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IDENTIFICATION OF RALSTONIA SOLANACEARUM CAUSING BACTERIAL WILT DISEASE IN TERUNG ASAM (*Solanum lasiocarpun* Dunal)

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ABSTRACT

Terung asam (Solanum lasiocarpum Dunal) or locally known as Terung Dayak is the most popular native vegetable in Sarawak. Its popularity can be witnessed by the fact that it has been widely planted throughout the state by local farmers. However, the farmers have been experiencing massive yield losses caused by a wilting disease directly attributed to a pathogen known as Ralstonia solanacearum. R. solanacearum is one of the most dreadful pathogens because it has a broad host range and causes lethal wilting to plants. More than 450 species of plants from 54 plant families have been reported to be infected by this pathogen. The objectives of this study were to first, identify and characterize the isolated R. solanacearum causing bacterial wilt disease in terung asam and second, to investigate the pathogenicity effect of inoculated R. solanacerum on the selected plant. In total, 30 pathogens were isolated on tetrazolium chloride (TZC) media, and it was found that 19 (63.33%) isolates were virulent while 11 isolates (36.67%) were avirulent. All of the isolates were gram negative. Out of 30 isolates, only 18 bacteria were found to have the ability to oxidize hexose and utilize disaccharides. Molecular identification of these isolates revealed that the pathogen belonged to Burkholderia solanacearum, Burkholderia sp. and Ralstonia solanacearum. The pathogenicity test for bacterial wilt disease found that 78 out of 100 plants inoculated with these bacteria showed wilting symptom disease 28 days post-inoculation. All of the pathogens isolated from terung asam showed symptoms of wilt disease on the plant as well as the tomato plants compared with the uninoculated ones. To the best of our knowledge, R. solanacearum which has a lethal effect on terung asam can cause equal destruction to other plants. Therefore, the findings and recommendations of this paper are useful for farmers to preventing their plants from being devastated by this pathogen.

Keywords: bacterial wilt disease, terung asam, Ralstonia solanacearum, pathogens, farmers,

1. INTRODUCTION

Terung asam (*Solanum lasiocarpum Dunal*) or locally known as Terung Dayak is the most popular native vegetable in Sarawak. The recent increase in its demand has seen its price soaring indicating that this crop has a commercial potential. However, its susceptibility to bacterial wilt disease caused by *Ralstonia solanacearum* has caused severe yield losses to farmers.

R. solanacearum is a dreadful pathogen that causes wilting in many plants. More than 450 species from 54 plant families have been reported to be infected by this pathogen [1]. Furthermore, this disease infects numerous important crops, such as pepper, tomato, potato, and banana, resulting in significant economic losses to farmers. Therefore, it is crucial that the pathogenicity and underlying mechanisms of *R. solanacearum* are investigated in order to find a solution for the farmers to deal with the disease.

The present study was aimed at identifying and characterizing *R. solanacearum* based on infected terung asam showing wilt symptoms as well as to confirm the virulence of *R. solanacearum* isolates on both tomato and terung asam plants. The study hypothesized that terung asam and tomato plants inoculated with *R. solanacearum* will show external and internal wilting symptoms compared with the control plant. The identified strains of *Ralstonia* are expected to belong to race 1. The findings of this study may assist farmers in obtaining an adequate supply of the crop, hence lowering food security concerns and indirectly aiding in wilt disease management measures in the country.

2. MATERIALS AND METHODS

2.1 Sample collection and sterilization

Terung asam infected with bacterial wilted disease were collected in three different places in Bintulu. Field diagnosis of the diseased plant samples was done by observing the symptoms. Each diseased plant's basal stem segments (about 10cm in length) were immediately collected in a sterile sampling bag and transported to the laboratory for storage. The samples were then washed under running tap water to remove any debris, and surface sterilization was done by soaking the samples inside 70% (v/v) ethanol for 1 minute, 2.5% (v/v) sodium hypochlorite for 4 minutes and washing it again with 70% (v/v) ethanol for 30 seconds. Finally, the samples were rinsed with sterile distilled water six times. The surface sterile sample was then immersed in sterile distilled water for 20 - 30 minutes to detect the presence of *R. solanacearum*.

2.2 Streaming test

Sterile test tube containing sterilized water was prepared and sections of the stem sample were placed in the test tube. A few minutes later, ooze or white slime stream of bacterial cells exuded from the stem were channeled into sterilized water which indicated presence of bacterium in the infected sample.

