Isolation and characterization of potential amylolytic, proteolytic, gelatinolytic and chitinolytic properties in bacterial antagonists obtained from chilli plants (*Colletotrichum* spp.)

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Abstract. Several bacteria are found to be potential as antagonists due to their capacity in producing extracellular enzymes amylase, protease, gelatinase, cellulose and chitinase. In this research, we collected bacteria from both healthy and anthracnose-infecting chili plants (root, stem and leaf). The results revealed that 27 bacterial isolates are non-pathogenic and non-hemolytic to plants, which are 10 bacteria isolated from roots, 8 bacteria isolated from leaves, 7 bacteria isolated from stems and 2 bacteria isolated from fruits. Morphology identification showed that most of non-pathogenic bacteria are circular, irregular and punctiform, with elevation flat, raised and umbonate. White and milky bacteria are dominant, with varied opacity (transparent, translucent and opaque) and textures (slimy, mucoid, shiny and dry). Enzymatic activity test revealed 11 isolates produced protease, amylase and chitinase enzymes (isolates 11, 34, 42, 48, 111,118, 139, 188, 215, 216 and 218).

1 Introduction

Chili (*Capsicum annuum* L.) is one of vegetable crops with high economic value and is widely used for household consumption and in the food industries. The demand of this commodity is increasing with the increasing of population and agricultural productivity every year. Chilli production in Indonesia recorded 1.55 million tons in 2023 with average consumption 2.42 kg/capita/year, revealing an increase 4.3% compared to 2022 with a population of 280,073 million [1]. These data showed that the chili production in Indonesia is still low.

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Colletotrichum spp. as causal agent of anthracnose disease is responsible for drastic yield loss in chilli production worldwide, in both tropical and subtropical regions. Typical symptoms of this disease in chili include dark spots on leaf and fruit, becoming darker with time, causing fruits to be softer and slightly sunken. This pathogenic fungus can be transmitted through infected seeds in nurseries and later infecting healthy plants in the field. This species is taking nutrients from living plants and it also can live on dead matter. This ability allows them to easily spread and to cause disease in the field [2]. High humidity and rainfall contribute to higher disease incidence compared to dry season. The loss can reach 50% to 100% [3–5] while in dry season the loss varied between 20%-35%.

Colletotrichum capsici, C. gloeosporioides, C. acutatum, C. dematium, C. atramentarium, C. coccodes and *C. nigrum* are the species found the most infesting chili plants in Indonesia [6–8]. In Indonesia, almost all chili plants are infected by anthracnose disease and *C. acutatum, C. capsici* and *C. gloeosporioidees* are dominant species found in each province. These 3 species are dominant found in chili in Indonesia. To overcome this problem, farmers often use synthetic fungicides by applying the synthetics 2-3 times a week, also with combining more than two active ingredients in one-time application [9,10]. Nevertheless, the excessive use of synthetics causes environment deterioration, higher crop residues problem, resistance development in pathogens and the loss of non-target and beneficial organisms [11].

The utilization of microbial antagonists obtained from plant roots, stems and leaves is expected to be the better approach to control anthracnose disease in chili. There are several antagonists have been used to replace the use of synthetics, such as *Bacillus amyloliquefaciens* and *B. velezensis* against *C. scovillei*, *B. tequilensis* against *C. acutatum*, *B. velezensis* against *C. gloeosporioides*, *Trichoderma* spp. against *C. capsici* and *Aspergillus niger* against *C. gloeosporioides* [12–16]. These microbes do have a variety of antagonistic traits, which they have the ability to produce antifungal secondary metabolites such as extracellular enzymes (chitinase, protease, cellulase, amylase and glucanase) and siderophores [17–19]. These enzymes play an important role in plant growth and development. Besides, these enzymes such as chitinase, protease, cellulase, amylase and lipase have also been applied in cosmetic industries [20,21].

Several agricultural industries have been used those antifungal enzymes to help them reducing the infection of anthracnose disease in chilli, replacing the application of synthetic fungicides. For this reason, studies and explorations on microbial antagonists are plenty nowadays. Therefore, this research also aimed at assessing several bacterial antagonists obtained from chili plants, which have potential producing important enzymes amylase, chitinase, gelatinase and proteolytic.

