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Potential of Novel Bacteria from Sidoarjo Hot Mud for Controlling Potato Soft Rot Caused by *Erwinia carotovora*

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Abstract

Soft rot is an important potato disease caused by the bacterial pathogen *Erwinia carotovora*. Although chemical bactericides are commonly used for control, biological control using antagonistic bacteria offers a promising and environmentally friendly alternative. Bacteria capable of surviving in extreme environments have been reported to show high efficiency and the ability to withstand various environmental conditions. This study aimed to isolate, evaluate the potential and activity, and identify the molecular basis of novel bacteria from Sidoarjo hot mud against *E. carotovora,* the causal agent of potato soft rot. The methods used included sample collection, bacterial isolation and screening, inhibition assays against *E. carotovora*, soft rot suppression assays on potato tubers, molecular identification using 16S rRNA gene sequencing, and antibiosis mechanism testing. Based on the research, 16 bacterial isolates were obtained, of which four isolates (BLS3, BLS6, BLS7, and BLS10) demonstrated antagonistic activity, as evidenced by the formation of clear inhibition zones on nutrient agar medium. Suppression assays further confirmed their ability to significantly reduce soft rot symptoms in potato tubers. Among these, BLS10 isolate exhibited the highest inhibition and suppression activity, with an inhibition zone of 2.03 cm and a soft rot mass of 0.52 grams. Molecular identification based on 16S rRNA gene sequencing showed that isolates of BLS3, BLS6, BLS7, and BLS10 were identified respectively as *Bacillus velezensis*, *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens*, and *Ochrobactrum intermedium*. All isolates demonstrated a bacteriostatic antibiosis mechanism, indicated by turbidity in peptone water, suggesting inhibition through secondary metabolite production.

Keywords: Antagonistic bacteria, biological control, BLS10, inhibition, *Ochrobactrum intermedium*

Introduction

The potato (*Solanum tuberosum* Linnaeus) is one of the key agricultural commodities in Indonesia, contributing significantly to national food security and holding substantial potential for domestic trade and export. Despite this, the development of potato cultivation remains suboptimal. According to Saptana et al. (2022), the national average productivity of potato is only 17.46 tons per hectare, which is significantly

lower than the global average ranging from 32.85 to 50.67 tons per hectare. This productivity gap highlights the urgent need for improvements in cultivation practices.

One of the main constraints on potato production is soft rot disease caused by *E. carotovora*. This disease can occur during cultivation, distribution, storage, and marketing, leading to postharvest yield losses of 15–30% (Agrios, 2005: Campos & Ortiz 2020). Chemical bactericides are commonly used for control, but they pose risks including harm to beneficial organisms, resistance development in pathogens, and long-term environmental damage (Pathak et al., 2022). Therefore, biological control using antagonistic bacteria offers a promising and environmentally sustainable alternative.

Recent study by Almasoudi et al. (2024) demonstrated that bacterial biocontrol agents *Bacillus bullata*, *B. siamensis*, and *B. velezensis* exhibited significant antagonistic activity against various isolates of *Pectobacterium carotovorum* subsp. *carotovorum*, the causative agent of soft rot disease in potatoes. The study revealed differences in the in vitro inhibition capacity among the tested strains. However, *B. velezensis* consistently showed superior performance in reducing soft rot symptoms during in vivo assays on inoculated potato tuber slices, indicating its strong potential as a biocontrol agent for managing bacterial soft rot in postharvest potato. Furthermore, a study by Compant et al. (2005) reported that *Pseudomonas fluorescens* also exhibits antagonistic activity against soft rot disease through the production of various secondary metabolite-derived antibiotic compounds, including siderophores, antibiotics, and surfactants.

Bacteria capable of surviving under extreme environmental conditions, such as high temperature and salinity, have been reported to possess high efficacy and adaptability. *Brevibacillus laterosporus* strain BPM3, isolated from a natural hot spring in Assam, India, exhibited strong inhibitory activity against several pathogenic fungi (*Fusarium oxysporum*, *F. semitectum*, *Magnaporthe grisea*, *Rhizoctonia oryzae*) and the bacterium *Staphylococcus aureus*. In greenhouse trials, this strain was able to reduce rice blast disease incidence by 30–67% (Saikia et al., 2011).

One of the extreme environments in Indonesia is the Sidoarjo hot mud, which has been classified as a national disaster since May 2006 (Davies et al., 2007). This area is characterized by high temperatures (45–70°C), alkaline pH levels (7.5–7.8), and elevated salinity reaching 30 ppt (30,000 ppm) (Dagdag et al., 2015). The eruption has significantly impacted the surrounding environment and led to the formation of a unique ecosystem, as indicated by microbial activity such as changes in mud coloration. Microbial analysis of Sidoarjo hot mud revealed the presence of bacteria at concentrations of approximately 5.1×10^4 cfu/g (Santosa et al., 2014).

Previous studies reported that *Pasteurella multocida*, isolated from Sidoarjo hot mud, exhibited antagonistic activity against *Pyricularia oryzae*, the causal agent of rice blast. In addition, thermophilic *Bacillus licheniformis*, a xylanase-producing bacterium, was also isolated from Sidoarjo hot mud and shown to suppress gray mold in strawberry caused by *Botrytis cinerea* (Habibi et al., 2014; Kim et al., 2007). However, to date, no systematic exploration has been conducted to assess the biocontrol potential of bacterial isolates from Sidoarjo hot mud against *E. carotovora*, the causative agent of soft rot disease in potatoes. This study aimed to isolate, evaluate the potential and activity, and identify the molecular basis of novel bacteria from Sidoarjo hot mud against *E. carotovora* the causal agent of potato soft rot.

Methods

The research was conducted at the Laboratory of Plant Pathology, Department of Plant Pest and Disease, Faculty of Agriculture,

Universitas Brawijaya, Indonesia, and the Laboratory of Genetics and Molecular Biology, Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Malang, Indonesia, from October 2015 to April 2016.