2.3 Isolation of *R. solanacearum*

The isolated *R. solanacaerum* was streaked on 2,3,5-triphenyltetrazolium chloride (TZC) agar. Prior to the streaking, a stem section from a wilting plant sample was immersed in sterilized distilled water for a few minutes until bacterial ooze was observed. The oozing solution was diluted 10X before being spread on 2,3,5-triphenyltetrazolium chloride (TZC) agar plates and then incubated at room temperature for 2 days. Bacterial colony that appeared dark red (avirulent) and white with a pink center (virulent) were streaked on fresh media until a single colony was obtained.

2.4 Biochemical Characterization Method

2.4.1 *Gram staining*

Bacterial colonies that grew on TZC media were subjected to gram staining to distinguish them from gram-positive and gram-negative bacteria. A loop full of the bacterium was smeared on a glass slide and allowed to air-dry and heat-fixed before staining. The bacterial smear was flooded with 0.5% aqueous crystal violet solution and left to stand for about 30 seconds. The slide was then washed under running tap water for 30 seconds. It was later flooded with iodine solution and left to stand for 3 minutes before washing with tap water to get rid of excess iodine solution. The slide was then dried overnight in a drying cabinet and examined using a compound microscope 100X magnification lens using immersion oil.

2.4.2 *Biovar determination*

Biovar determination was conducted and carried out based on the ability of the isolates to oxidize hexose alcohol, i.e., mannitol, sorbitol and dulcitol, or to utilize disaccharides, i.e., lactose, maltose and cellobiose [2]. For disaccharide, sucrose, lactose and maltose were used, whereas for sugar, alcohol, mannitol, dulcitol and sorbitol were used. 10 mL of each sugar was added into separate sterilized test tubes and heated at 100°C for 30 minutes to sterilize the solutions. Bottles of semi-solid TZC media were melted in the water bath and cooled to 70°C. After that, 10 mL of carbohydrate solution was added and mixed in TZC media. 5 mL of the mixed solution was transferred into another test tube that had been labelled and stored at room temperature. Distilled water was used as a control. Each pure culture from TZC media was added into 5 mL of sterile distilled water and adjusted to 10^8 CFU/ml using a spectrometer measuring OD600 = 0.3.1 and 2 drops of bacterial suspension were inoculated into each test tube containing the sugar solutions and control and incubated at 30°C. Changes of color from olivaceous green to orange on the surface of the medium indicated solubilization of carbohydrate solution. Each experiment was done in triplicate.

2.5 Molecular identification of endophytic bacteria

The boiling method described by [3, 4] was used to obtain DNA from a selected isolate. A single colony was inoculated in 10 mL TZC broth and incubated overnight. The bacteria culture broth was centrifuged (10,000 rpm for 10 minutes) to obtain pallet on the following days which were later mixed with sterile water and boiled for 10 minutes, and then cooled for 5 minutes at -20°C. The DNA is obtained from supernatant after being centrifuged (12,000 rpm for 10 minutes). 16S RNA gene were amplified using universal primers 27F and 1492R. The PCR products were sent for sequencing and validation. They were later identified using NCBI database.

2.6 Plant inoculation

2.6.1 ***Seed germination of tomato and terung asam seedling***

For seed germination, the seedlings were uniformly placed on wet tissue paper for 6 to 7 days in the dark [5]. The germinated seedlings were selected and transferred into polybags (12 x 14 inches) containing sterile topsoil and compost with a ratio 2:1 and grown in the greenhouse. Prior to transferring the seedlings into the polybags, the percentage of vigor index (VI) was calculated using the following formula:

Vigor index (VI) = [Plant height (Root length + Shoot length)] / Germination rate

2.6.2 ***Pathogenicity test of bacterial wilt disease***

Pathogenicity of *R. solanacearum* was tested on tomato (*Solanum lycopersicum*) and terung asam (*Solanum lasiocarpum Dunal*) seedlings. The bacteria cultures were freshly streaked on TZC media and incubated for 2 days. Each pure culture from TZC media was transferred into 5 mL of sterile distilled water, adjusted to 10^8 CFU/ml using a spectrometer, and measured at OD600 = 0.3. The inoculation was done on plants at four- to six-leaves stage. The leaf of each plant was sprayed with bacterial suspension and immediately covered with plastic sheet for 24 hours. Sterile distilled water was used for the control treatment. The wilting symptoms were later observed at 7-, 14-, 21-, and 28-days post inoculation. The disease severity was scored using a 0 to 5 rating scale as shown in Table 1 [6]. The disease index percentage (% DI) percentage was calculated based on the scores 28 days after inoculation. The percentage of disease index was calculated using the formula:

$$\%DI = \frac{\varepsilon(ni \times vi)}{V \times N} \times 100$$

Where: ni = number of plants with the particular disease

vi: disease score

V: the highest disease score

N: the number of plants observed

disease score, V = the highest disease score and N = the number of plants observed.