2 Materials and Methods

2.1 Sample collection

Plant parts such as roots, stems and leaves were obtained from chili plantations in lowlands and highlands in 4 regencies in Sumatera Utara, namely Deli Serdang, Langkat, Karo and Simalungun. The lowlands are located in Deli Serdang and Langkat. In Deli Serdang Regency, we observed chili plantations in 2 different villages, Emplasmen Kualanamu (3.1 m asl) and Sidodadi Ramunia (3.79 m asl). In Langkat Regency, we also observed 2 chilli plantations in 2 different villages, Kwala Bingai (3.79 m asl) and Padang Brahrang (3.81 m asl). We also collected samples from highlands, Karo and Simalungun regencies. In Karo Regency, we obtained samples from villages Merek-Suka (1,116 m asl). and Negeri

Tongging (1200 m asl). In Simalungun Regency, samples were obtained from villages Seribudolok (1,380 m asl) and Siboras (1400 m asl). The selected sampling sites are 0.5 ha minimum with 50% infection rate, with one village displays healthy plants (to be collected as control/comparison). Employing diagonal design method, 3 (three) chili plants were collected from each location. The roots were collected by digging the soils 30 cm from the surface. Healthy and infected stems and leaves were also collected using sterilized equipment. The samples of roots, stems and leaves were separated and were put in plastic (with labels for each) and then were put in a cooled box at 4^0C for further use. The bacterial isolation and characterization were carried out at Plant Disease Laboratory, Agriculture Faculty, Universitas Sumatera Utara and at Quarantine Laboratory I, Kualanamu, Medan from July 2023-July 2024.

2.2 Initial screening for bacterial antagonist

The plant parts were soaked in 50 ml alcohol 70% for a minute, continued by soaking them with hypochloride 2% for a minute and re-soaked with alcohol for 30 seconds before cleaning them using distilled water 3 times. These sterilized plant parts were cut in exact size 1x1 cm air-dried before grounding them with mortar for the experiments involving suspension 10^{-5} on PDA and NA media using spreader.

Each petri dish was labelled with codes containing the place where the isolates obtained from. The successful isolates posing transparent zones were replaced to new NA media through streak quadrant method (3 times) to produce pure bacterial culture. We obtained 218 bacteria isolates for hemolysis test using blood agar. Transparent zones produced by bacteria showed that they are pathogenic to human [22,23].

Microbes (bacteria and fungi) with no hemolytic activities on blood agar were assessed on 3-week-old cabbage. They were cultured on NA (for bacteria) and PDA (fungi) media and the bacteria produced in NA were applied beneath two leaf epidermis on cabbage in the form of suspension ($\pm 10^7$ CFU ml^{-1}). Macroscopic characterization were then applied to bacterial with no pathogenic activities on plants and human with several variables observed: shape, elevation, margin, size, color, opacity and texture with the guideline of Bergey's Manual of Determinative Bacteriology by [24].

Amylolytic test was employed by culturing the bacterial isolates on amylum- suspended NA media and were incubated at 37^0C for 48 hours. The sodium was then applied on the bacteria to observe the transparent zones produced. These isolates then were re-cultured on NA media with modification [25–27]. For proteolytic test, bacterial isolates were cultured on Skim Milk Agar (SM Agar) added with tryptone, yeast extract and dextrose, incubated at 37^0C for 48 hours [25–27]. Proteolytic, amylolytic and chitinolytic activities were recorded using this following formula [28]:

$$S = \frac{\pi}{4} (dc^2 - dco^2) \quad (1)$$

Where:
S = enzyme intensity (mm^2)
dc = diameter of clear zone (mm)
dco = diameter of colonization (mm)

Bacterial pathogenic activity, bacterial haemolytic activity, macroscopic characteristics of the selected bacteria, amylolytic, proteolytic, gelatinase and chitinase activities demonstrated by the selected bacteria were observed in this study.

3 Results

3.1 Pathogenic and hemolytic activity of single bacterial isolates

From a total 218 isolates obtained from field, there were 42 isolates assessed on PDA media and then were re-cultured on NA for their pathogenic activity through hemolytic and pathogenic tests. When there is no hemolytic activity, the bacteria is non-pathogenic. These non-pathogenic bacteria were then infested in cabbage for further assessment by injecting the bacterial suspension beneath its leaf epidermis. The plants were daily observed for 1 week. Leaf cabbage posing necrotic symptom showed that the bacteria are pathogenic.

