

Identification of Bacterial Soft Rot in Potatoes from Batu City, Indonesia

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Abstract. Soft rot is a plant disease that can reduce both the quality and quantity of potato production, primarily caused by *Pectobacterium carotovorum*. This study aims to isolate and identify the bacteria responsible for soft rot disease in potato tubers using molecular methods. Bacterial isolation was performed on a diseased potato tuber collected from Sumber Brantas Village, followed by a pathogenicity test, PCR, 16S rRNA gene sequencing, and phylogenetic analysis. Based on macroscopic and microscopic examination, four isolates exhibiting soft rot symptoms were identified: P1, P2, K5, and K7. The 16S rRNA sequencing results and BLAST analysis showed that isolate K5 was closely related to *Serratia rubidua*, isolate P2 was closely associated with *Pectobacterium carotovorum*, isolate P1 was closely related to *Pseudomonas koreensis*, and isolate K7 was closely associated with *Ralstonia pseudosolanacearum*. This study presents the first molecular characterization of bacteria responsible for soft rot in potato tubers originating from Batu City, East Java. The findings provide foundational insights that may support the development of molecular-based diagnostic tools and region-specific disease management strategies for potato cultivation in Indonesia.

Keywords: molecular identification; PCR; *Pectobacterium carotovorum*; potato; soft rot

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world, ranking fourth after rice, wheat, and corn. Potato tubers play a crucial role as a source of carbohydrates and contribute to food security (Su & Wang, 2019). Potato production has the potential to significantly enhance the economy by offering a sustainable source of income and contributing to the well-being of farming communities (Noor & Hanani, 2025). According to data from the Badan Pusat Statistik (2023), potato production in Indonesia from 2018 to 2022 increased in line with total agricultural output, reaching 1.28–1.42 million tons. However, in 2020, there was a decline, with total production reaching only 1.28 million tons. One of the main constraints on potato production is potato plant diseases, especially soft rot disease, which causes plant tissue to become soft, rotten, and is accompanied by an unpleasant odor. This disease is prevalent both in the field and during storage of the tubers. It has

the broadest host range compared to other bacterial diseases (Davidsson *et al.*, 2013). The infection invades plant tissue through natural wounds and openings (Joko *et al.*, 2011).

The primary bacteria responsible for soft rot disease belong to the genera *Pectobacterium* and *Dickeya* (Czajkowski *et al.*, 2011). One of the most commonly found bacterial species is *Pectobacterium carotovorum*, a pathogenic bacterium that causes soft rot disease in potatoes, leading to a reduction in both the quality and quantity. *Pectobacterium carotovorum* is considered as a complex species because its strains show divergent characteristics (Li *et al.*, 2019). *P. carotovorum* is a facultative anaerobe with strong pectin-degrading activity, which contributes to soft rot in plants. It produces several powerful plant cell wall-degrading enzymes (PCWDEs), such as pectinases, cellulases, and proteases, which result in tissue decay, the release of plant nutrients, and bacterial proliferation (Pritchard *et al.*, 2015). *P. carotovorum* is a gram-negative



bacterium that thrives at 20–25°C, can survive on plant residues and in soil, has a broad host range, and exhibits high genetic variation (Mariano & Souza, 2011).

Research on soft rot bacteria has been extensively conducted, focusing on identification, virulence factors, mechanisms, and environmental factors that influence pathogen development. One such study by Charkowski *et al.* (2012) examined virulence factors and pathogenicity. Several bacterial genera are capable of rotting plants and causing soft rot symptoms (Charkowski, 2018). One of the primary challenges in controlling this disease is early detection and pathogen identification, particularly in potato seeds that are often infected without exhibiting symptoms. Such infections make soft rot disease difficult to control. Additionally, commercial potato varieties lack resistance to pathogen infections. Control measures are primarily limited to seed sanitation, including treatment programs and seed certification, which help reduce the spread of pathogens in seeds (Czajkowski *et al.*, 2011). The primary source of disease spread comes from latently infected seeds. Latent infections can persist under various temperature, environmental, and other conditions that stimulate bacterial growth and spread. The presence of latent infections compromises the accuracy of seed sanitation and certification processes, necessitating additional practices such as crop management, site selection, and storage (Van Gijsegem *et al.*, 2021). Therefore, early detection and accurate identification are strongly required to prevent pathogen spread.

