB Darmaga Campus, Bogor 16680
* Corresponding Author:
Email: nani010@brin.go.id

METHODS

Re-Culture of Marine Actinomycetes Isolates

In this work, marine actinomycetes were isolated from sediments, saltwater, and sponges from the Lembeh Strait in North Sulawesi (Atikana *et al*. 2025). In this investigation, 20 marine actinomycetes were re-cultivated from glycerol stock by streaking one loop needle of each isolate on the surface of marine ISP2 agar media (1% malt extract, 0.4% yeast extract, 0.4% D-glucose, 3% Bacto agar, and 3% Marine ART SF-1). The isolates were then cultured at 28°C for four days. These four-day-old isolates were re-cultured in 10 mL of liquid ISP2 medium (1% malt extract, 0.4% yeast extract, 0.4% D-glucose, and 3% marine ART SF-1). This pre-culture phase was carried out over three days in a shaker incubator at 190 rpm and 28°C.

Culture/Fermentation of Marine Actinomycetes

The culture (fermentation) technique involved taking 1 mL of the three-day-old pre-cultured isolates and placing them in each test tube containing 10 mL of liquid ISP2 culture media with 2.5% bagasse as a substrate. The test tube was then sealed and incubated in a shaker incubator at 190 rpm and 28°C for seven days. The supernatant was then harvested seven times by transferring 1.5 mL of culture results to 1.5 mL Eppendorf tubes. The culture results were centrifuged at 12,000 rpm at 4°C for 20 min. The supernatant was collected and transferred to a sterile 1.5 mL Eppendorf tube for further analysis at 4°C.

Preparation of Double-Layered Pectin Agar Media

Prior to pectinase screening, a double-layered pectin agar media was created in two steps: the bottom layer and the top layer. The lower layer contains 0.5% Bacto agar and 50 mM sodium phosphate buffer pH 7.0, whereas the upper layer contains 1.7% soft agar, 50 mM sodium phosphate buffer pH 7.0, and 0.5% apple pectin substrate.

Preliminary Screening of Pectinase-Producing Actinomycetes

To screen for pectinase producers among marine actinomycetes, 5 µL of supernatant was inoculated on double-layered pectin agar media and incubated for 3 days at 37°C. This screening was performed using a previously described procedure (Rahmani *et al*. 2018). After incubation, the Lugol dye was applied, and a clear zone formed to demonstrate enzyme activity. The clear zone results were promptly photographed and documented.

Re-culturing actinomycetes BLH 1−20 and producing pectinase. The preliminary screening selects the most likely pectinase manufacturers. The chosen isolate (BLH 1-20) was recultivated from glycerol stock using ISP2 agar media. This culture was incubated at 28°C for four days. Prior to pectinase synthesis, the BLH 1-20 was precultured by transferring a single colony from the four-day-old culture to 50 mL of liquid ISP2 media. The fermentation took place in a shaker incubator with 190 rpm agitation at 28°C for three days. A total of 10 mL of preculture was mixed into 100 mL of liquid ISP2 culture media. The fermentation took place in a shaker incubator with 190 rpm agitation at 28 °C for four days. The supernatant was collected by centrifugation at 12,000 rpm and 4 °C for 15 min.

Pectinase Activity of BLH 1–20

The activity of pectinase was evaluated using a previously reported methodology. 250 µL of diluted enzyme was mixed with 250 µL of 0.5% pectin substrate in 50 mM sodium acetate buffer (pH 6.0). The enzyme reaction was carried out at 30°C for 15 min. To stop the reaction, add 750 µL of DNS solution to the enzyme mixture and heat it in a water bath at 99°C for 10 min. The enzyme combination was then incubated in ice water for 10 min before being quantified using a spectrophotometer at 540 nm.

Pectinase Characterization of BLH 1-20

Characterization of pectinase comprises determining the optimal pH and temperature for BLH 1-20. Enzyme activity was measured using previously reported techniques (Miller 1959; Rahmani *et al*. 2017). The pH optimum was determined using pH buffers ranging from 3.0 to 10.0, including pH buffers 3.0−5.0 (50 mM citrate buffer), 4.0−6.0 (50 mM acetate buffer), 6.0−8.0 (50 mM phosphate buffer), 7.0−9.0 (50 mM Tris-HCl buffer), and 8.0−9.0 (50 mM glycine-NaOH buffer). The temperature test range used, from 30 C to 90°C, was conducted out at the optimal pH.