Table 1. Pathogenicity and haemolysis tests of bacterial isolates

No	Isolate Code	hemolytic activity	pathogenic activity
1	29	-	-
2	42	-	-
3	58	-	-
4	60	+	-
5	117	+	-
6	118	-	-
7	129	+	-
8	131	+	-
9	136	+	-
10	137	+	-
11	139	-	-
12	163	-	-
13	207	-	-
14	214	+	-
15	215	-	-
16	216	-	-
17	217	+	-
18	218	-	-
19	11	-	-
20	30	+	-

21	33	-	-
22	37	-	-
23	48	-	-
24	49	+	-
25	73	+	-
26	111	-	-
27	116	-	-
28	120	-	-
29	133	-	-
30	157	+	-
31	184	+	-
32	202	+	-
33	34	-	-
34	82	-	-
35	90	-	-
36	101	-	-
37	138	-	-
38	161	-	-
39	187	-	-
40	188	-	-
41	190	+	-
42	199	-	-

As explained in the result above, we found that 27 out of 42 bacterial isolates are non-pathogenic, which are 10 bacteria isolated from roots, followed by 8 bacteria isolated from leaves, 7 bacteria isolated from stems and 2 bacteria isolated from fruits. This result showed that chili plant root contains good bacteria, beneficial for protecting plants, plant growth and for providing nutrients including N, P and K, compared to antagonists found in stems, leaves and fruit.

3.2 Characterization and identification of bacterial isolates

Macroscopic identification was carried out on these bacterial isolates on NA media to observe bacterial shape, color, size, elevation and edge. This information was presented in Table 2.

Table 2. Macroscopic identification of bacterial antagonists

Isolate code	source	gram	Morphology						
			Shape	elevation	margin	Size	color	opacity	Texture
29	root	-	circular	flat	entire	Small	white	transparent	slimy
42	root	+	circular	flat	entire	moderate	milky	circular	mucoid
58	root	+	circular	flat	entire	Small	white	circular	hiny
60	root	+	circular	raised	entire	Small	white	circular	slimy
117	root	-	circular	flat	entire	Small	white	transparent	shiny
118	root	+	circular	raised	entire	Small	white	opaque	slimy
129	root	+	punctiform	flat	entire	punctiform	white	transparent	mucoid
131	root	+	circular	flat	entire	Small	white	transparent	shiny
136	root	-	circular	raised	filamentous	Small	white	translucent	slimy
137	root	-	irregular	flat	filiform	small	white	transparent	mucoid
139	root	-	circular	flat	entire	small	white	transparent	shiny
163	root	+	circular	flat	entire	small	white	transparent	slimy
207	root	+	circular	flat	undulate	small	white	transparent	shiny
214	root	-	punctiform	flat	entire	small	white	transparent	mucoid
215	root	+	circular	raised	entire	small	milky	translucent	mucoid
216	root	+	circular	raised	entire	small	yellow	translucent	mucoid
217	root	+	circular	flat	undulate	small	white	opaque	slimy
218	leaf	+	circular	flat	entire	small	white	transparent	slimy
11	leaf	+	punctiform	flat	entire	punctiform	white	transparent	shiny
30	leaf	+	circular	flat	entire	small	white	transparent	slimy
33	leaf	+	irregu-lar	raised	undulate	small	milky	translucent	slimy
37	leaf	-	punctiform	flat	entire	punctiform	white	transparent	slimy
48	leaf	+	circular	umbonate	entire	small	milky	translucent	slimy

49	leaf	+	circular	flat	undulate	small	white	opaque	dry
73	leaf	+	circular	flat	entire	small	white	transparent	slimy
111	leaf	+	circular	raised	entire	small	milky	transparent	slimy
116	leaf	-	Rhizoid	flat	filiform	small	white	transparent	shiny
120	leaf	+	punctiform	raised	filamentous	punctiform	white	transparent	slimy
133	leaf	+	circular	convex	entire	small	white	translucent	mucoid
157	leaf	+	circular	flat	undulate	moderate	white	transparent	shiny
184	leaf	+	circular	flat	undulate	small	white	opaque	slimy
202	stem	-	circular	raised	entire	small	milky	transparent	mucoid
34	stem	-	Rhizoid	flat	filiform	small	milky	opaque	mucoid
82	stem	+	circular	flat	undulate	small	white	translucent	dry
90	stem	-	circular	flat	entire	small	milky	translucent	slimy
101	stem	-	circular	flat	entire	small	white	transparent	slimy
138	stem	-	circular	raised	entire	small	white	translucent	slimy
161	stem	+	circular	raised	entire	small	white	opaque	slimy
187	stem	-	circular	flat	entire	small	white	transparent	slimy
188.	fruit	+	circular	raised	entire	small	white	transparent	slimy
190	fruit	-	circular	raised	lobate	small	milky	opaque	mucoid
199	fruit	+	circular	flat	undulate	small	white	transparent	shiny