Detection and identification of plant diseases can be performed using two methods: direct and indirect. Direct disease detection can be used to analyze a large number of samples, including molecular and serological methods (Fang & Ramasamy, 2015). One of the detection and identification methods often used to prevent the spread of soft rot is the *Polymerase Chain Reaction* (PCR) method. PCR is a scientific technique in molecular biology that amplifies one or

more copies of DNA to generate thousands to millions of copies of a specific DNA sequence. This method was developed for the rapid and sensitive detection and identification of plant pathogenic bacteria.

Recently, the potato soft rot disease has escalated in Sumber Brantas village, Batu city, one of largest centers of potato production in East Java. Based on field surveys and interviews with farmers, this disease was found both in the field and during storage. Potato tubers are the most affected by soft rot disease, particularly during storage in warehouses. The infection rate of this disease is relatively high, impacting harvest quality and market value, which can affect the local economy. To date, no comprehensive studies have investigated the pathogens responsible for soft rot disease in potatoes in Sumber Brantas Village, Batu City, one of the major potato production centers in East Java. Soft rot disease in potatoes not only threatens potato production in Sumber Brantas Village but also represents a global challenge in plant disease management, highlighting the need for broadly applicable control strategies. Although potatoes play a crucial role in supporting the economy of East Java, no molecular identification of the causal pathogen has been conducted previously in Sumber Brantas Village, Batu City. This study addresses the lack of molecular data on soft rot pathogens in the region by providing species-level identification through 16S rRNA analysis, thereby strengthening efforts for accurate disease diagnosis and control in this central potato-producing area.

METHODS

Bacterial Isolation and Pathogenicity Test

This research was conducted from October 2023 to February 2024. Bacterial soft rot on potato tubers was collected from Sumber Brantas Village, Bumiaji District, Batu City, East Java, located at a 7°45'19"S, 112°32'25"E. A total of 20 symptomatic potato tubers were collected from the field for subsequent bacterial isolation and

identification of the causal agent. The isolation process was carried out in the Plant Disease Laboratory of the Plant Pest and Disease Department, Faculty of Agriculture, Brawijaya University. Tubers with soft rot symptoms were surface-sterilized by washing them with running water, followed by soaking in a 1% sodium hypochlorite (NaOCl) solution for 2 minutes and rinsing twice with sterile distilled water for 1 minute. The sterilized tubers were then cut into two parts using a sterile scalpel, and the bacterial exudate was collected using a sterile inoculating loop and streaked onto Yeast Peptone Agar (YPA) medium. After colony growth, purification was carried out by streaking onto fresh YPA medium using a sterile inoculating loop to produce well-isolated single colonies.

Bacterial identification was based on the color and shape of the colonies. Each bacterial colony with a distinct color and shape was further purified on YPA media using the four-quadrant streaking technique to obtain a single colony. After 48 hours, each single colony as a purified isolate was observed for morphological characteristics, including shape, size, color, elevation, surface texture, and edges (Maghfiroh *et al.*, 2022). Each bacterial isolate was subsequently inoculated into healthy potato tubers for pathogenicity testing.

The pathogenicity test aims to determine whether the bacterial isolates found are pathogens that cause soft rot in tubers. Healthy potato tubers free from disease and damage were selected for testing to serve as controls in the pathogenicity assay. Healthy potato tubers were sterilized sequentially by soaking them in 1% sodium hypochlorite for 10 minutes, followed by 70% alcohol, and then three times washing with sterile distilled water. They were air-dried. A sterile micropipette tip was used to make two holes in the tubers, and 1 mL of a 10^8 cfu/mL bacterial suspension was then added. As a control treatment, the tubers were wounded and injected with sterile distilled water. Observations were

conducted for 7–14 days in a sterile container and incubated at room temperature. The tuber exhibits rot symptoms, indicating a positive reaction (Nawar & Al-Juboory, 2023).