Pathogen Preparation

The pathogenic bacteria *E. carotovora* was obtained from the collection of the Plant Disease Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya. The *E. carotovora* isolate was cultured on nutrient agar (NA) and incubated at room temperature for 24 hours. Subculturing and propagation were carried out using the same medium and method. To confirm its pathogenicity, the *E. carotovora* isolate was tested for its ability to induce soft rot disease in potato tubers. The pathogenicity test followed the method described by Haque et al. (2009). The surface of the potato tubers was sterilized by immersion in 1% sodium hypochlorite for 10 minutes, followed by rinsing three times with sterile distilled water and air-drying. The tubers were then punctured using the tip of a micropipette and inoculated with 1 mL of *E. carotovora* bacterial suspension at a concentration of 10^9 cfu/mL. For the control treatment, tubers were inoculated with sterile distilled water. The inoculated tubers were incubated in a humid chamber at room temperature for 7 days.

Sample Collection, Isolation, and Screening of Bacteria from Sidoarjo Hot Mud

Sample collection was conducted using a purposive sampling method at 10 representative points around the eruption center. The selected sampling sites were characterized by high temperatures, elevated salinity, and visible discoloration indicative of microbial activity (Table 1, Figure 1). High temperature areas were targeted to isolate thermotolerant bacteria, saline areas for halotolerant bacteria, and discolored zones for other potentially unique microorganisms.

The collected samples were transported to the laboratory, and bacterial isolation was performed using the serial dilution method described by Sanders (2012). One gram of the sample was suspended in 900 µL of sterile distilled water and homogenized. Serial dilutions were conducted by transferring 100 µL of the suspension into Eppendorf tubes containing 900 µL of sterile distilled water, and this process was repeated up to a 10^{-9} dilution. Subsequently, 100 µL of the 10^{-9} dilution was spread onto nutrient agar (NA) plates and incubated at room temperature for 24–48 hours. Distinct bacterial colonies were then purified to obtain single and pure isolates.

Table 1. Location and environmental conditions of the sample collection site

Sample Code	Coordinates	Elevation (m)	Temperature (°C)	Description
LS-1	S 07.52977° E 112.71278°	31	31	High salinity
LS-2	S 07.31844° E 112.42556°	25	38	Discoloration
LS-3	S 07.52857° E 112.70908°	26	59	High temperature
LS-4	S 07.31810° E 112.42575°	25	39	Discoloration
LS-5	S 07.52674° E 112.70849°	26	30	Discoloration
LS-6	S 07.52798° E 112.70854°	29	38	Discoloration
LS-7	S 07.52782° E 112.70841°	27	37	Discoloration
LS-8	S 07.52886° E 112.70885°	27	32	High salinity
LS-9	S 07.52874° E 112.70886°	28	56	High temperature
LS-10	S 07.31332° E 112.42865°	33	39	Discoloration

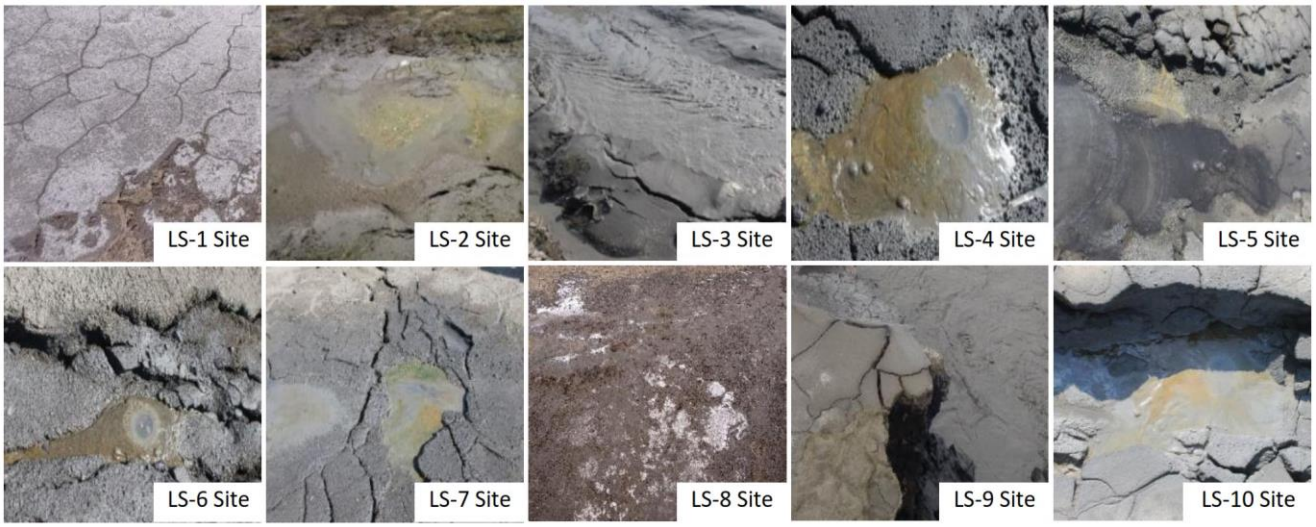


Figure 1. Environmental conditions at the sampling area of Sidoarjo hot mud

A total of 16 bacterial isolates were screened for antagonistic activity against *E. carotovora* using the method described by Kawaguchi et al. (2008). Bacterial suspensions were adjusted to a concentration of 10^9 cfu/mL. Sterile filter paper discs (5 mm in diameter) were immersed in the bacterial suspensions for approximately 1 minute, air-dried for 2 hours, and then placed on nutrient agar (NA) plates. The plates were incubated at 37°C for 24 hours. To inactivate the antagonistic bacteria, the plates were inverted, and chloroform was added to the lid for 1–2 hours until it fully evaporated. After inactivation, the plates were sprayed with a suspension of *E. carotovora* at 10^9 cfu/mL and incubated at 37°C for 48 hours. The formation of clear inhibition zones was observed, and isolates showing such zones were selected for further testing.