Table 2 presented the results of macroscopic identification of bacterial isolates. From 42 screened bacteria, we found 27 gram-positive bacteria and 15 gram-negative bacteria. Based on the morphology characterization, there is similarity in shape between bacteria (circular and punctiform), but with varied elevation, margin, size, color, opacity and texture.

3.3 Potential bacterial antagonists based on their enzymatic activities

Generally, enzymes are obtained from several microbes. These microbes secrete enzymes in varied amounts. Bacteria are known to be a source of enzyme production. The results of amylolytic, protease, gelatinase and chitinase test on bacterial isolates were given in Table 3.

Table 3. Enzymatic test for assessing bacterial antagonists

No	Isolate Code	amylolitic	protease	gelatinase	chitinase
1	29	+	+	-	-
2	42	+	+	+	+
3	58	+	+	+	-
4	118	+	+	+	+
5	139	+	+	+	+
6	163	+	+	+	-
7	207	+	+	+	-
8	215	+	+	+	+
9	216	+	+	+	+
10	218	+	+	+	+
11	11	+	+	+	+
12	33	+	+	+	-
13	37	+	+	+	-
14	48	+	+	+	+
15	111	+	+	+	-
16	116	+	+	+	-
17	120	+	+	-	-
18	133	+	+	+	+
19	34	+	+	+	+
20	82	+	+	+	-
21	90	+	+	+	+
22	101	+	+	+	-
23	138	+	+	-	-
24	161	+	+	-	-
25	187	+	+	+	-
26	188	+	+	+	+
27	199	+	+	+	-

Table 3 explained that there are total of 27 bacterial isolates exhibited amyloltic, proteolytic, gelatinolytic and chitinolytic activities and all isolates possessed amylolytic and proteolytic activities. However, only 24 isolates exhibited gelatinase activity and 12 isolates expressed chitinolytic activity. The amylolytic activity by bacteria can be tested by applying some iodine as an indicator on the surface of the media. The appearance of transparent zones indicates that the bacteria produce amylolytic enzyme, and if there is no transparent zone, it shows no amylolytic enzymes produced. Similar results on proteolytic activity, transparent zones indicated that bacteria produced protease enzyme.

3.4 Enzymatic activities

The tests conducted for assessing the potential of bacterial antagonists confirmed that bacterial isolates obtained from chili plants (root, stem, leaf and fruit) demonstrated the ability of bacteria to produce extracellular enzymes such as protease, amylase and chitinase. According to [29,30], the production of transparent zones by bacteria depends on their ability to breakdown the complex molecules into simple molecules. Our experiments revealed 11 isolates expressed the ability to produce those 3 enzymes (isolates 11, 34, 42, 48, 111, 118, 139, 188, 215, 216 and 218).

Table 4. Enzymatic activities of bacterial isolates

No	Code	Proteolytic (mm^2)	amylolytic (mm^2)	chitinase (mm^2)
1	29	8.52	26.35	-
2	42	62.47	108.19	42.2
3	58	23.64	63.35	-
4	118	29.12	47.73	13,42
5	139	19.19	33.88	23.88
6	163	37.92	75.11	-
7	207	52.21	99.93	-
8	215	88.20	113.13	18.88
9	216	94.12	109.16	39.12
10	218	81.11	114.46	20.26
11	11	97.14	123.37	32.24
12	33	89.12	59.94	-
13	37	70.10	62.23	-
14	48	59.82	110,72	15.30
15	111	99.18	68.72	49.27

16	116	55.13	40.10	-
17	120	42.22	79.64	-
18	133	72.43	101.54	-
19	34	77.15	121.12	34.12
20	90	67.78	65.19	-
21	82	28.92	40,02	-
22	101	45.32	66.22	-
23	138	39.63	55.18	-
24	161	60.10	52.74	-
25	187	68.10	90.90	-
26	188	97.10	135.67	46.82
27	199	92.11	66.82	-