Macroscopic Identification of Bacteria

Macroscopic identification of bacteria was divided into two stages: morphological characterization and Gram staining. Morphological characterization was performed by observing single colonies after 24 hours of incubation on YPA medium. The Gram staining procedure was initiated by transferring a loopful of bacterial colonies onto a sterile microscope slide, followed by the addition of sterile distilled water and spreading evenly across the slide. The smear was then heat-fixed by briefly passing the underside of the slide over a Bunsen burner flame until the surface was dry. Subsequently, the smear was flooded with crystal violet solution for 1 minute, rinsed with running water, and air-dried. Decolorization was carried out using ethanol, followed by rinsing and air-drying. Safranin solution was then applied for 1 minute, followed by a quick rinse with running water and air-drying. The stained preparations were examined under a light microscope at 1000× magnification. Gram-negative bacteria appeared red, whereas Gram-positive bacteria appeared purple.

Molecular Identification of Bacteria DNA Extraction

Bacteria were cultured on nutrient agar (NA) medium for 48 h to obtain single colonies, which were inoculated into nutrient broth (NB) medium and incubated with shaking at 125 rpm for 48 h. The culture was harvested by centrifugation at 6,000 rpm for 2 min, and the pellet was resuspended in 300 µL STESS lysis buffer. The suspension was incubated at 65 °C for 30 min with intermittent vortexing, followed by centrifugation at 13,000 rpm for 10 min. The supernatant was transferred into a new tube, mixed with an equal volume of chloroform: isoamyl alcohol (24:1), and centrifuged at

13,000 rpm for 5 min to separate the phases. The aqueous layer was collected and mixed with cold isopropanol (1:1, v/v) to precipitate genomic DNA (gDNA). The mixture was incubated at -20°C for 2 h to overnight before further analysis.

After the incubation period, the tube was centrifuged at 13,000 rpm for 10 minutes to obtain a DNA pellet. The supernatant was discarded, and 70% EtOH was added to the DNA pellet while gently inverting the tube to mix with the suspension volume. The tube was then centrifuged at 13,000 rpm for 7 minutes. The supernatant was discarded, and the DNA pellet was dried by inverting the tube for 30 minutes to 1 hour. The dried DNA pellet was resuspended in 30–50 μL of TE buffer to obtain template DNA.

All quality control procedures were applied throughout the DNA extraction and PCR stages. Experimental procedures were conducted under sterile conditions using pre-sterilized equipment to minimize the risk of contamination. According to Gupta (2019), DNA isolation during the extraction stage must be performed efficiently to yield DNA of good quantity and quality pure and free from contaminants such as RNA and proteins.

Amplification and Sequencing

Amplification of bacterial genomic DNA was conducted using the 16S rRNA primers 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3'). The PCR mixture contained the DNA template, nuclease-free H_2O , GoTaq polymerase, and forward and reverse primers. PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 15 min, with a final extension at 72°C for 5 min. DNA bands were visualized by agarose gel electrophoresis. Briefly, 0.5 g of agarose was dissolved in 50 mL of TBE buffer, homogenized, and heated until boiling. After cooling slightly, 1 μL of DNA stain was

added, and the solution was poured into a gel tray with a comb to form wells. Once solidified, the gel was placed in the electrophoresis chamber, and samples were loaded with a DNA ladder as the size marker. Electrophoresis was carried out at 80 V for 45 min, and DNA fragments were observed under ultraviolet illumination. A no-template control (NTC) was included to monitor potential contamination during PCR and sequencing. Sanger sequencing was subsequently performed to identify the bacterial species, and sequencing was carried out at PT Science Indonesia. The sequence data were then analyzed using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to compare with the NCBI database (Stover & Cavalcanti, 2017). Furthermore, a phylogenetic tree construction using the neighbor-joining (NJ) method with 100 bootstrap replications, and evolutionary distances were calculated based on the Kimura 2-parameter model in MEGA 11 software. One outgroup sample used for sequence comparison is *Bacillus amyloliquefaciens* (JN366747.1).