3. RESULTS

3.1 Sample collection of infected terung asam

Terung asam affected by bacterial wilt disease displays symptoms of sudden leaf withering due to nutrient and water scarcity caused by bacterial obstruction (Figure 1). In this study, 96 samples of infected terung asam were obtained from 3 different locations in Bintulu, Sarawak namely University Putra Malaysia Bintulu Campus (UPMKB), Kampung Jepak, and Kampung Jabai.

3.2 Streaming test

The diagnosis of *R. solanacearum* infection was further validated through a streaming test in which infected samples presented an ooze coming out from the stem (Figure 2a). Out of the 96 samples collected, 30 of them (31.25%) showed the presence of a milky white strand or threadlike ooze of bacteria, indicative of the existence of *R. solanacearum* in the sample (Figure 2b).

3.3 Isolation and morphological characterization of bacteria isolates

A total of 30 bacteria isolates were obtained from the infected terung asam through the cultivation of ooze on TZC media between 24 and 48 hours at room temperature. The morphological characteristics of the bacterial colonies indicated that the bacterial isolates were divided into two categories of infection, namely virulent and avirulent, as depicted in Figure 3. The bacterial composition, as described in Figure 5, comprised 19 virulent isolates, accounting for 63.33% of the total while the remaining isolates (36.67%) were avirulent, as indicated in Table 2.

3.4 Biochemical characterization

The bacterial isolates were confirmed through gram staining technique and Biovar differentiation test.

3.4.1 *Gram stain*

One of *R. solanacearum's* characteristics is it is gram-negative and rod shaped. All of the bacteria isolates exhibited reddish-pink color indicating that these bacteria were gram-negative (Figure 4).

3.4.2 *Biovar test*

Identification of the biovar of *R. solanacerum* was achieved through the utilization of disaccharides and the oxidation of hexose alcohols. The biovar determination test revealed that only 18 isolates of *R. solanacearum* were capable of oxidizing disaccharides, such as sucrose, lactose, and maltose as well as hexose alcohols, such as dulcitol, sorbitol, and mannitol, within 28 days (Figure 5). The results of the experiment indicated that the disaccharides and hexose alcohol underwent a reaction, as evidenced by a change in color on the surface of the solution, as shown in Table 3. Biovar-III can be identified in the culture based on the positive reaction of disaccharides and alcohol sugars.

3.5 Molecular identification of *R. solanacearum* using 16s rDNA gene amplification

The 16s primer was utilized to perform PCR amplification on all bacterial isolates that were suspected to be *R. solanacearum* resulting in a 1500bp amplification as described in Figure 6. The absence of a band in the Control DNA template provides evidence that PCR amplification was not contaminated. Table 4 presents the BlastN outcomes of the bacterial isolates which were identified as *Burkholderiales solanacearum* (B19, B20, B23, B24, and B27), Burkholderiales sp. (B21, B22, B25, B26, and B2), and *Ralstonia solanacearum* (A7, A8, A13, A16,

B56, B18, C10, and C22). The investigation delineated three discrete assemblages of microorganisms predicated on their genomic constitution (Figure 7).

3.6 Plant inoculation

3.6.1 *Seed germination*

The evaluation of seed vigor was carried out through the germination efficiency test, which was performed prior to the plant inoculation test. This is shown in Figure 8. The selected tomato and terung asam seeds showed a germination rate of 83%. The tomato seeds demonstrated a vigor index (VI) of 1047.59 while those of terung asam displayed a VI of 456.63. The findings indicated a notable vigor index (VI) in the tomato and terung asam seeds, suggesting their robust characteristics. Table 5 describes the Vigor Index (VI) pertaining to the germination of tomato and terung asam seeds. The vigor rate was calculated based on the vigor index formula by Abdul Baki (1973): Vigor index (VI) = [Plant height (Root length + Shoot length)] / Germination rate.

3.6.2 *Pathogenicity test of bacterial wilt disease*

The virulence level of five distinct bacterial strains was evaluated on tomato (A) and terung asam (B) plants as shown in Figure 9. The study discovered that 78% of plants treated with the selected bacteria strains (A7, A13, B5, C10, and C22) displayed withering symptoms after 28 days demonstrating pathogenicity when compared with the control group. Table 6 shows the percentage of disease index (DI) for both plants.

4. DISCUSSION

The goal of this study was to find and isolate the strain of *R. solanacearum* that infects terung asam in Bintulu, as well as to test the isolates' pathogenicity on terung asam and tomato plants. Combining molecular and traditional characterization approaches can provide a comprehensive understanding of *R. solanacearum* strains infecting terung asam. The plants' reaction to various *R. solanacearum* isolates can be used to determine if they cause the same or different disease symptoms in the plant species investigated. These aid in our understanding of *R. solanacearum* infection in terung asam and how it may influence crops associated with it. The findings can be utilized to guide future research and to develop effective management strategies to stop the pathogen's spread and reduce its detrimental impact on crop yield.