4 Discussions

Gram staining is a common technique to separate bacteria into two groups: gram-positive organisms resulted from basic pH of the chromophore (color bearing ion) and gram-negative organisms, resulted from acidic pH of the chromophore. Majority of the stains used for staining bacteria are of the basic pH as negative ions dominated bacterial cell walls, cytoplasm and membrane cell, which attract the positive ions, e.g methylene blue and crystal violet. Positive ions, basic stain, negative ions and acidic stains influence the results of bacterial observation [31,32]. From total 42 selected bacterial isolates we have, gram-negative bacteria are more dominant as a result of lower peptidoglycan in the bacterial cell wall, but high of phospholipid and protein layer.

Generally, gram-negative bacteria are resistant to acids and metals. Gram-negative bacteria are more tolerant to heavy metals compared to positive gram bacteria due to they have more complex cell walls and dense capsules, which may be essential in metal adsorption, allowing them to have higher tolerance towards metal concentration [33]. Gram-positive bacteria have a thick layer of peptidoglycan and lack of outer membrane compared to gram-negative bacteria [34]. Interestingly, gram-positive bacteria exhibit higher peptidoglycan than gram-negative bacteria, functioning as protective barrier against extreme changes in environment [35]. They also produce few of teichoic and teichuronic acids. Teichoic acids such as galactose, mannose, rhamnose, arabinose and glucosamine provide high density of negative charge in the cell wall, contributing to virulence and antibiotic resistance in pathogenic species [36]. Non-bacterial pathogens, both positive and negative-gram bacteria play an important role as biocontrol agents.

The bacterial isolates we assessed during this study shared similar morphology and characteristics as illustrated in guideline book for bacterial identification. According to [37], bacteria classification relies on gram staining, the temperature, the type of environment in

which they inhabit and more importantly, their growth patterns, which provide varied colony structures. Conventional method through colony or cell morphology characterization still reliable to use nowadays [38]. Morphology observation is an important step, facilitating easier further identification of bacterial colony. This observation includes identifying their shapes, margins, elevations and colour of the colony [39,40].

Enzymes are complex protein molecules, acting as biological catalysts by accelerating chemical reactions inside cells. These enzymes such as amylase, protease and chitinase are found in all living organisms, including plants, posing a significant role in matter decomposition and pathogen growth inhibition. Bacteria producing exoenzyme amylase are able to hydrolyse amylum (starch) into glucose [41,42]. These groups of bacteria are essential in environment as probiotic agents and bioremediation. In general, amylolytic bacteria produce extracellular enzyme inside cells and function outside the cells [43,44]. Amylolytic test is to determine bacteria activity in hydrolysing amylum. This amylolytic activity is employed by applying iodine as a pathogenicity indicator. The formation of transparent zones around the fungal colony indicates amylolytic enzymes exhibited by bacteria [45]. Carbon requirement in bacteria also determines the appearance of this transparent zone.

Microbial protease is an important key for enzymatic breakdown of proteins, generating smaller peptides and amino acids [46–48]. These enzymes are crucial and have been used in various industrial domains such as detergent industry for stain removal during laundry, food industries (bakery, milk, beer), pharmacies and other chemical industries [49–51]. Proteolytic activity can be identified when the transparent zones (halo zones) are created in a growth media [52]. This research used Using SM Agar Media to observe the proteolytic activity, by the appearance of clear zone. In agricultural settings, protease-producing bacteria offer potential biocontrol capabilities against phytopathogens. Temperature and pH temperature plays important role for proteolytic activity of *Stenotrophomona* sp [53]. The microbe showed optimum activity at pH 8 and temperature 60^0C (419.68 U/mL and 519.86 U/mL). Seven bacterial strains capable of producing protease enzymes, with *Bacillus* spp. being predominant among the isolates [54]. Proteolytic activity posed by isolates s6-19 and S4-19 of *Streptomyces* spp. facilitated 63% and 56% disease protection against downy mildew caused by *Sclerospora graminicola* in pearl millet [55]. Drought-sensitive plants exhibit higher proteolytic activities, protecting the plants from water-deficit condition [56].