This study did not employ inferential statistical analysis, as its primary objective was the qualitative identification of bacterial species through molecular analysis. A descriptive approach was applied by evaluating the sequence similarity of the 16S rRNA gene and the evolutionary relationships among isolates. Taxonomic classification was based on widely accepted similarity thresholds, specifically $\geq 97\%$ for species-level and $\geq 95\%$ for genus-level identification, as outlined by Stackebrandt and Goebel (1994).

The research focused on identifying the pathogens responsible for soft rot in potato tubers, using samples obtained exclusively from Sumber Brantas Village. This limited sampling scope may restrict the representation of pathogen diversity across other regions or temporal scales. The isolation technique relied solely on culture-based methods with YPA medium, which may have failed to detect certain species that

are unculturable or require extended incubation. These limitations should be considered when interpreting the results, underscoring the need for further studies with broader geographic coverage.

All procedures were conducted in accordance with applicable ethical standards. Infected potato tuber samples were collected with the consent and assistance of local farmers. All laboratory activities followed standard safety and sterilization protocols to prevent cross-contamination. As this research did not involve human or animal subjects, ethical approval was not required.

RESULTS AND DISCUSSION

Isolation and Pathogenicity Test of Bacterial Isolates

Bacterial isolation from potato tubers with soft rot symptoms from Sumber Brantas Village, Bumiaji, Batu City resulted in 20 bacterial isolates. The bacterial isolation of potato tubers with soft rot symptoms from Sumberbrantas Village, Bumiaji, Batu City resulted 20 bacterial isolates. The bacteria were then streaked on YPA media to observe single morphological characters. Each isolate was subsequently tested for pathogenicity to determine whether the bacteria were pathogenic to potato tubers. According to [Firmansyah et al. \(2024\)](#), the pathogenicity test aims to determine whether the isolates found can cause disease in the host plant. Based on the pathogenicity test results, four bacterial isolates P1, P2, K5, and K7 were identified as causing soft rot disease in potato tubers. Several challenges were encountered

during the research process. One of the main difficulties was the rapid growth of bacterial colonies on YPA medium, which hindered morphological identification. To overcome this, a four-quadrant streaking technique was applied to obtain single colonies. Another challenge was the risk of cross-contamination during sampling from decayed potato tubers. To address this, all samples were thoroughly surface-sterilized prior to laboratory isolation. Additionally, the limited number of tubers exhibiting soft rot symptoms posed a constraint, resulting in only a subset of isolates being suitable for subsequent molecular analysis.

The pathogenicity test revealed initial symptoms of brownish or cream-colored tubers, which later turned black, soft, and wet, emitting a rot odor, and became slimy ([Figure 1](#)). Rot symptoms begin on the surface of the tuber and progressively extend inward. Infected tissue is clearly visible with dark brown or black borders. This finding is consistent with those of [Czajkowski et al. \(2011\)](#), who stated that soft rot of tubers originates in lenticels, stolon tips, and/or wounds under wet conditions. Lesions can spread throughout the tuber. The tuber tissue macerates to a cream color, parts of the tuber turn black upon exposure to air, and an unpleasant odor is emitted when secondary microorganisms attack. According to [Muturi et al. \(2019\)](#), *P. carotovorum* produces plant cell wall-degrading enzymes, one of which is pectinase, which contributes to plant tissue damage.