The affected terung asam plants exhibited susceptibility to further diseases caused by other living organisms, such as fungi and insects, thereby indicating their weakened state. The streaming test revealed that a minor proportion of the samples (31.25%) exhibited stem oozing. The ooze yielded a predominant population of virulent *R. solanacearum* strains, which were identified as gram-negative. Out of the total isolates, a mere 18 were identified as *R. solanacearum* and were classified under Biovar-III. These isolates exhibited the capacity to oxidize disaccharides and hexose alcohols. The selected strains displayed signs of wilting in both tomato and terung asam plants, indicating their ability to cause disease in multiple host plants. The discoveries furnish insights into the characteristics of *R. solanacearum* strains and their impact on plant well-being and disease control. Additionally, they shed light on the susceptibility of terung asam plants that have been infected with secondary infections. The streaming test is an early identification approach for *R. solanacearum* isolation on bacterial wilted disease plants. However, despite having bacterial wilt disease symptoms, only a minority of the disease plant samples had ooze and this could be caused by early infection. There is no severe harm to the host plant in the early stages of infection because the quantity of *R. solanacearum* in the stem is still too low and it has not clogged the stem to deprive it from nutrition and water transportation. *R. solanacearum* was discovered colonizing the tomato root in the early stages of infection [7]. As a result, isolating the pathogen from the root of the terung asam can be improved. Ooze from the infected sample was confirmed to include *R. solanacearum* which shows a similar tendency to prior studies on infected Solanaceae plants. 20 isolates of *R. solanacearum*

was successfully amplified using distinct *R. solanacearum*-specific primers, yielding specific amplicon sizes of 280 and 140 bp [8]. Similarly, *R. solanacearum* was discovered utilizing *R. solanacearum* specific primers Y2 and OLI1 and yielded a 288 bp specific band size [9].

All of the bacterium isolates were gram-negative, which is consistent with previous research on *R. solanacearum* identification [10][11][12]. The majority of the pathogens were gram negative, which contributes to significant losses in food production due to antibiotic resistance [13]. The Gram's staining reaction was done using crystal violet, and the microscopic results revealed that all *R. solanacearum* isolates did not maintain violet color, but rather counter stain, which is pink [14]. All isolates of *R. solanacearum* from each group are gram negative and straight or curved, rod-shaped [15]. Other report has found that 7 groups of *R. solanacearum* isolated from *Solanum melongena* L. were also gram-negative bacteria [16]. Similarly, 10 strains gram-negative bacteria of *R. solanacearum* were isolated from infected *Eucalyptus urograndis* hybrids [17].

Only 18 isolates identified as Biovar-III were capable of oxidising disaccharides and hexose alcohols. *R. solanacearum* was divided into various groups in study based on its ability to utilize disaccharides and oxidize hexose alcohol [18]. The data showed that Biovar I could only oxidize hexose alcohol sugar while Biovar II could only oxidize disaccharides. Biovar III on the other hand, was capable of oxidizing both disaccharides and hexose alcohol while Biovar IV was only capable of oxidizing alcohols. Changes in color from green to yellow can reflect sugar and alcohol sugar utilization and oxidation. The differentiation of *R. solanacearum* biovars based on carbohydrate utilization were also reported previously in several study [19]. 25 isolates of *R. solanacearum* from wilted potato, pepper, aubergine, tobacco, and tomato plants used hexose alcohol (mannitol, sorbitol, and dulcitol) and disaccharides (cellobiose, lactose, and maltose) [20].

Pathogenicity testing of R. solanacearum on terung asam and tomato plants supported findings of previous studies. Abiotic factor such environment condition can cause *R. solancearum'*s physiological condition shift from virulent to avirulent [21]. Other than that, it was observed that *R. solancearum* may lose its virulence as a result of culturing [22]. However, virulent strains of *R. solanacearum* were inoculated on 7-year-old tomato seedlings, 100% of the treated seedlings died after a week [23]. Determination of *R. solanacearum* race based on the host range, phylotype and biovar [24]. This study's findings were also validated by in other study [25] which classified

R. solanacearum from *Curcuma alismatifolia* (Patumma) as biovar 4 and phylotype 1. Other report also stated that *R. solanacearum* was classified into races and biovars based on host range variability [26].