Inhibition Assay Against *E. Carotovora*

The inhibition test followed the same procedure as the initial screening. The experiment consisted of five treatments: one control using a bactericide (streptomycin sulfate 20%) and four selected antagonistic bacterial isolates. All treatments were incubated at 37°C for 48 hours, and the diameters of the inhibition zones were measured using a digital caliper. The experiment was designed as a completely randomized design with a single factor, five treatments, and four replications (Table 2). The quantitative variable observed was the diameter of the inhibition zone, measured 48 hours post-incubation. The inhibition zone was calculated to assess the antagonistic potential of bacterial isolates derived from Sidoarjo hot mud against *Erwinia carotovora*, using the following formula.

$$IZ\ (cm) = Ø\ cz\ (cm) - Ø\ c\ (cm)$$

Notes: IZ = inhibation zone, cz = clear zone, c = colony

Tabel 2. Inhibition assay treatments

Treatment	Description
P1	Bactericide and *E. carotovora* 10^9 cfu/mL (control)
P2	Sidoarjo mud isolate BLS3 isolate 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL
P3	Sidoarjo mud isolate BLS6 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL
P4	Sidoarjo mud isolate BLS7 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL
P5	Sidoarjo mud isolate BLS10 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL

Soft Rot Disease Suppression Assay

This assay aimed to evaluate the ability of Sidoarjo hot mud bacteria to suppress soft rot disease in potato tubers caused by *E. carotovora*. The Granola potato cultivar was used. The method was adapted from Haque et al. (2009). Tubers were washed under running water and air-dried, followed by surface sterilization using 1% sodium hypochlorite (NaOCl) for 10 minutes. Tubers were rinsed three times in sterile distilled water and air-dried. A small wound was made on one side of the tuber, and 50 µL of a 10^9 cfu/mL suspension of antagonistic bacteria was inoculated into the wound and left for 1–2 hours. For control treatments, sterile distilled water and streptomycin-based bactericide were applied. Subsequently, the same wound was inoculated with 50 µL of *E. carotovora* (10^9 cfu/mL). The inoculated tubers were incubated under humid conditions at room temperature for 7 days. The experimental design followed a completely randomized design with six treatments and four replications (Table 3).

Observation variables included both qualitative and quantitative parameters. Qualitative data included documentation of symptom development and soft rot suppression, while quantitative data consisted of the mass of rotten tissue. On day 7 post-inoculation, tubers were halved, and the rotten tissue was scooped out and weighed to assess disease severity.

Table 3. Treatments for the suppression of soft rot disease in potato

Treatment	Description
P1	Sterile distilled water and *E. carotovora* 10^9 cfu/mL (Negative control)
P2	Bactericide and *E. carotovora* 10^9 cfu/mL (Positive control)
P3	Sidoarjo mud isolate BLS3 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL
P4	Sidoarjo mud isolate BLS6 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL
P5	Sidoarjo mud isolate BLS7 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL
P6	Sidoarjo mud isolate BLS10 10^9 cfu/mL and *E. carotovora* 10^9 cfu/mL

Molecular Identification Using 16S rRNA

Molecular identification of the antagonistic bacterial isolates was carried out through 16S rRNA gene sequencing. Genomic DNA was extracted using the Presto™ Mini gDNA Bacteria Kit, followed by PCR amplification using universal primers uinF (5'-AGAGTTTGATCATGGGTCAG-3') and uinR (5'-TACGGCTACCTTGTTACGA-3'), as described by Aris et al. (2013). The PCR was performed using GoTaq® Green Master Mix under standard thermal cycling conditions. Amplified PCR products were separated via electrophoresis on a 1.9% agarose gel and visualized using a Gel-Doc XR 1000 imaging system. DNA sequencing was conducted by 1st BASE. The resulting sequences were analyzed using BioEdit for sequence editing and aligned with homologous sequences through BLAST (NCBI). Phylogenetic relationships among the isolates were inferred by constructing a phylogenetic tree using MEGA6 software.

Antibiosis Mechanism Test

The antibiosis mechanism of the antagonistic bacteria was evaluated by assessing the type of inhibitory effect exhibited. A sample from the inhibition zone was transferred into a test tube containing 5 mL of 0.5% peptone water and incubated on a shaker for 24 hours, following the method of Djatmiko et al. (2007). The analysis was qualitative, based on visual observations of turbidity or color changes in the peptone water, which were interpreted as indicators of bacterial growth or inhibition.

Data Analysis

Quantitative data obtained from the experiments were analyzed using Analysis of Variance (ANOVA) at a 5% level of significance. When significant differences were detected

among treatments, the analysis was followed by Duncan's Multiple Range Test (DMRT) to compare means, also at the 5% significance level. All statistical analyses and graphical representations were performed using Microsoft Excel and DSAASTAT version 1.101.

Result

Isolation and Screening of Bacteria

Bacterial isolation from 10 Sidoarjo hot mud samples resulted in 16 distinct bacterial isolates. Specifically, 4 isolates (BLS1.1, BLS1.2, BLS1.3, BLS1.4) were obtained from sample LS-1; 3 isolates (BLS2.1, BLS2.2, BLS2.3) from LS-2; 2 isolates (BLS8.1, BLS8.2) from LS-8; and one isolate each from samples LS-3 through LS-7, LS-9, and LS-10 (Table 4).