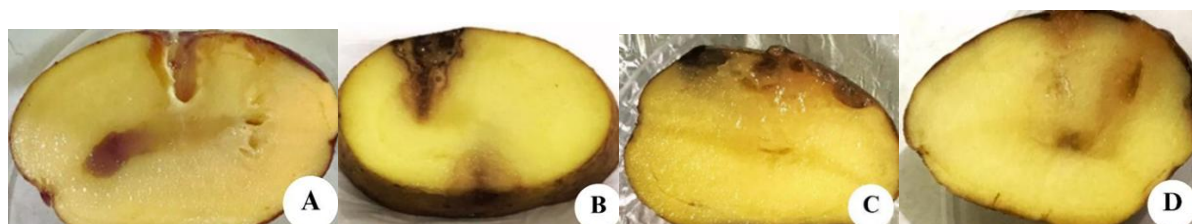


Figure 1. Potato soft rot (pathogenicity) test on potato tubers at 7 days after inoculation, Isolate of (A) P1; (B) P2; (C) K5; (D)K7

Macroscopic Characterization of Bacterial Colony

Bacterial isolates that have been tested for pathogenicity were then identified macroscopically and molecularly to determine their species. Macroscopic characterization was distinguished based on single colony characteristics, including color, colony shape, cell shape, margin, and elevation (Hikmawati *et al.*, 2019). Bacterial morphology exhibited similar characteristics based on macroscopic identification results in Table 1; however, each isolate has a different colony color (Figure 2). Bacterial isolates

have similarities in shape, cell morphology, margin, and elevation.

Gram staining was performed to classify the isolates as Gram-negative or Gram-positive based on the staining results. Isolates P1, P2, K5, and K7 were classified as Gram-negative because they appeared red after staining (Figure 3). According to Schaad *et al.* (2001), Gram-negative cells have a higher lipid content in their cell walls. Lipids generally dissolve in alcohol, causing the cell wall to break down during the alcohol rinsing step, which results in the loss of the crystal violet stain.

Table 1. Morphological Characteristics of Bacterial Colony

Isolate	Color	Shape	Cell Morphology	Margin	Elevation
P1	white	round	bacilli	entire	convex
P2	white	round	bacilli	entire	convex
K5	yellow	round	bacilli	entire	convex
K7	white	round	bacilli	entire	convex

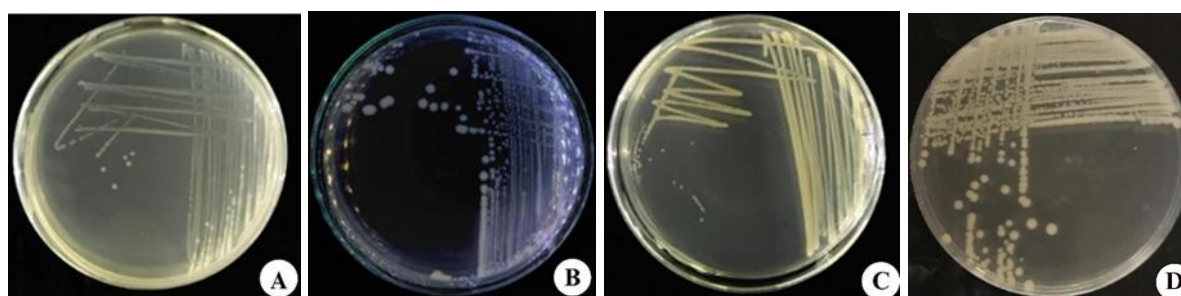


Figure 2. Morphology characteristics of bacterial colony, Isolate of (A) P1; (B) P2; (C) K5; (D) K7

Molecular Characterization of Bacteria

Bacterial isolates were identified using 16S rRNA gene sequence amplified with universal primer (Suárez *et al.*, 2022). The bacterial identification process consists of several stages, namely bacterial DNA extraction, DNA amplification using PCR, nucleotide base sequence analysis (sequencing), and phylogenetic kinship analysis. DNA extraction refers to the Quick-DNA Fungal/Bacteria Miniprep Kit protocol (Zymo Research, 2021), DNA amplification using the 2X My Taq HS Red Mix protocol

with an amplicon target of 1,500 base pairs (bp). The nucleotide base sequence (sequencing) obtained was then analyzed using the Basic Local Alignment Search Tool (BLAST). Phylogenetic analysis was conducted using the Molecular Evolutionary Genetics Analysis (MEGA 11) application (Tamura *et al.*, 2021).