Future research on the pathogenicity factors of *R. solanacearum* in terung asam is needed to have a comprehensive understanding of the bacterium's virulence. Researchers can learn more about the pathogenicity of *R. solanacearum* by studying the processes and virulence factors it employs. The identification and characterization of significant genes and proteins implicated in pathogenesis can provide additional crucial insights into the molecular foundations of disease development. Similarly, studying the molecular and physiological interactions between terung asam plants and *R. solanacearum* will help us understand disease progression. This research sheds light into the bacterium's routes of infection, resistance to plant defenses, and its physiological impacts. Using these data, specific methods to control the bacterial wilt disease caused by *R. solanacearum* in terung asam can be developed. Future research initiatives focusing on these areas of study can improve our understanding of disease and result in the adoption of sustainable management practices and better disease control approaches.

R. solanacearum has been identified as the pathogenic bacteria isolated from bacterial wilt-infected terung asam, which is dominated by virulent strains that inflict significant harm to host plants. Furthermore, *R. solanacearum* isolated from terung asam is capable of infecting other *Solanacea* plants, such as tomato, which exhibit comparable wilting signs. As a result, a better understanding of their interaction and pathogenic factors may help to improve disease management measures.

4. CONCLUSION

Bacterial wilt disease caused by *R. solanacearum* has resulted in significant agricultural loss due to the severity of infection which can lead to sudden death of the crop. Due to poor knowledge of the pathogen, disease management has been largely ineffective in mitigating the spread and severity of infection. Identification of bacteria isolated from bacterial wilt-diseased terung asam in Bintulu pointed to *R. solanacearum* race 1 and biovar-III as the culprits. Inoculation of these pathogens on tomato plant also causing similar symptom of bacterial wilt disease proved that *R. solanacearum* is the causal agent in both crops. However, isolation techniques need improvement to obtain the target pathogen. The current findings will be useful to study *R. solanacearum* population structures using molecular approaches with a special emphasis on its integrated management. This information will guide future research aimed at finding management strategies for bacterial wilt in this region, particularly for plant improvement and production. Lastly, further experiments using potassium hydroxide, catalase oxidase tests, and levan production from sucrose can be carried out in the future to confirm the presence of *R. solanacearum*.

Figure and Table

Figure 1. Symptoms of infected terung asam in each location: a. symptom of wilting and sudden death, b. yellowing and wilting symptom leaf, and c. cross-section of infected leaf.

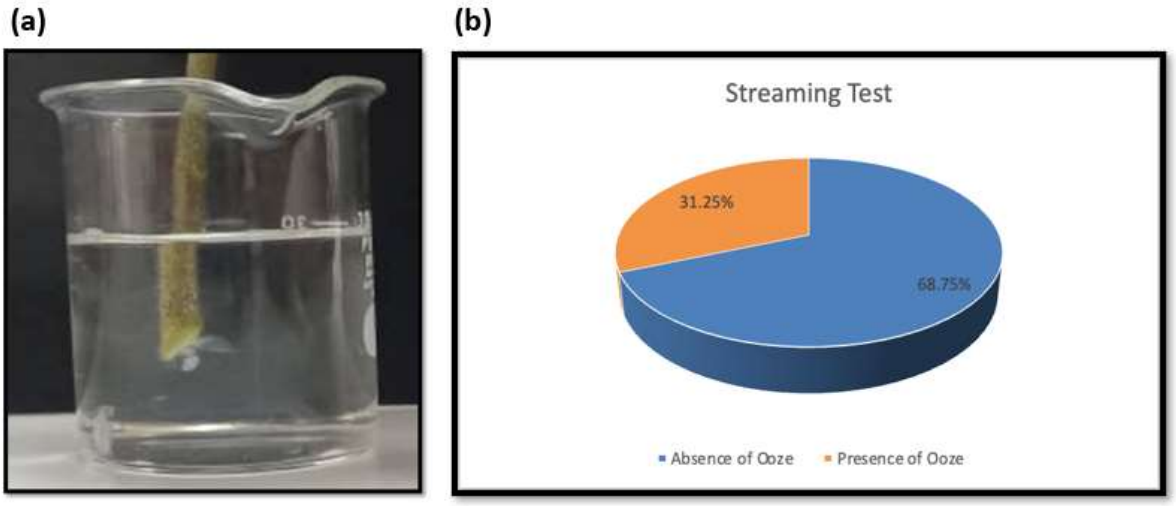

Figure 2. thread-like ooze of bacterial from infected stem (a) The emergence of filamentous formations from the infected plant serves as a clear indication of the existence of *R. solanacearum*. (b) Sample of infected terung asam showing bacterial ooze.