Table 4. Bacterial isolates obtained from Sidoarjo hot mud

Sample Code	Number of Isolates	Isolate Codes
LS-1	4	BLS1.1, BLS1.2, BLS1.3, BLS1.4
LS-2	3	BLS2.1, BLS2.2, BLS2.3
LS-3	1	BLS3
LS-4	1	BLS4
LS-5	1	BLS5
LS-6	1	BLS6
LS-7	1	BLS7
LS-8	2	BLS8.1, BLS8.2
LS-9	1	BLS9
LS-10	1	BLS10

Screening of the 16 isolates revealed that 4 isolates (BLS3, BLS6, BLS7, and BLS10) were capable of inhibiting the growth of *E.carotovora*, as evidenced by the formation of clear zones on nutrient agar (NA) medium (Table 5). These four isolates were subsequently selected for further analysis and molecular identification.

Table 5. Screening results of Sidoarjo mud bacteria with inhibitory activity against *E. carotovora*

Isolate Code	Inhibiton	Isolate Code	Inhibiton
BLS1.1	-	BLS4	-
BLS1.2	-	BLS5	-
BLS1.3	-	BLS6	+
BLS1.4	-	BLS7	+
BLS2.1	-	BLS8.1	-
BLS2.2	-	BLS8.2	-
BLS2.3	-	BLS9	-
BLS3	+	BLS10	+

A positive sign (+) indicates the presence of a clear inhibition zone; a negative sign (-) indicates no inhibition zone.

Inhibition of *E. carotovora* Growth

The inhibition assay demonstrated that four selected antagonistic bacterial isolates from Sidoarjo hot mud were able to inhibit the growth of *E. carotovora*, though with varying levels of effectiveness (Table 6). Among them, BLS10 isolate exhibited the strongest inhibitory effect, forming a 2.03 cm inhibition zone, followed by BLS7 isolate (1.93 cm) and BLS6 isolate (1.80 cm). BLS3 isolate showed the weakest activity, with an inhibition zone of 1.51 cm. Although BLS10 isolate displayed notable antagonistic potential, its efficacy was still lower than that of the positive control, streptomycin sulfate 20%

(2.93 cm). Overall, the results indicate that while all isolates exhibit inhibitory activity against *E. carotovora*, BLS10 isolate shows the greatest potential as a biocontrol agent and warrants further investigation.

Table 6. Inhibition of *E. carotovora* growth by antagonistic bacteria at 48 hours after incubation

Treatment	Mean Inhibition Zone (cm)
P1 (streptomycin sulfate 20%, control)	2.93 a
P2 (BLS3 isolate)	1.51 c
P3 (BLS6 isolate)	1.80 bc
P4 (BLS7 isolate)	1.93 b
P5 (BLS10 isolate)	2.03 b

Values in the same column with the same letter are not significantly different (DMRT, $p < 0.05$)

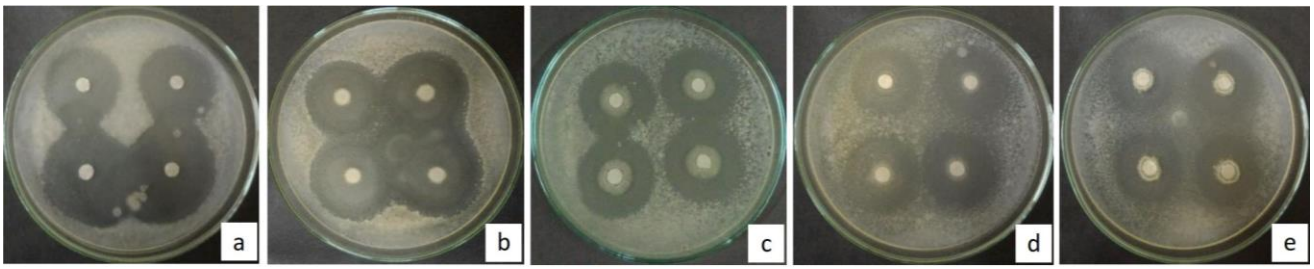


Figure 2. Inhibition zones formed by each treatment at 48 hours after incubation. (a) Streptomycin sulfate 20%, (b) BLS3 isolate, (c) BLS6 isolate, (d) BLS7 isolate, and (e) BLS10 isolate.

Suppression of Soft Rot in Potato Tubers

The suppression assay demonstrated that the application of antagonistic bacterial isolates resulted in varying levels of effectiveness in reducing soft rot severity in potato tubers (Table 7, Figure 3). The negative control, treated with sterile distilled water, resulted in the highest average soft rot mass (2.15 g), indicating no suppression activity. In contrast, the positive control (P2), using 20% streptomycin sulfate, showed the lowest soft rot mass (0.47 g), confirming its strong antibacterial efficacy. Among the tested isolates, BLS10 (P6) showed the highest suppressive effect, with a soft rot mass of 0.52 g. Isolate BLS6 (P4) demonstrated intermediate suppression, with a soft rot mass of 1.23 g, which was significantly lower than the negative control but higher than BLS10 and P2. Meanwhile, BLS3 (P3) and BLS7 (P5) showed moderate suppression, with soft rot masses of 1.46 g and 1.45 g, respectively. However, these values were not significantly different from the negative control, indicating limited antagonistic activity.

Table 7. Average mass of soft rot in potato tubers 7 days after incubation

Treatent	Soft Rot Mass (g)
P1 (sterile distilled water, negative control)	2,15 a
P2 (streptomycin sulfate 20%, positive control)	0,47 c
P3 (BLS3 isolate)	1,46 ab
P4 (BLS6 isolate)	1,23 bc
P5 (BLS7 isolate)	1,45 ab
P6 (BLS10 isolate)	0,52 c

Values in the same column with the same letter are not significantly different (DMRT, $p < 0.05$



Figure 3. Development of soft rot symptoms in potato tubers caused by each treatment 7 days after incubation. (a) sterile distilled water (negative control), (b) streptomycin sulfate 20% (positive control), (c) BLS3 isolate, (d) BLS6 isolate, (e) BLS7 isolate, and (f) BLS10 isolate

Molecular Identification

PCR amplification targeting the 16S rRNA gene was successfully conducted on all selected bacterial isolates. Visualization of the PCR products on a 1.9% agarose gel revealed distinct DNA bands of approximately 1,500 bp, consistent with the expected size for 16S rRNA gene amplification, when compared to the 1 kb DNA ladder (Figure 4). The clarity and intensity of the bands indicated that the selected primers and annealing temperature were optimal for the amplification process.