DNA extraction is a method used to separate DNA from cell membranes, proteins, and other cellular components. This process aims to isolate DNA by removing other components that may interfere with amplification and sequencing, ensuring the

purity of the extracted DNA. One of the key stages in DNA extraction is cell lysis (Gupta, 2019). Cell lysis is the process of breaking down or rupturing the bacterial cell membrane, allowing the DNA to be released

and separated from other cellular components. The DNA extraction process utilizes a lysis buffer to facilitate the disruption of cell membranes and tissues while minimizing contamination.

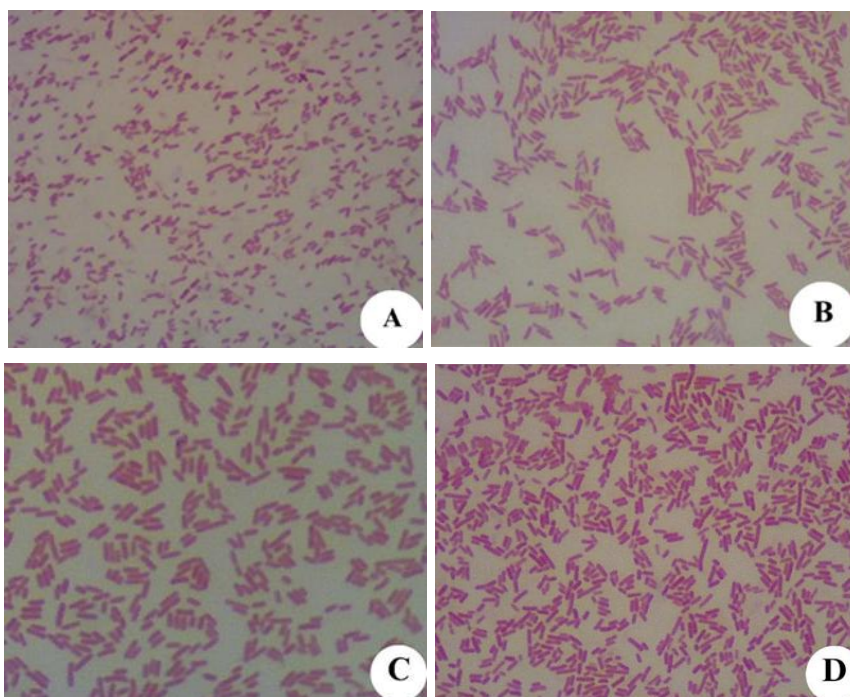


Figure 3. Gram test characteristics of bacterial colony, Isolate of (A) P1; (B) P2; (C) K5; (D) K7

The results of electrophoresis on agarose gel showed that all samples (P1, P2, K5, and K7) were successfully amplified for the 16S rRNA gene (Figure 4). The bands were observed at approximately 1,000 bp based on the DNA marker (M). Based on the electrophoresis results, no contamination occurred during the PCR amplification process, as evidenced by the absence of bands in the negative control (NTC). Isolate P1 displayed a transparent, thick, and smear-free DNA band. Isolates P2, K5, and K7 showed thick DNA bands with slight smearing. The success of amplification can be assessed from the

quality of the resulting DNA bands. A transparent, thick, and smear-free DNA band on agarose gel indicates that the bacterial isolate has high quality, with a higher concentration of DNA produced. This is consistent with the statement of Ramlan *et al.* (2024), which states that high-quality DNA bands are not degraded and are free of contamination. If a DNA band exhibits smearing, it suggests poor DNA purity. The smear pattern observed on the agarose gel indicates that the DNA extraction results contain contaminants, including proteins, organic salts, and RNA molecules (Syah *et al.*, 2024).