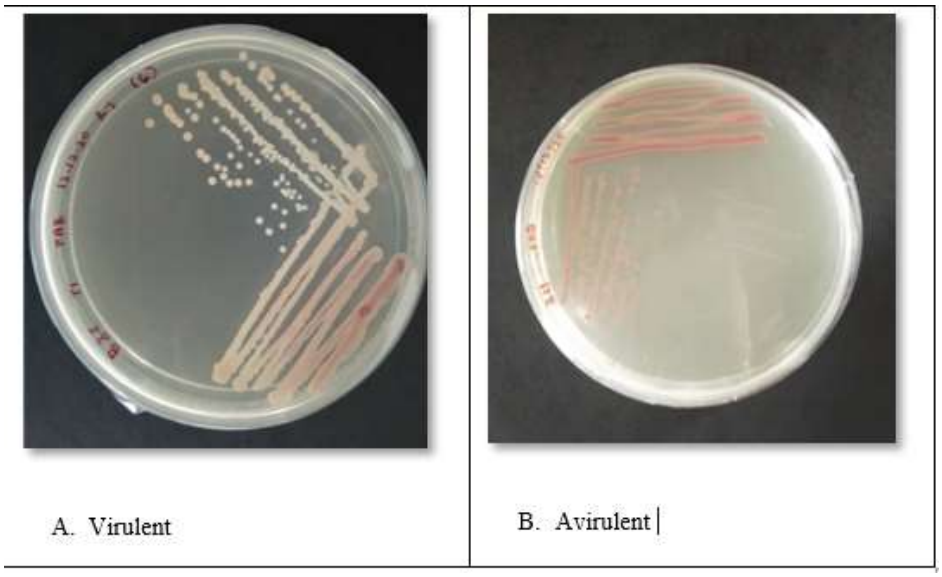

Figure 3. Virulent (a) and avirulent (b) bacteria isolates on TZC media.

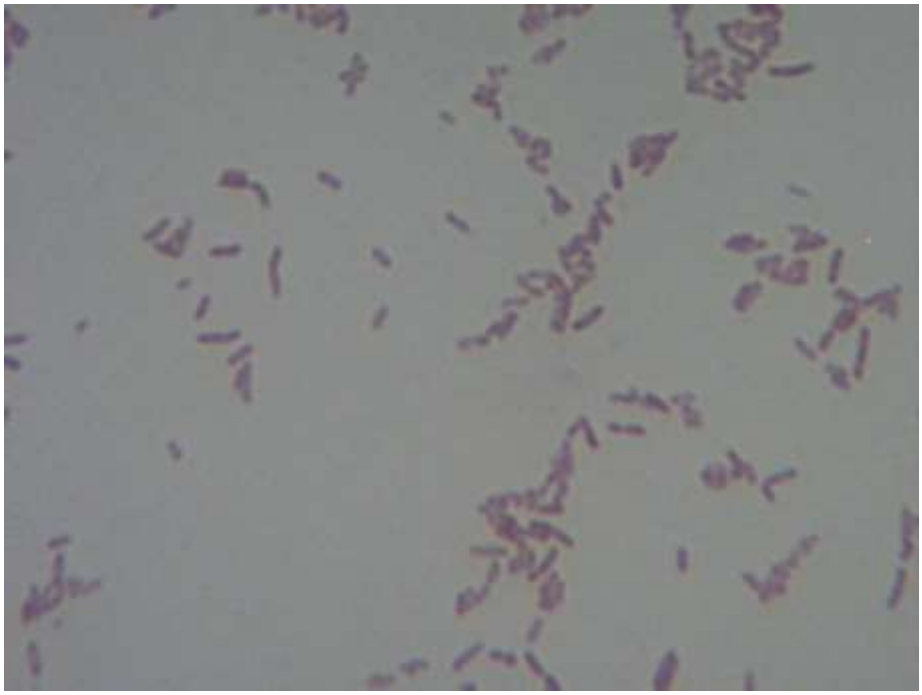

Figure 4. Gram's stain response to *R. solanacearum.* All isolates showed negative reactions on the gram staining test resulting in pink color and presence of rod shaped bacteria.