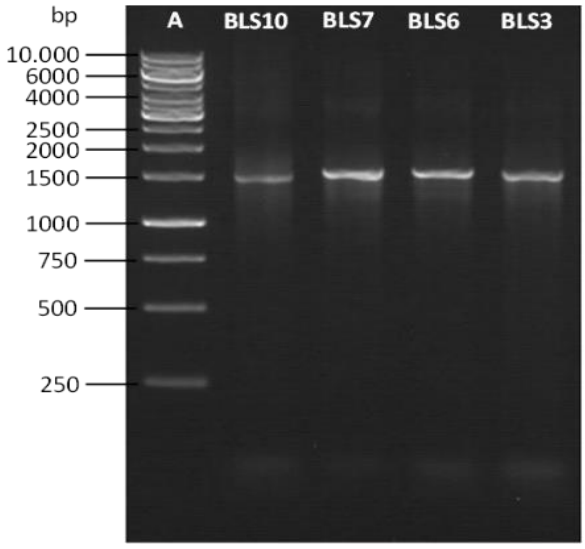


Figure 4. Agarose gel electrophoresis of PCR products targeting the 16S rRNA gene. A: DNA marker (1 kb DNA ladder).

Sequence analysis using the BLAST tool (NCBI) demonstrated that isolate BLS3 shared 98% sequence homology with *Bacillus velezensis* strain DB-1 (KX056902.1); BLS6 had 99% homology with *Bacillus methylotrophicus* strain MER 171 (KT719754.1); BLS7 showed 99% homology with *Bacillus amyloliquefaciens* strain ML471 (KC692205.1); and BLS10 shared 99% homology with *Ochrobactrum intermedium* strain PFM-ABF (KU885897.1) (Table 8).

Phylogenetic analysis was conducted for isolates BLS6 and BLS7, as only these isolates had high quality and sufficient sequence length for tree construction. The 16S rRNA sequences of BLS6 and BLS7 were aligned and compared with reference sequences of selected Gram-positive bacteria from the genus Clostridium and Gram-negative bacteria from the genus Pseudomonas. Phylogenetic tree construction using MEGA6 software indicated that BLS6 and BLS7 clustered closely with *B. methylotrophicus* strain MER 171 and *B. amyloliquefaciens* strain ML471, respectively (Figure 5).

Antibiosis Mechanism

Qualitative analysis based on visual observations of turbidity or color changes in the peptone water medium showed that all selected antagonistic bacterial isolates exhibited a bacteriostatic mechanism against *E. carotovora*. This was indicated by the presence of turbidity in the peptone water medium after 24 hours of incubation (Table 9, Figure 6).

Table 8. BLAST analysis results of 16S rRNA gene sequences

Isolate Code	Query Length	Closest BLAST Match	Max Score	Query Coverage	Identity
BLS3	262	*Bacillus velezensis* strain DB-1 (KX056902.1)	468	100%	98%
BLS6	1421	*Bacillus methylotrotrophicus* strain MER 171 (KT719754.1)	2610	100%	99%
BLS7	1422	*Bacillus amyloliquefaciens* strain ML471 (KC692205.1)	2610	100%	99%
BLS10	437	*Ochrobactrum intermedium* strain PFM-ABF (KU885897.1)	800	100%	99%

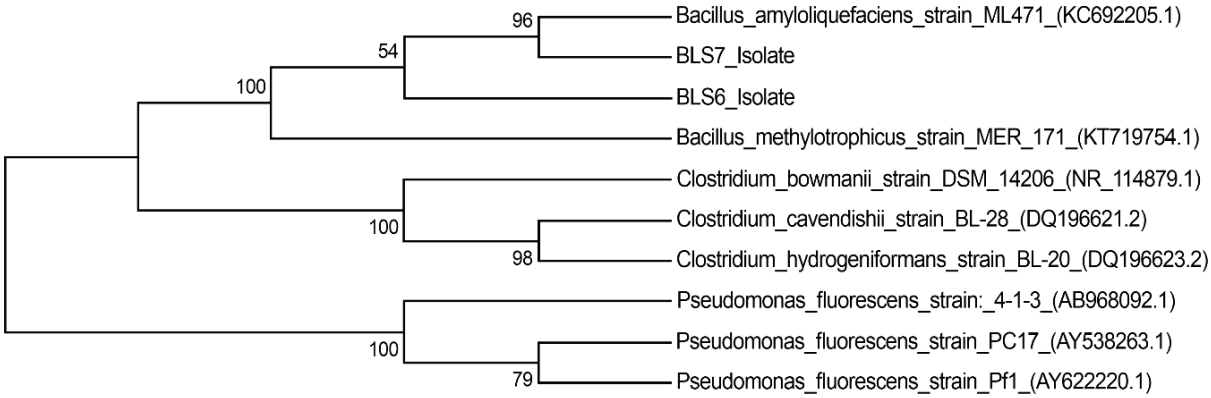


Figure 5. Phylogenetic tree of bacterial isolates BLS6 and BLS7

Table 9. Antibiosis Mechanism of Antagonistic Bacteria Isolated from Sidoarjo Hot Mud

Bacterial Isolate	**Antibiosis Mechanism**
BLS3 (*Bacillus velezensis*)	Bacteriostatic
BLS6 (*Bacillus methylotrophicus*)	Bacteriostatic
BLS7 (*Bacillus amyloliquefaciens*)	Bacteriostatic
BLS10 (*Ochrobactrum intermedium*)	Bacteriostatic