Hydrolysis of Pectin Polysaccharides with Pectinase Derived from Isolate BLH 1-20

Pectinase hydrolysis was performed on commercial substrates (orange and apple) and pectin biomass obtained from cocoa peel, sugarcane bagasse, and orange peel. The pectinase hydrolysis technique began by preparing a 1% substrate solution from each pectin substrate, up to 2.5 mL, which was then vortexed until evenly mixed. The substrate solution was combined with 7.5 mL of pectinase to create a reaction volume of 10 mL. The enzymatic hydrolysis procedure used a 1:3 ratio, and the reaction mixture was incubated at 30°C with 150 rpm agitation for 72 h. Hours 1, 4, 24, 68, and 72 were sampled serially with 0.1 mL. Hour 0 was completed without the addition of enzymes or incubation on the substrate. The hydrolysis findings were inactivated at 90°C for 10 min before being centrifuged at 13,000 rpm at 4°C for 15 min. The liquid was taken for TLC analysis.

Thin Layer Chromatography Analysis

TLC is a qualitative study of a sample that detects components depending on their polarity. The pectin standards used in TLC testing were galacturonic acid

(D1), digalacturonic acid (D2), and trigalacturonic acid (D3). The TLC method used in this work follows a previously published procedure (Rahmani *et al*. 2017). 5 µL of hydrolysis sample and 5 µL of standard solution (1000 ppm) were put to a silica plate, followed by TLC analysis to determine the product. The pattern on the silica plate shows the type of oligosaccharide generated by pectinase.

Molecular Identification of Isolate BLH 1-20

Actinomycetes BLH 1−20 were identified using 16S rRNA sequences, following the Hamada *et al*. (2015) technique. DNA was extracted using Promega's Wizard Genomic DNA Purification kit. The extracted DNA was used as a template for PCR with Emerald Amp GT PCR Master Mix and a total reaction volume of 100 µL. The primers used were: 9F (5'AGRGTTTGATCMTGGCTCAG3') and 1510R (5'TACGGYTACCTTGTTAYGACTT3'). The sequencing analysis was carried out with the cooperation of Apical Scientific Laboratory (Selangor, Malaysia), and PT Genetics Science (Tangerang, Banten, Indonesia). The sequences of the forward and reverse sequencing findings for the 16S rRNA gene were aligned with BioEdit software version 7.2.6. The identity of the BLH 1−20 isolate was validated using BLAST on the GeneBank/NCBI database, and the resulting nucleotide sequence is registered under accession number MT280078.

MegaX version 10.2.5 (Kumar *et al*. 2018) was used to construct a phylogenetic tree by comparing the genomes of BLH 1−20 to multiple sequences from different typical strains for Streptomyces species, with *Deferribacter thermophilus* serving as the outgroup. Several representative strains in this investigation were chosen based on the list of prokaryotic names in one nomenclature (LPSN; http://www.bacterio.net, Parte 2014). The phylogenetic tree was created using the Maximum Likelihood (ML) approach, 100 bootstraps, and the Tamura-Nei model (Nei and Kumar 2000; Guindon and Gascuel 2003; Tamura *et al*. 2004; Wiens *et al*. 2008; Yoshida and Nei 2016). Before creating the phylogenetic tree, the best phylogenetic model was chosen using MegaX's statistical analysis (Kumar *et al.* 2018).

RESULTS AND DISCUSSION

Marine Actinomycetes from Indonesia are Potential Producers of Pectinase

The National Research and Innovation Agency's Research Center for Applied Microbiology gathered 20 marine actinomycete isolates to conduct bioprospecting for pectinase-producing bacteria. The screening method was carried out by staining iodine-Lugol dye on double-layer pectin agar medium. In addition, a commercial pectin substrate derived from apple peel was used in the screening procedure.

Pectin polysaccharides consist of D-galacturonic acid units linked by α-(1,4) linkages (Bharadwaj and Udupa 2019). After three days of incubation at 37°C, a brown tint will appear and can be stained using Lugol's iodine solution. The brown color is caused by a complex combination containing pectin polysaccharides from the substrate and Lugol's iodine. The pectin hydrolysis process in the crude extract inoculation region degrades the pectin into monomer units that are unable to form a color complex, resulting in a clear zone.