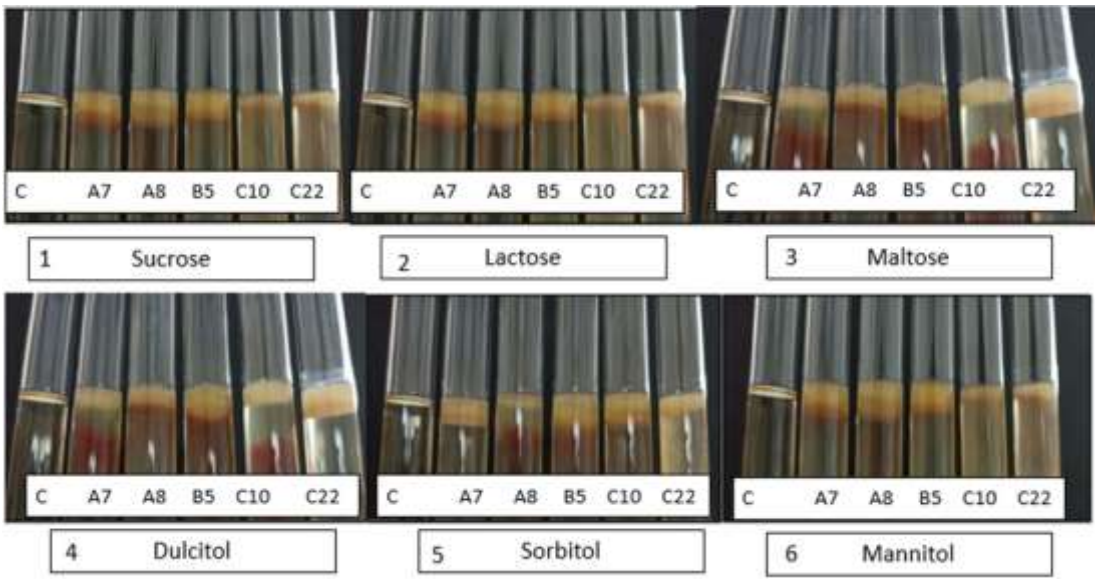

Figure 5: Differentiation of *R. solanacearum* strains into biovars based on their ability to utilize disaccharides and oxidize hexose alcohols, and producing acid when positive (+). Control: bacteria inoculated in medium supplement with sterile distilled water; 1: bacteria inoculated in medium supplement with sucrose; 2: bacteria inoculated in medium supplement with lactose; 3: bacteria inoculated in medium supplement with maltose; 4: bacteria inoculated in medium supplement with dulcitol; 5: bacteria inoculated in medium supplement with sorbitol, and 6: bacteria inoculated in medium supplement with mannitol.

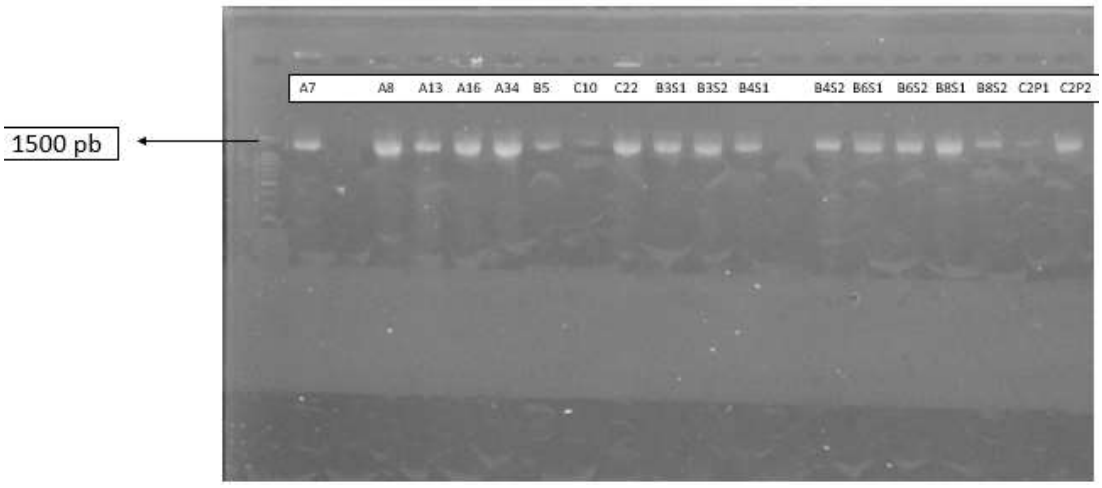

Figure 6. Electrophoretic analysis of PCR amplified DNA from 5 isolates of *R. solanacearum* using the 16s primers.