Figure 6. Condition of peptone water medium at 24 hours after incubation

Discussion

Based on the results, out of 16 bacterial isolates obtained from the Sidoarjo hot mud, four isolates (BLS3, BLS6, BLS7, and BLS10) demonstrated antagonistic activity against *Erwinia carotovora*. These isolates produced clear inhibition zones on nutrient agar (NA), indicating their ability to suppress the growth of the pathogenic bacterium. The presence of inhibition zones suggests the production of antimicrobial compounds by the antagonistic isolates. Such antibiosis mechanisms are often associated with the production of secondary metabolites including alkaloids, phenolics, flavonoids, glycosides, and phytoalexins. Furthermore, antibiosis is closely linked to the ability of antagonistic bacteria to produce hydrolytic enzymes such as chitinases, proteases, and cellulases (Hallman et al., 1997).

In the soft rot suppression assay, all four bacterial isolates significantly reduced the severity of soft rot symptoms on potato tubers. The level of suppression varied among the isolates, likely reflecting differences in the types and quantities of bioactive compounds they produce. This specific disease suppression is typically attributed to one or several microbial antagonists acting through direct mechanisms. Such suppression is thought to occur when biocontrol agents are introduced and exert disease inhibition through the production of bioactive substances (Pal and Gardener, 2006). Similar findings have been reported in studies using *Pseudomonas fluorescens*, which produces the antibiotic 2,4-diacetylphloroglucinol and suppresses a broad range of soilborne pathogens (Weller et al., 2002).

Molecular identification revealed that the four selected isolates were *Bacillus velezensis* strain DB-1, *Bacillus methylotrophicus* strain MER 171, *Bacillus amyloliquefaciens* strain ML471, and *Ochrobactrum intermedium* strain PFM-ABF. Members of the genus *Bacillus* are well-known for their antagonistic capabilities against plant pathogens, mainly due to their ability to produce various antibiotics (Sumi et al., 2014). For instance, *B. velezensis* strain G341 has been reported to suppress plant pathogens by producing bioactive compounds such as bacillomycin D, fengycin, and (oxy) difficidin (Lee et al., 2015). In addition, *B. velezensis* has been shown to control Fusarium wilt in strawberry plants under field conditions (Hyeon et al., 2009), and root rot in hydroponic vegetable crops by inhibiting the mycelial growth of *Pythium spp.* through surfactant production (Kanjanamaneesathian et al., 2013; Ruiz-Garcia et al., 2005).

B. methylotrophicus is widely distributed in the rhizosphere and is recognized as a plant growth-promoting rhizobacterium (PGPR). It exhibits biocontrol potential by producing secondary metabolites or antibiotics. For example, *B. methylotrophicus* FKM10, isolated from the rhizosphere of apple trees in Shandong, China, showed antagonistic activity against soilborne pathogens such as *Fusarium oxysporum*, *F. solani*, and *F. proliferatum* (Wang et al., 2016). Another strain, *B. methylotrophicus* BC79, isolated from forest soil in Qinling, China, was found to suppress rice blast disease by producing phenaminomethylacetic acid (Shan et al., 2013).

B. amyloliquefaciens is a well-established biocontrol agent for various plant diseases. It has demonstrated effectiveness in suppressing soft rot caused by *E. carotovora* in postharvest vegetables such as green peppers, Chinese cabbage, and potatoes. Its antagonistic mechanism involves the production of antimicrobial compounds and competition for space and nutrients (Zhao et al., 2012). In addition, strains CM-2 and T-5 of

B. amyloliquefaciens, when formulated as bioorganic fertilizers, significantly reduced bacterial wilt incidence in tomato plants by 63–74%, promoted plant growth, and reduced *Ralstonia solanacearum* populations in the rhizosphere (Tan et al., 2013).

O. intermedium is a beneficial bacterium from the genus *Ochrobactrum* (Barquero-Calvo et al., 2009). The strain NH-5 of *O. intermedium* was reported to effectively suppress *Colletotrichum falcatum*, the causal agent of red rot in sugarcane (varieties SPF-234 and Co-1148), under field conditions. This inhibitory activity is linked to its production of siderophores and the broad-spectrum antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) (Hassan et al., 2014).

Antibiosis mechanism tests revealed that all isolates *B. velezensis* (BLS3), *B. methylotrophicus* (BLS6), *B. amyloliquefaciens* (BLS7), and *O. intermedium* exerted bacteriostatic effects against *E. carotovora*. According to Dias et al. (2014), the mode of action of antibiosis can be determined based on changes in turbidity or color in the test medium (peptone water). A bacteriostatic mechanism is indicated by the development of turbidity in the medium, signifying bacterial growth, whereas a bactericidal effect is evidenced by the medium remaining clear, indicating the absence of bacterial proliferation. In this study, the appearance of turbidity in all treatments confirmed that the isolates inhibited *E. carotovora* growth without causing complete cell death.

The ability of these antagonistic bacteria to suppress *E. carotovora* through bacteriostatic means suggests that their antagonism may involve the production of inhibitory metabolites, such as lipopeptides, organic acids, or enzymes, which interfere with pathogen metabolism or replication. Bacteriostatic compounds act by inhibiting DNA replication, RNA synthesis, cell wall formation, protein synthesis, plasma membrane function, or the synthesis of essential metabolites (Kohanski et al., 2010). Typical bacteriostatic agents include tetracycline, doxycycline, chloramphenicol, erythromycin, and fusidic acid, which act by binding to the 30S and 50S ribosomal subunits to inhibit protein synthesis (Ocampo et al., 2014). Although this mechanism does not eliminate the pathogen entirely, it may be sufficient to reduce disease severity under natural conditions. These findings highlight the potential of bacteriostatic agents as biocontrol candidates, especially in integrated disease management systems where sustained suppression is desirable without the risk of disrupting beneficial microbiota.