This work demonstrated the value of diameter of the clear zone of 20 marine actinomycetes from Indonesia that have capacity to produce pectinase (Table 1).

Table 1 Diameter of the clear zone of pectinase producing marine actinomycetes at three pH levels

Isolates code	Sources	Diameter of the clear zone at pH levels (mm)		
		pH 5.0	pH 6	pH 7
BLH 1–1	Marine sediment	10.55	14.25	32.9
BLH 1–3	Marine sediment	10.05	13.4	31
BLH 1–3(1)	Marine sediment	10.35	15.7	30.85
BLH 1–5	Marine sediment	9.7	13.2	31.5
BLH 1–11	Marine sediment	10.05	14.2	36.65
BLH 1–7	Marine sediment	7.4	18.65	39.1
BLH 1–12	Marine sediment	9.15	14.25	33.6
BLH 1–20	Marine sediment	11.2	16.3	36.75
BLH 3–7	Marine sediment	9.5	14.7	32.1
BLH 3–8	Marine sediment	10.05	15.9	32.8
BLH 3–16	Marine sediment	9.75	15.1	30.95
BLH 3–25(2)	Marine sediment	9.05	14.25	31.8
BLH 5–26	Marine sediment	11.45	15.35	31.05
BLH 5–31	Marine sediment	10	15.85	30.75
BLH 5–36	Marine sediment	9.8	35.2	41.55
BLH 9–3	Marine sediment	6.1	8.4	16.55
BLH 12–1(K)	Marine sediment	9.9	14.4	29.4
BLB 8–1	Sea water	–	5.15	11.6
BLC 17–1	Sponge animal	–	7	13.6
BLC 17–3	Sponge animal	–	8.55	13.15

Among all, isolate BLH 1-20 was the most promising producer, hence it was chosen for further investigation. This work validates prior research that found actinomycetes from the Lembeh Strait to be prospective enzyme producers (Agustriana *et al*. 2023, Nadhifah *et al*. 2023, Putri *et al.* 2024, Jimat *et al*. 2024).

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Characterization of Isolate BLH 1-20

The best pH for isolate BLH 1-20 growth was obtained in media with acetic acid buffer pH 6.0, with an activity value of 5.14 U/mL (Figure 1A). The confirmation test identified the pectinase enzyme generated by BLH 1−20 as an acid pectinase. This finding is consistent with another study that found that the pectinase enzyme produced from the bacteria *Klebsiella oxytoca* af-Ga had an optimal pH of 6.0 (Ganeshprasad *et al*. 2022). Furthermore, acid pectinase enzymes have been widely used in the food industry, particularly for clarifying fruit juice and creating wine. Hence, the enzyme developed in this work has additional applications in the food sector.

The optimal temperature for isolated BLH 1−20 growth was 30°C, with an activity value of 5.4 U/mL (Figure 1B). Similar findings were reported in a study of pectinase activity in *Enterobacter* sp. MF84, which was also optimal at 30°C (Abdollahzadeh *et al*. 2020). However, the pectinase activity of isolate BLH 1-20 decreased at temperatures ranging from 40°C to 90°C (Figure 1B).

Thin Layer Chromatography Analysis

Oligosaccharide compounds arising from pectinase hydrolysis of pectin were determined on a variety of substrates including apple peel/biomass, oranges, chocolate, and sugarcane bagasse, as well as commercial pectin such as apple pectin (Sigma) and orange pectin (Sigma). The isolate BLH 1−20 degraded pectinase on both commercial and biomass pectin substrates (Figures 2 and 3). However, to provide the best conditions for pectin degradation, the pectin oligosaccharide products must be optimized using the BLH 1-20 isolate. A thick area in the standard range D2 indicates degradation of commercial oranges after one hour. Spots D2 and D4 formed because of cocoa peel pectin biomass degradation after 1 and 4 h, respectively. While apple pectin degradation spot D2 appeared at 4 and 24 h, sugarcane bagasse biomass developed spot D1 at 1 to 48 h (Figure 3).