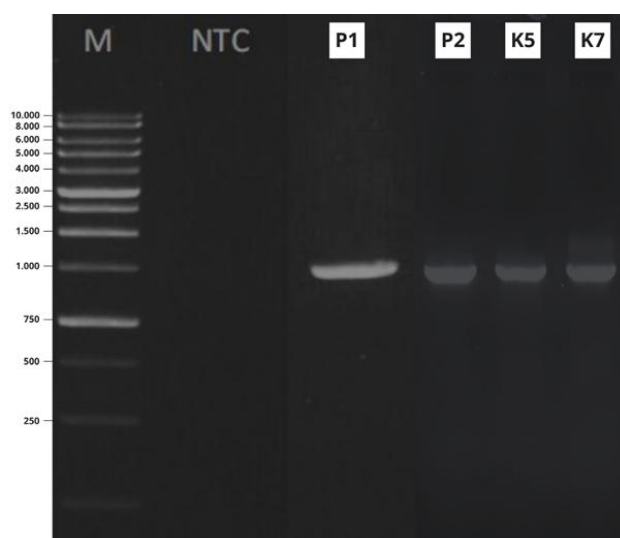


Figure 4. Bacterial DNA bands amplified by PCR and visualized by agarose gel electrophoresis, M: DNA Marker; NTC: No Template Control

16S rRNA Gene Nucleotide Sequence Analysis

The phylogenetic analysis of 16S rRNA gene nucleotide sequences aims to determine the evolutionary relationships between bacterial samples and bacterial species. The

16S rRNA gene can be used for comparison between organisms at the genus, phylum, strain, species, and subspecies levels. The BLAST results of the 16S rRNA gene nucleotide sequence analysis are presented in [Table 2](#).

Table 2. Similarity index of 16S rRNA bacterial strains as a result of BLAST-N analysis

Strain	Nearest Hit Species	Similarity (%)	Accession Number
P1	<i>Pseudomonas koreensis</i>	99.78	OM319779.1
P2	<i>Pectobacterium carotovorum</i>	98.00	MN394022.1
K5	<i>Serratia rubidaea</i> .	98.97	MK503535.1
K7	<i>Ralstonia pseudosolanacearum</i>	100.00	NR_134148.1

A total of 20 bacterial isolates obtained from symptomatic potato tubers, four isolates (25%) were confirmed as pathogens based on pathogenicity tests and 16S rRNA gene nucleotide sequence analysis. These four isolates were identified as follows: P1 as *Pseudomonas koreensis*, P2 as *Pectobacterium carotovorum*, K5 as *Serratia rubidaea* and K7 as *Ralstonia pseudosolanacearum*. This indicates variability in bacterial species responsible soft rot symptoms in potato tubers from Sumber Brantas Village. Homology or percentage identity varied among the isolates. According to [Stackebrandt and Goebel](#)

(1994), the similarity threshold for 16S rRNA gene identification at the genus level is 95%, while at the species level, the minimum similarity threshold is 97%. Sequence analysis of all isolates showed an E-value of 0.0, indicating that the identification results were highly reliable and nearly identical. Query coverage for all isolates was 100%, except for isolate K5, which had 99%, confirming that the DNA sequences analyzed covered nearly all target sequences in the NCBI database.

The results of the 16S rRNA gene sequences were then analyzed to determine their phylogenetic relationships using a

phylogenetic tree approach. Phylogenetic trees aim to connect taxonomic units, such as species and genes, which serve as links between the analyzed organisms. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method with 1,000 bootstrap replications, and evolutionary distance calculations were performed based on the Kimura 2-parameter model. The outgroup species

used was *Bacillus amyloliquefaciens* strain 30N2-6. The phylogenetic tree identification results showed that isolate K5 is closely related to *Serratia rubidaea*, isolate P2 is closely related to *Pectobacterium carotovorum*, isolate P1 is closely related to *Pseudomonas koreensis*, and isolate K7 is closely related to *Ralstonia pseudosolanacearum* (Figure 5).