Conclusion

Based on the research, 16 bacterial isolates were obtained from the Sidoarjo hot mud. Among them, four isolates (BLS3, BLS6, BLS7, and BLS10) demonstrated antagonistic potential by inhibiting the growth of *E. carotovora*, with inhibition zones ranging from 1.43 cm to 2.03 cm, and suppressing soft rot disease in potato tubers, with average soft rot mass ranging from 0.52 g to 1.46 g. Notably, isolate BLS10 exhibited the strongest antagonistic activity, achieving an inhibition zone of 2.03 and reducing soft rot mass to 0.52 g. Molecular identification revealed that these isolates correspond to *Bacillus velezensis*, *B. methylotrophicus*, *B. amyloliquefaciens*, and *Ochrobactrum intermedium*. All isolates exerted a bacteriostatic effect on *E. carotovora*, indicating that suppression likely occurred through the production of inhibitory secondary metabolites.

Declaration

Author contribution

Ahmad Muhidin is the main contributor of this paper and the corresponding author. Femita Hapsari, Luqman Qurata Aini, Muhammad Akhid Syib'li, and Restu Rizkyta Kusuma are co-authors who contributed to the research execution, data analysis, and manuscript preparation. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Agrios GN. 2005. Plant pathology. Fifth edition. Elsevier Academic Press. San Diego.

Almasoudi NM, AD Al-Qurashi, MI Elsayed, KAM Abo-Elyousr. 2024. Native bacterial bioagents for management of potato soft rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum*. *Egyptian Journal of Biological Pest Contro*l (34)31. 1-8. DOI: 10.1186/s41938-024-00794-4.

Aris M, Sukenda, E Harris, MF Sukadi, M Yuhana. 2013. Identifikasi molekuler bakteri patogen dan desain primer PCR. Jurnal Budidaya Perairan 1(3): 43-50. DOI: 10.35800/bdp.1.3.2013.2733.

Barquero-Calvo E, R Conde-Alvarez, C Chacón-Díaz, L Quesada-Lobo, A Martirosyan, C Guzmán-Verri, M Iriarte, M Mancek-Keber, R Jerala, JP Gorvel, I Moriyón, E Moreno, E Chaves-Olarte. 2009. The differential interaction of *Brucella* and *ochrobactrum* with innate immunity reveals traits related to the evolution of stealthy pathogens. *PLoS One* 4(6): 1-17. DOI: 10.1371/journal.pone.0005893.

Campos H, O Ortiz. 2020. *The potato crop: Its agricultural, nutritional and social contribution to humankind*. Cham. hlm. 351–388. Springer. DOI: 10.1007/978-3-030-28683-5_10

Compant S, B Duffy, J Nowak, C Clément, EA Barka. 2005. Use of plant growth-promoting bacteria for biocontrol of plant disease: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology*. 71(9): 4951-4959. DOI: 10.1128/AEM.71.9.4951-4959.2005.

Dagdag EE, Sukoso, WP Asthervina. 2015. Isolation and characterization of isolate thermophilic bacteria from water and solid sediment of Lapindo mud. Resources and Environment 5(2): 66-71. DOI: 10.5923/j.re.20150502.03.

Davies RJ, RE Swarbrick, RJ Evans, M Huuse. 2007. Birth of a mud volcano: East Java, 29 May 2006. GSA Today 17(2): 4-9. DOI: 10.1130/GSAT01702A.1.

Dias C, A Aires, MJ Saavedra. 2014. Antimicrobial activity of isothiocyanates from cruciferous plants against methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Mol Sci* 15(11): 19552-19561. DOI: 10.3390/ijms151119552.

Djatmiko HA, T Arwiyanto, B Hadisutrisno, BH Sunarminto. 2007. Potensi tiga genus bakteri dari tiga rizosfer tanaman sebagai agensia pengendali hayati penyakit lincat. *Jurnal Ilmu-ilmu Pertanian Indonesia* 9(1): 40-47. DOI: 10.31186/jipi.9.1.40-47.

Habibie FM, AK Wardani, M Nurcholis. 2014. Isolasi dan identifikasi molekuler mikroorganisme termofilik penghasil xilanase dari lumpur panas Lapindo. *Jurnal Pangan dan Agroindustri* 2(4): 231-238.https://jpa.ub.ac.id/index.php/jpa/article/view/95/113.

Hallmann J, A Quadt-Hallmann, WF Mahaffee, JW Kloepper. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of*

Microbiology 43(10): 895-914. DOI: 10.1139/m97-131.

Haque MM, MS Kabir, LQ Aini, H Hirata, S Tsuyumu. 2009. SlyA, a MarR family transcriptional regulator, is essential for virulence in *Dickeya dadantii* 3937. *Journal of Bacteriology* 191(17): 5409-5418. DOI: 10.1128/JB.00240-09.

Hassan MN, S Afghan, ZU Hassan, FY Hafeez. 2014. Biopesticide activity of sugarcane associated rhizobacteria: *Ochrobactrum intermedium* strain NH-5 and *Stenotrophomonas maltophilia* strain NH-300 against red rot under field conditions. *Phytopathologia Mediterranea* 53(2): 229-239. DOI: 10.14601/Phytopathol_Mediterr-11927.

Hyeon NM, MS Park, HG Kim, SJ Yoo. 2009. Biological control of strawberry *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *fragariae* using *Bacillus velezensis* BS87 and RK1 formulation. *Journal of Microbiology and Biotechnology* 19(5): 520–524. DOI: 10.4014/jmb.0805.333.

Kanjanamaneesathian M, R Wiwattanapatapee, W Rotniam, A Pengnoo, W Wongpetkhiew, V Tanmala. 2013. Application of a suspension concentrate formulation of *Bacillus velezensis* to control root rot of hydroponically-grown vegetables. *New Zealand Plant Protection* 66: 229-234. DOI: 10.30843/nzpp.2013.66.5556.