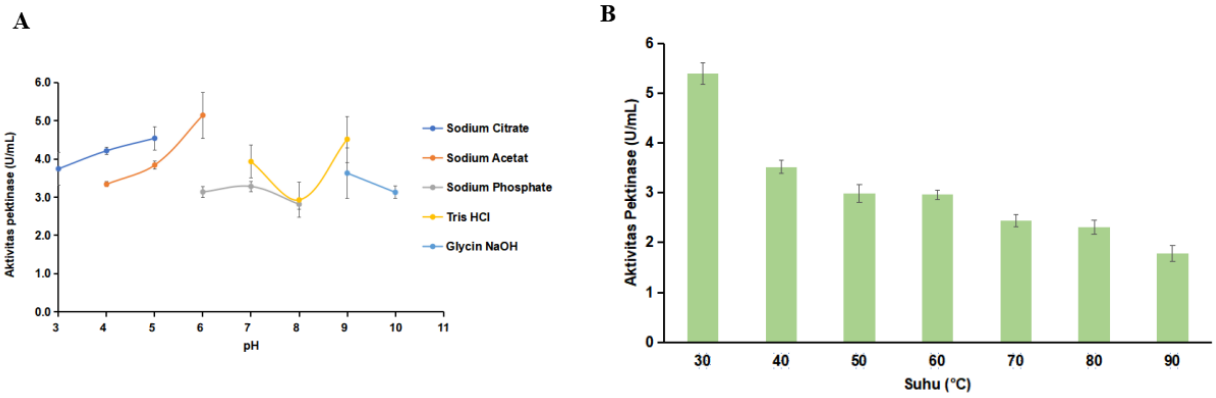
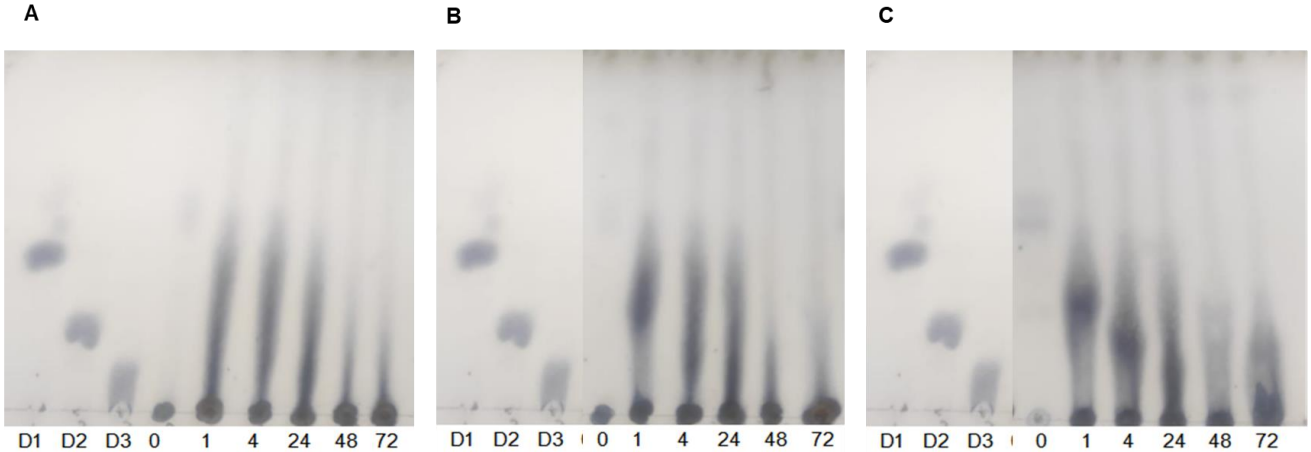


Figure 1 The optimum pH (A) and temperature (B) for pectinase activity from BLH Isolate 1–20. The optimal pH was determined using 0,5% pectin substrate at temperature 30ºC while the optimal temperature was determined using 0,5% pectin substrate in acetic acid at a pH of 6.0