Gelatin is usually made of animal bones, cartilage and skin and is often employed as an ingredient of fertilizer and is used to stimulate plant growth and protection [57–59]. Gelatinase enzymes are also secreted by several bacteria which effectively hydrolize gelatines into peptides, providing nitrogen, allowing the plants to use it for their development. Earlier studies documented various bacteria indicate gelatinase activity. [60] found 86% bacteria isolated from *Lessertia frutescens* produced gelatinase and amylase, enabling these bacteria to have potential for sustainable agriculture and ecosystem management. [61] reported gelatinase activity exposed by *Lysobacter capsici* has successfully reduced the eggs and juveniles of root-knot nematode in tomato plants in Korea by using them as a medium for their enhanced gelatinase activity in *in-vitro* experiments. [62] confirmed that a gelatinase and chitinase-producing bacterium, *Lysobacter antibioticus* HS124 exhibit an important role in controlling stink bug and improving the soybean yield by 17.0, 20.3, 19.0 and 25.6% at different experimental sites.

Chitinolytic bacteria are able to degrade chitin into oligomers and its derivatives, where they have been used in many fields such as medical, agrochemical food and cosmetic industries. This enzyme is not only produced by bacteria, but also fungi, insects, plants, animals and humans [63]. Chitinase can be utilized as bio-control, protecting plants from phytopathogens by causing lysis to the cell walls of those fungi. Previous studies showed several applications of chitinase produced by endophytic bacteria to control diseases caused by phytopathogens [64]. [65] investigated that bacteria *Pseudomonas stutzeri* isolated from plant *Wheithania*

somnifera exhibits chitinase which controls the growth of *Fusarium oxysporum* and *Rhizoctonia solani.* Also, [66] described that several chitinolytic bacterial strains such as *Paenibacillus elgii* HOA73, *Lysobacter capsici* HS124, *Streptomyces griseus, Pseudomonas fluorescens,* and *Paenibacillus ehimensis* MA2012 demonstrated the ability to control several strains of *C. gloeosporioides* in pepper.

5 Conclusions

There are 27 non-pathogenic bacterial isolates obtained from pathogenicity and hemolysis tests, which the higher number of species found in roots, 10 isolates, followed by 8 isolates obtained from the leaves, 7 isolates from stems and 2 isolates from fruits Morphology identification showed that most of non-pathogenic bacteria are circular, irregular and punctiform, with elevation flat, raised and umbonate. White and milky bacteria are dominant, with varied opacity (transparent, translucent and opaque) and textures (slimy, mucoid, shiny and dry). Enzymatic activity test revealed that 11 isolates expressed the ability to produce protease, amylase and chitinase enzymes (isolates 11, 34, 42, 48, 111, 118, 139, 188, 215, 216 and 218).

References

1. Sekretariat Jenderal Kementerian Pertanian, *Outlook Cabai: Komoditas Pertanian Subsektor Hortikultura* (Jakarta, 2023)
2. M. A. Syabana, A. Saylendra, and D. Ramdhani, Agrologia **4**, 288706 (2015)
3. S. Kumar, G. Stecher, and K. Tamura, Molecular Biology and Evolution **33**, 1870 (2016)
4. P. P. Than, H. Prihastuti, S. Phoulivong, P. W. J. Taylor, and K. D. Hyde, Journal of Zhejiang University Science B **9**, 764 (2008)
5. M. A. Balendres and F. Dela Cueva, (2020)
6. D. D. de Silva, J. Z. Groenewald, P. W. Crous, P. K. Ades, A. Nasruddin, O. Mongkolporn, and P. W. J. Taylor, IMA Fungus **10**, 1 (2019)
7. M. Guevara-Suarez, M. Cárdenas, P. Jiménez, L. Afanador-Kafuri, and S. Restrepo, Agronomy **12**, 548 (2022)
8. S. K. Sudirga, I. M. S. Wijaya, A. A. K. Darmadi, and N. P. A. Astiti, SABRAO J. Breed. Genet **55**, 2239 (2023)
9. S. H. Widodo & Hidayat, Asian J Plant Pathol **12**, 7 (2018)
10. A. K. Pandey, A. Kumar, E. K. Mbeyagala, M. J. Barbetti, A. Basandrai, D. Basandrai, R. M. Nair, and J. R. Lamichhane, Critical Reviews in Plant Sciences **42**, 177 (2023)
11. T. Islam, Danishuddin, N. T. Tamanna, M. N. Matin, H. R. Barai, and M. A. Haque, Plants **13**, 2737 (2024)
12. X. Zou, J. Ning, X. Zhao, H. Lv, N. Qin, H. Yin, and L. Ren, Biological Control **192**, 105480 (2024)
13. H.-T. Kwon, Y. Lee, J. Kim, K. Balaraju, H. T. Kim, and Y. Jeon, Frontiers in Microbiology **13**, 826827 (2022)
14. V. Choub, S.-J. Won, H. B. Ajuna, J.-H. Moon, S.-I. Choi, H.-I. Lim, and Y. S. Ahn, Horticulturae **8**, 557 (2022)
15. S. N. S. P. K. Agnihorti, Y. Kumar, J. Pharm. Innov. **12**, 13 (2023)