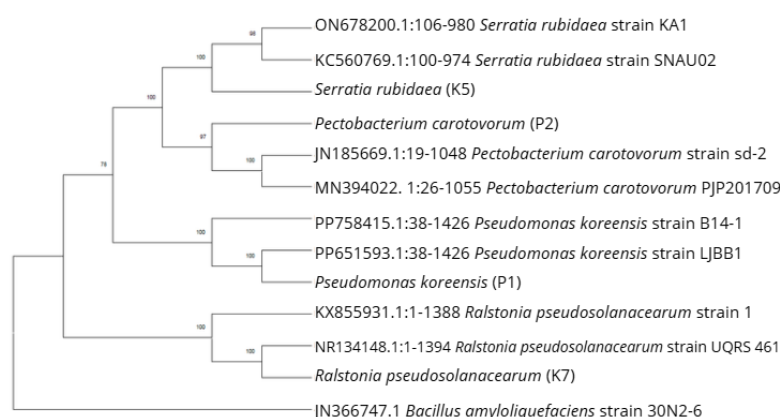


Figure 5. Phylogenetic tree of isolate bacterial strains and reference sequences from GenBank

The bacterial nucleotide sequence obtained in this study shared a high degree of identity with GenBank reference sequences, supporting its classification as the same species (Newell *et al.*, 2013). Molecular data obtained through PCR amplification, sequencing, and phylogenetic tree construction served as key evidence in identifying the bacterial species associated with soft rot. PCR confirmed the successful amplification of the target 16S rRNA region, while BLAST analysis and phylogenetic reconstruction revealed both the species identity and the evolutionary relationships of the isolates.

Together, these findings provided the foundation for the taxonomic interpretations presented in this study. In addition to geographic and temporal limitations in sample collection, this study also faced methodological constraints that may have influenced the results. The selection of isolates was based solely on differences in colony morphology, which may have led to

the exclusion of strains that are morphologically similar but genetically distinct. Therefore, the use of non-culture-based molecular techniques, such as metagenomics or qPCR, is recommended for future studies to provide a more comprehensive understanding of the bacterial community responsible for soft rot in potatoes.

This study did not involve statistical testing because its primary objective was to identify bacterial species through molecular analysis of 16S rRNA gene sequences. The classification of isolates relied on widely accepted similarity thresholds, where values of $\geq 97\%$ indicate species-level identity and $\geq 95\%$ for genus-level (Stackebrandt and Goebel (1994). As such, descriptive evaluation based on sequence similarity and query coverage was deemed adequate to support the conclusions drawn from the data.

Based on the findings regarding soft rot disease in potato tubers from Sumber Brantas Village, Batu, the following practical

measures are recommended for disease management: (1) early detection during the potato seed certification process; (2) the application of sanitation practices during storage and transportation to minimize contamination; and (3) the use of disease-resistant seed varieties. These recommendations are consistent with those of Czajkowski *et al.* (2011), who reported that soft rot can be managed through contamination prevention, physical treatment of seed tubers, and the application of biological control methods. Although this study successfully identified the pathogen associated with soft rot in potato tubers, further research employing rapid detection techniques such as Fourier transform infrared (FTIR) spectroscopy, quantitative PCR (qPCR), or amplification-based methods (AMP) is needed to facilitate early detection and prevent disease spread.

CONCLUSION

This study successfully identified four bacterial species responsible for soft rot disease in potato tubers from Sumber Brantas Village, Batu City, through 16S rRNA gene analysis and the construction of a phylogenetic tree. The identified species were *Serratia rubidaea*, *Pectobacterium carotovorum*, *Pseudomonas koreensis*, and *Ralstonia pseudosolanacearum*. These findings make a significant contribution to the development of early detection systems, seed certification, and the management of soft rot disease in potato production centers across Indonesia. Given the limitations of this study, further research is recommended, incorporating broader geographic sampling and the use of advanced molecular methods, such as metagenomics, qPCR, and FTIR spectroscopy. These approaches are expected to enhance pathogen identification. Moreover, the research framework applied in this study can also be adapted for use in agricultural regions, both locally and internationally.

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