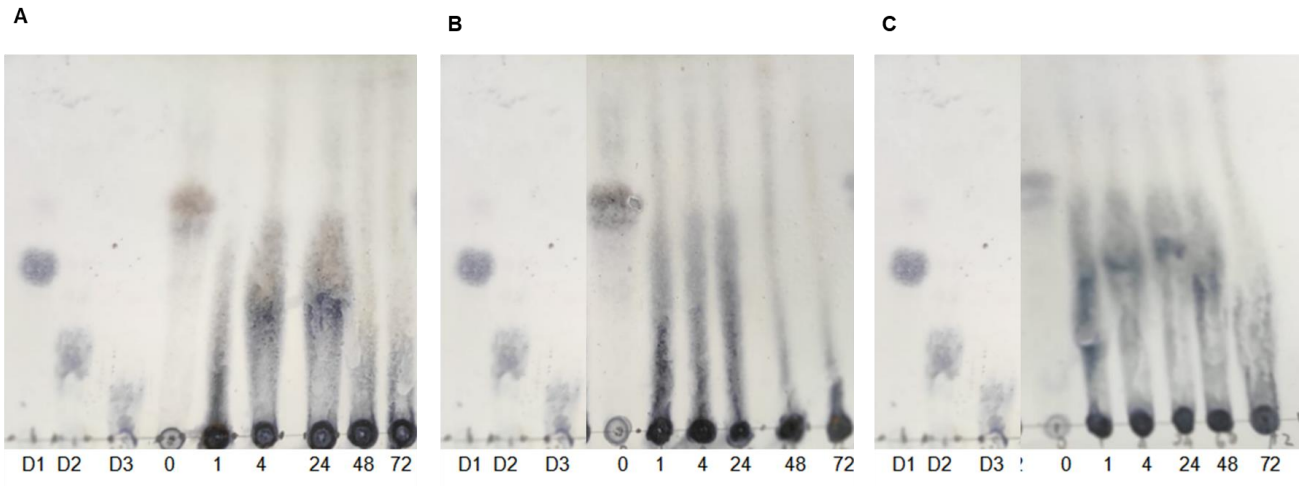


Figure 3 Thin layer chromatograms of pectinase hydrolysis results on various pectin sources for 72 h: (A) apple pectin biomass, (B) citrus pectin biomass, and (C) sugarcane bagasse pectin biomass

Molecular Identification Confirms Isolate BLH 1–20 as *Streptomyces sampsonii*

The Sanger sequencing of the 16S rRNA gene revealed that the isolate BLH 1–20 was 99.70% similar to *Streptomyces sampsonii*. The identification was also verified by the phylogenetic tree, which places BLH 1-20 in the same branch as *Streptomyces sampsonii* (Figure 4). Previous research has shown that maritime Streptomyces from Indonesia have the potential to create essential enzymes (Agustriana *et al*. 2023, Nadhifah *et al*. 2023: Putri *et al*. 2024: Jimat *et al*. 2024).

CONCLUSION

Marine actinomycetes found in Indonesian seas have the potential to manufacture pectinase. This study chose Streptomyces BLH 1-20 as the most promising enzyme producer since it demonstrated the highest pectinase activity. Characterization of BLH 1-20 revealed that isolates grew well at a sodium phosphate buffer pH of 6.0 and a temperature of 30 °C, with an activity of 5.4 U/mL. To achieve properly degraded products, the reaction conditions in the pectinase hydrolysis process must be optimized using various pectin polysaccharides (commercial and biomass).

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REFERENCES

Abdollahzadeh R, Pazhang M, Najavand S, Fallahzadeh–Mamaghani V, Amani GAR. 2020. Screening of pectinase–producing bacteria from farmlands and optimization of enzyme production from selected strain by RSM. *Folia Microbiol. (Praha).* 65(4): 705–719. https://doi.org/10.1007/s12223-020-00776-7

Agustriana E, Rahmani N, Rachmayati R, Nuryati, Andriani A, Yulianti SE. 2023. Lipase–producing marine Actinomycetes having potential to degrade PET films. *In*: IOP Conference Series: Earth and Environmental Science. 1163 (012008): 1-8. https://doi.org/10.1088/1755-1315/1163/1/012008

Al–Dhabi NAGA, Esmail AKM, Ghilan, Arasu MV. 2020. Isolation and screening of *Streptomyces* sp. Al–Dhabi–49 from the environment of Saudi Arabia with concomitant production of lipase and protease in submerged fermentation. *Saudi Journal of Biological Sciences*. 27(1): 474–479. https://doi.org/10.1016/j.sjbs.2019.11.011