Kawaguchi A, K Inoue, Y Ichinose. 2008. Biological control of crown gall of grapevine, rose, and tomato by nonpathogenic *Agrobacterium vitis* strain VAR03-1. *Phytopathology* 98(11): 1218-1225. DOI: 10.1094/PHYTO-98-11-1218.

Kim JH, SH Lee, CS Kim, EK Lim, KH Choi, HG Kong, DW Kim, SW Lee, BJ Moon. 2007. Biological control of strawberry gray mold caused by *Botrytis cinerea* using *Bacillus licheniformis* N1 formulation. *J Microbiol Biotechnol* 17(3): 438–444. PMID. 18050947.

Kohanski MA, DJ Dwyer, JJ Collins. 2010. How antibiotics kill bacteria: from targets to networks. *National Review of Microbiology* 8(6): 423-435. DOI: 10.1038/nrmicro2333.

Lee HH, J Park, JY Lim, H Kim, GJ Choi, JC Kim, YS Seo. 2015. Complete genome sequence of *Bacillus velezensis* G341, a strain with a broad inhibitory spectrum against plant pathogens. *Journal of Biotechnology* 211: 97-98. DOI: 10.1016/j.jbiotec.2015.07.005.

Ministry of Agriculture. 2015. Rencana Strategis Kementerian Pertanian Tahun 2015-2019. Kementerian Pertanian Republik Indonesia. Jakarta.

Ocampo PS, V Lázár, B Papp, M Arnoldini, P Abel zur Wiesch, R Busa-Fekete, G Fekete, C Pál, M Ackermann, S Bonhoeffer. 2014. Antagonism between bacteriostatic and bactericidal antibiotics is prevalent. *Antimicrobial Agents and Chemotherapy* 58(8): 4573–4582. DOI: 10.1128/AAC.02463-14.

Pal KK, BM Gardener. 2006. Biological control of plant pathogens. *The Plant Health Instructor* 2: 1117-1142. DOI: 10.1094/PHI-A-2006-1117-02.

Pathak VM, VK Verma, BS Rawat, B Kaur, N Babu, A Sharma, S Dewali, M Yadav, R Kumari, S Singh, A Mohapatra, V Pandey, N Rana, JM Cunill. 2022. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. *Frontiers in Microbiology* (13)1. 1-29. DOI : 10.3389/fmicb.2022.962619.

Ruiz-Garcı´a C, V Be´jar, F Martı´nez-Checa, I Llamas, E Quesada. 2005. *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Ve´lez in Ma´laga, southern Spain. *International Journal of Systematic and Evolutionary Microbiology* 55(1): 191–195. DOI: 10.1099/ijs.0.63310-0.

Saikia R, DK Gogoi, S Mazumder, A Yadav, RK Sarma, TC Bora, BK Gogoi. 2011. *Brevibacillus laterosporus* strain BPM3, a potential biocontrol agent isolated from a natural hot water spring of Assam, India. *Microbiological Research* 166(3): 216-225. DOI: 10.1016/j.micres.2010.03.002.

Sanders ER. 2012. Aseptic laboratory techniques: plating methods. *J Vis Exp*. 63: 1-18. DOI: 10.3791/3064.

Santosa S, Z Kusuma, B Prasetya, B Yanuwiadi. 2014. The effect of Sidoarjo's mud soil with organic materials toward the growth of Albizian (*Samanea saman* Merr). *Natural and Applied Science* 5(1): 57-65.

http://www.savap.org.pk/journals/ARInt./Vol.5(1)/2014(5.1-08).pdf.

Saptana, AL Sayekti, AD Perwita, B Sayaka, E Gunawan, SG Sukmaya, NQ Hayati, Y Sumaryanto, MP Yufdy, S Mardianto, AD Pitaloka. 2022. Analysis of competitive and comparative advantages of potato production in Indonesia. *PLoS ONE* 17(2): 1-21. DOI: 10.1371/journal.pone.0263633.

Shan H, M Zhao, D Che, J Cheng, J Li, Z Feng, Z Ma, D An. 2013. Biocontrol of rice blast by the phenaminomethylacetic acid producer of *Bacillus methylotrophicus* strain BC79 . *Crop Protection* 44: 29-37. DOI: 10.1016/j.cropro.2012.10.012.

Sumi CD, BW Yang, IC Yeo, YT Hahm. 2015. Antimicrobial peptides of the genus *Bacillus*: a new era for antibiotics. *Can J Microbiol* 61(2): 93-103. DOI: 10.1139/cjm-2014-0613.

Tan S, Y Dong, H Liao, J Huang, S Song, Y Xu, Q Shen. 2013. Antagonistic bacterium *Bacillus amyloliquefaciens* induces resistance and controls the bacterial wilt of tomato. *Pest Manag Sci* 69(11): 1245-1252. DOI: 10.1002/ps.3491.

Wang C, X Hu, K Liu, Q Hou, Q Yang, Y Ding, B Du. 2016. Draft genome sequence of *Bacillus methylotrophicus* FKM10, a plant growth-promoting rhizobacterium isolated from apple rhizosphere. *Genome Announc* 4(1): 10-1128. DOI: 10.1128/genomeA.01739-15.

Weller DM, JM Raaijmakers, BB Gardener, LS Thomashow. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40: 309-348. DOI: 10.1146/annurev.phyto.40.030402.110010.

Zhao Y, P Li, K Huang, Y Wang, H Hu, Y Sun. 2012. Control of postharvest soft rot caused by *Erwinia carotovora* of vegetables by a strain of *Bacillus amyloliquefaciens* and its potential modes of action. *World J Microbiol Biotechnol* 29(3): 411-420. DOI: 10.1007/s11274-012-1193-0.

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