16. G. E. Dawwam and A. E. Sehim, Egyptian Journal of Biological Pest Control **32**, 144 (2022)
17. A. Nurmalinda, N. R. Mubarik, and L. Sudirman, Jurnal Ilmu Pertanian Indonesia **25**, 35 (2020)
18. W. Wang, Y. Lu, Y. Wang, Y. Zhang, B. Xia, and J. Cao, Annals of Translational Medicine **8**, (2020)
19. R. S. Riseh, M. Vatankhah, M. Hassanisaadi, and E. A. Barka, Frontiers in Bioscience-Landmark **29**, 105 (2024)
20. A. Dukare, S. Paul, and A. Arambam, Egyptian Journal of Biological Pest Control **30**, 56 (2020)
21. C.-H. Kuo, H.-M. D. Wang, and C.-J. Shieh, Catalysts **14**, 162 (2024)
22. E. F. Sharaf, W. S. El-Sayed, and R. M. Abosaif, Journal of Taibah University for Science **8**, 207 (2014)
23. D. K. T. Sukmadewi, I. Anas, R. Widyastuti, and A. Cintaresmini, Jurnal Ilmu Tanah Dan Lingkungan **19**, 68 (2017)
24. D. H. Bergey, *Bergey's Manual of Determinative Bacteriology* (Lippincott Williams & Wilkins, 1994)
25. A. Hammami, N. Fakhfakh, O. Abdelhedi, M. Nasri, and A. Bayoudh, International Journal of Biological Macromolecules **108**, 56 (2018)
26. C. Masi, G. Gemechu, and M. Tafesse, Annals of Microbiology **71**, 1 (2021)
27. R. D. Agustiani, N. Rahmani, and N. Ekowati, *Isolation and Characterization of Rhizospheric Bacteria Associated with Canna Plant: For Production of Maltooligosaccharide Amylase* (Asadel Publisher, 2023)
28. U. Hasanah, T. Ardyati, and P. D. Fitriasari, in *AIP Conference Proceedings* (AIP Publishing, 2020)
29. S. Banerjee, T. K. Maiti, and R. N. Roy, Annals of Microbiology **70**, 1 (2020)
30. N. S. Ilyasu, N. H. Adams, R. Umar, S. Ishaya, O. D. Nweke, S. Usman, A. H. Jagaba, and H. M. Yakasai, Case Studies in Chemical and Environmental Engineering **9**, 100637 (2024)
31. G. A. O'Toole, Journal of Bacteriology **198**, 3128 (2016)
32. A. A. Paray, M. Singh, M. Mir, and A. Kaur, Int J Res Rev **10**, 336 (2023)
33. X. Stachurska, B. Środa, K. Dubrowska, J. Jabłońska, M. Roszak, and J. Karakulska, Acta Scientiarum Polonorum Zootechnica **19**, 63 (2020)
34. E. R. Chellaiah, Applied Water Science **8**, 154 (2018)
35. S. J. Kim, J. Chang, and M. Singh, Biochimica et Biophysica Acta (BBA)-Biomembranes **1848**, 350 (2015)
36. F. K. K. Li, L. J. Worrall, R. T. Gale, E. D. Brown, and N. C. J. Strynadka, Science Advances **10**, eadj3864 (2024)
37. R. Kshikhundo and S. Itumhelo, World News of Natural Sciences **3**, (2016)
38. M. V Holderman, E. De Queljoe, and S. B. Rondonuwu, Jurnal Ilmiah Sains **17**, 13 (2017)
39. O. T. Kurahman, A. Yuliawati, L. Haerunnisa, A. Supriyatna, T. Cahyanto, Y. Suryani, A. Supriadin, C. Hidayat, and M. Masri, Elkawnie: Journal of Islamic Science and Technology **6**, 222 (2020)
40. N. K. E. S. Dewi, I. G. P. Wirawan, and M. Sritamin, International Journal of Biosciences and Biotechnology **9**, 31 (2021)
41. D. P. L. M. Karel, S. Hilyana, J. Perikanan **9**, 125 (2019)
42. R. A. Putri, N. Nursyirwani, and F. Feliatra, Asian Journal of Aquatic Sciences **4**, 98 (2021)
43. A. R. K. W. S. Adnan, J. Sains Dan Teknol. Pangan **2**, 759 (2017)