Al–Dhabi NAGA, Esmail V, Duraipandiyan, Arasu. MV. 2019. Chemical profiling of *Streptomyces* sp. Al–Dhabi–2 recovered from an extreme environment in Saudi Arabia as a novel drug source for medical and industrial applications. *Saudi Journal of Biological Sciences*. 26(4): 758–766. https://doi.org/10.1016/j.sjbs.2019.03.009

Amador ML, Jimeno J, Paz–Ares L, Cortes–Funes H, Hidalgo M. 2003. Progress in the development and

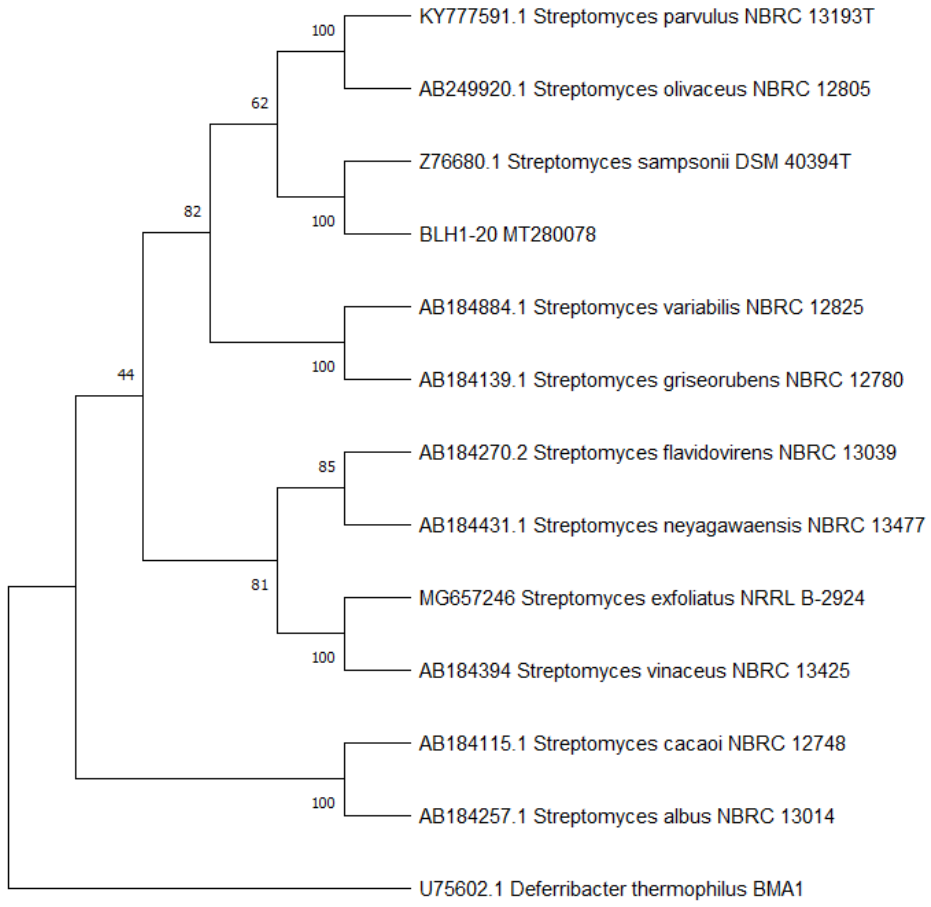

Figure 4 Neighbor–joining phylogenetic tree analysis of Streptomyces BLH 1–20 based on 16S rRNA gene analysis with a bootstrap value of 1000 repetitions on its branching, *Actinospica robiniae* is used as an outgroup.

acquisition of anticancer agents from marine sources. *Annals of Oncology*. 14(11): 1607–1615. https://doi.org/10.1093/annonc/mdg443

Atikana A, Gutleben J, de Voogd N, Tjalsma T, Nio SL, Versluis D, Indraningrat AAG., Arbi UY, Smidt H, Lisdiyanti P, Sipkema D. 2025. Sponges from Lembeh Strait: Antimicrobial activity and prokaryotic diversity. *Journal of Applied Microbiology*. 136(6): 1-13. https://doi.org/10.1093/jambio/lxaf129

Bharadwaj PS, Udupa PM. 2019. Isolation, purification and characterization of pectinase enzymes from *Streptomyces thermocarboxydus. Journal of Clinical Microbiology and Biochemical Technology*. 5(1): 1–6.

Boovaragamoorthy GM, Anbazhagan M, Piruthiviraj P. 2019. Clinically important microbial diversity and its antibiotic resistance pattern towards various drugs. *Journal of Infection and Public Health*. 12(6): 783–788. https://doi.org/10.1016/j.jiph.2019.08.008

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systems Biology*. 52: 696–704. https://doi.org/10.1080/10635150390235520

Hamada M., Shibata C., Nurkanto A., Ratnakomala S., Lisdiyanti P, Tamura T, Suzuki K. 2015. *Serinibacter tropicus* sp. nov., an actinobacterium isolated from the rhizosphere of a mangrove, and emended description of the genus Serinibacter. *International Journal Systems Evolution Microbiology*. 65: 1151–1154. https://doi.org/10.1099/ijs.0.000068

Jimat RT, Ethica SN, Rahmani N, Yulianti SE, Rachmayati R, Nuryati N. 2024. Alginate lyase from *Streptomyces olivaceus* is a safe and effective antibiofilm in male Wistar rats (*Rattus norvegicus*). *Iranian Journal of Toxicology*. 18: 1–16. https://doi.org/10.61186/IJT.18.1.45

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution.* 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Miller GL. 1959. Use of dinitrosalicylic reagent for determination of reducing sugar. *Analytical Technology*. 31: 426–428. https://doi.org/10.1021/ac60147a030

Nadhifah H, Rahmani N, Mangunwardoyo W, Yopi, Atikana A, Ratnakomala S, Lisdiyanti P. 2023. Xylanopectinolytic enzymes by marine actinomycetes from sediments of Sarena Kecil, North Sulawesi: High potential to produce galacturonic acid and xylooligosaccharides from raw biomass. *Journal of Genetic Engineering and Biotechnology.* 21(31): 1−11. https://doi.org/10.1186/s43141-023-00488-8

Nei M, Kumar S. 2000. *Molecular Evolution and Phylogenetics*. New York (US): Oxford University Pr. https://doi.org/10.1093/oso/9780195135848.001.0001

Parte AC. 2014. LPSN: List of prokaryotic names withstanding in nomenclature. *Nucleic Acids Research*. 42: 613–616. https://doi.org/10.1093/nar/gkt1111

Putri AZ, Rahmani N, Rachmayati R, Agustriana E, Yulianti, SE, Wijaya H. 2024. Production and purification of high activity β-mannanase from *Streptomyces rochei* and enzymatic hydrolysis of sugar palm fruit for galactomannan oligosaccharide (GMOS) production. *Bioresource Technology*. 25: 1−10. https://doi.org/10.1016/j.biteb.2023.101744

Rahmani N, Kashiwagi N, Lee J, Niimi–Nakamura S, Matsumoto H, Kahar P, Lisdiyanti P, Yopi, Prasetya B, Ogino C, Kondo A. 2017. Mannan endo–1,4–β–mannosidase from *Kitasatospora* sp. isolated in Indonesia and its potential for production of mannooligosaccharides from mannan polymers. Applied *Microbiology and Biotechnology Express*. 7(100): 1−11. https://doi.org/10.1186/s13568-017-0401-6

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor–joining method. *The Proceedings of the National Academy of Sciences.*101: 11030–11035. https://doi.org/10.1073/pnas.0404206101

Wiens JJ, Kuczynski CA., Smith SA., Mulcahy DG, Sites JW, Townsend TM, Reeder TW. 2008. Branch lengths, support, and congruence: testing the phylogenomic approach with 20 nuclear loci in snakes. *Systems Biology*. 57: 420–431. https://doi.org/10.1080/10635150802166053

Yopi. 2013. Purification and characterization of pectinase from *Aspergillus ustus* Bl5 *Berita Biologi*. 2(3): 375−381.

Yoshida R, Nei M. 2016. Efficiencies of the NJp, maximum likelihood, and bayesian methods of phylogenetic construction for compositional and noncompositional genes. *Molecular Biology and Evolution*. 33: 1618–1624. https://doi.org/10.1093/molbev/msw042