44. Q. Huang, H. Liu, J. Zhang, S. Wang, F. Liu, C. Li, and G. Wang, BMC Microbiology **22**, 205 (2022)
45. S. Abouamama, B. Anis, S. Abir, H. Maroua, and B. Sirine, Heliyon **9**, (2023)
46. S. Wettstadt and M. A. Llamas, Frontiers in Molecular Biosciences **7**, 586497 (2020)
47. M. Kieliszek, K. Pobiega, K. Piwowarek, and A. M. Kot, Molecules **26**, 1858 (2021)
48. T. J. Ashaolu, C. C. Lee, J. O. Ashaolu, O. Tarhan, H. Pourjafar, and S. M. Jafari, Food Reviews International **40**, 1875 (2024)
49. J. Fernández-Lucas, D. Castañeda, and D. Hormigo, Trends in Food Science & Technology **68**, 91 (2017)
50. I. García-Cano, D. Rocha-Mendoza, J. Ortega-Anaya, K. Wang, E. Kosmerl, and R. Jiménez-Flores, Applied Microbiology and Biotechnology **103**, 5243 (2019)
51. F. Sun, Y. Hu, X. Yin, B. Kong, and L. Qin, Process Biochemistry **89**, 37 (2020)
52. E. Fachrial, V. KRISDIANILO, H. HARMILENI, I. N. E. Lister, T. T. Nugroho, and S. SARYONO, Biodiversitas Journal of Biological Diversity **22**, (2021)
53. Y. S. Soeka and S. Sulistiani, Berita Biologi **16**, 203 (2017)
54. S. Afrin, T. Tamanna, U. F. Shahajadi, B. Bhowmik, A. H. Jui, M. A. S. Miah, and M. N. I. Bhuiyan, The Microbe **4**, 100123 (2024)
55. H. G. Pushpalatha, J. Naveen, N. Geetha, G. Hithamani, and H. S. Shetty, Indian Phytopathology **76**, 521 (2023)
56. S. J. Moloi and R. Ngara, Frontiers in Plant Science **14**, 1165845 (2023)
57. F. F. M. H. Khirzin, S. Ton, JSPI **14**, 119 (2019)
58. J. A. Rather, N. Akhter, Q. S. Ashraf, S. A. Mir, H. A. Makroo, D. Majid, F. J. Barba, A. M. Khaneghah, and B. N. Dar, Food Packaging and Shelf Life **34**, 100945 (2022)
59. S. Park, M. Safdar, W. Kim, J. Seol, D. Kim, K. Lee, H. Il Son, and J. Kim, Small **20**, 2402899 (2024)
60. S. Tsipinana, L. Obi, S. Amoo, and R. Adeleke, South African Journal of Botany **174**, 768 (2024)
61. Y. S. Lee, X. H. Nguyen, K. W. Naing, Y. S. Park, and K. Y. Kim, Indian Journal of Microbiology **55**, 74 (2015)
62. Y.-S. Lee, H.-D. Jeon, Y.-T. Kim, S. Monkhung, and K.-Y. Kim, Korean Journal of Soil Science and Fertilizer **50**, 71 (2017)
63. E. A. Veliz, P. Martínez-Hidalgo, and A. M. Hirsch, AIMS Microbiology **3**, 689 (2017)
64. R. Rosyida, M. Martosudiro, and A. Muhibuddin, Research Journal of Life Science **9**, 131 (2022)
65. A. Kumar, S. P. J. Kumar, A. D. Chintagunta, D. K. Agarwal, G. Pal, A. N. Singh, and J. Simal-Gandara, Archives of Phytopathology and Plant Protection **55**, 1 (2022)
66. M. Anees, M. Abid, S. U. Rehman, N. Ahmed, M. Ashraf, L. Zhang, and K. Y. Kim, Plant Protection Science **55**, (2019)