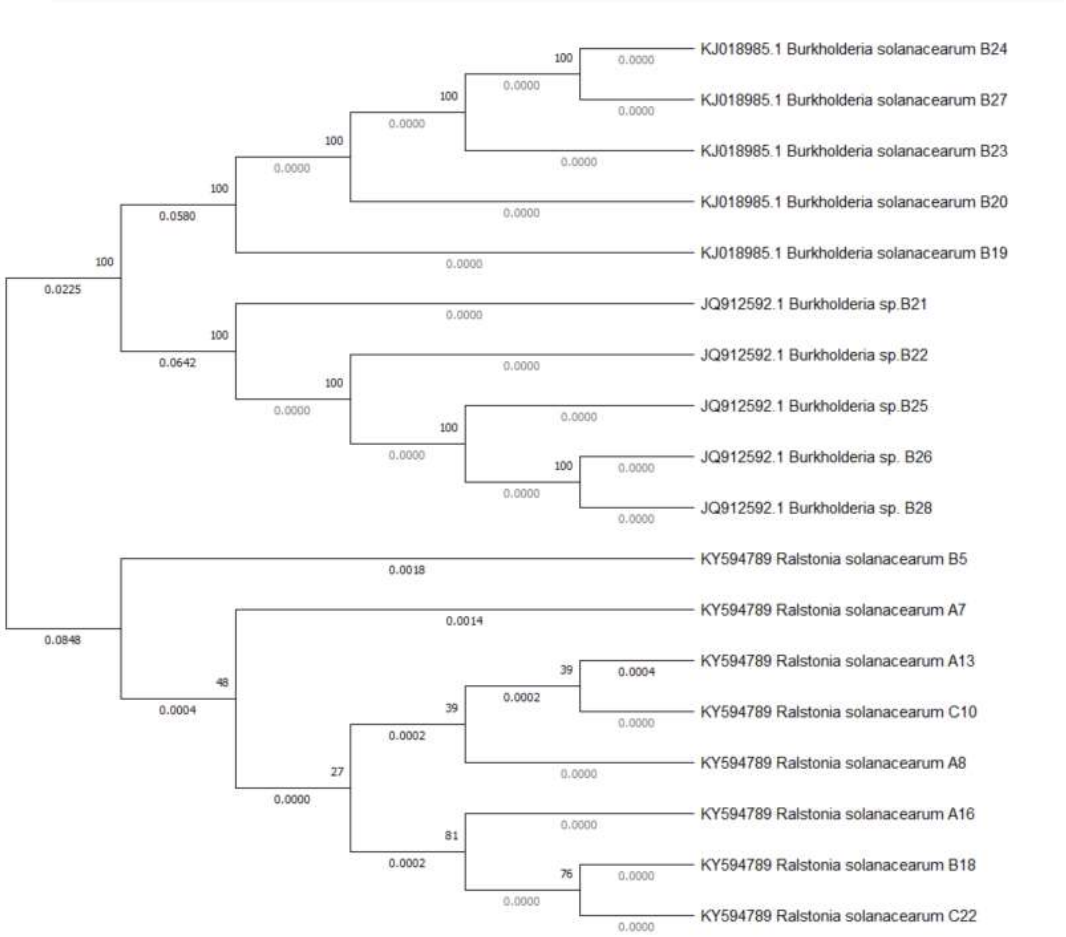

Figure 7. The phylogenetic tree was constructed by analyzing the 16S rRNA sequences of bacterial isolates obtained from National Centre for Biotechnology Information (NCBI). The MEGA software was utilized to perform the analysis.

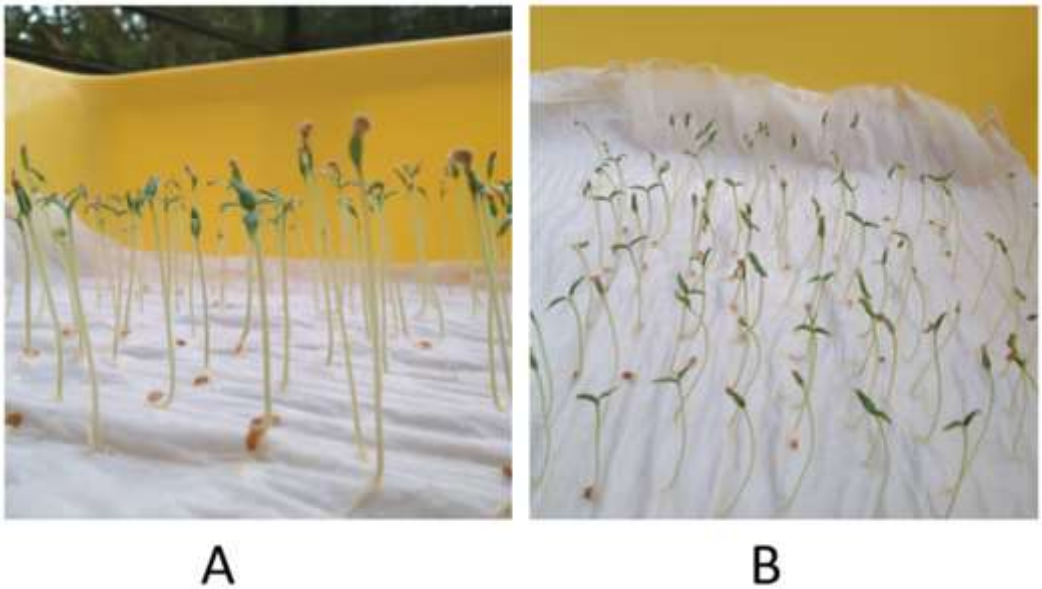

Figure 8. Seed germination of (A) tomato and (B) terung asam seedlings after 7 days of sowing.

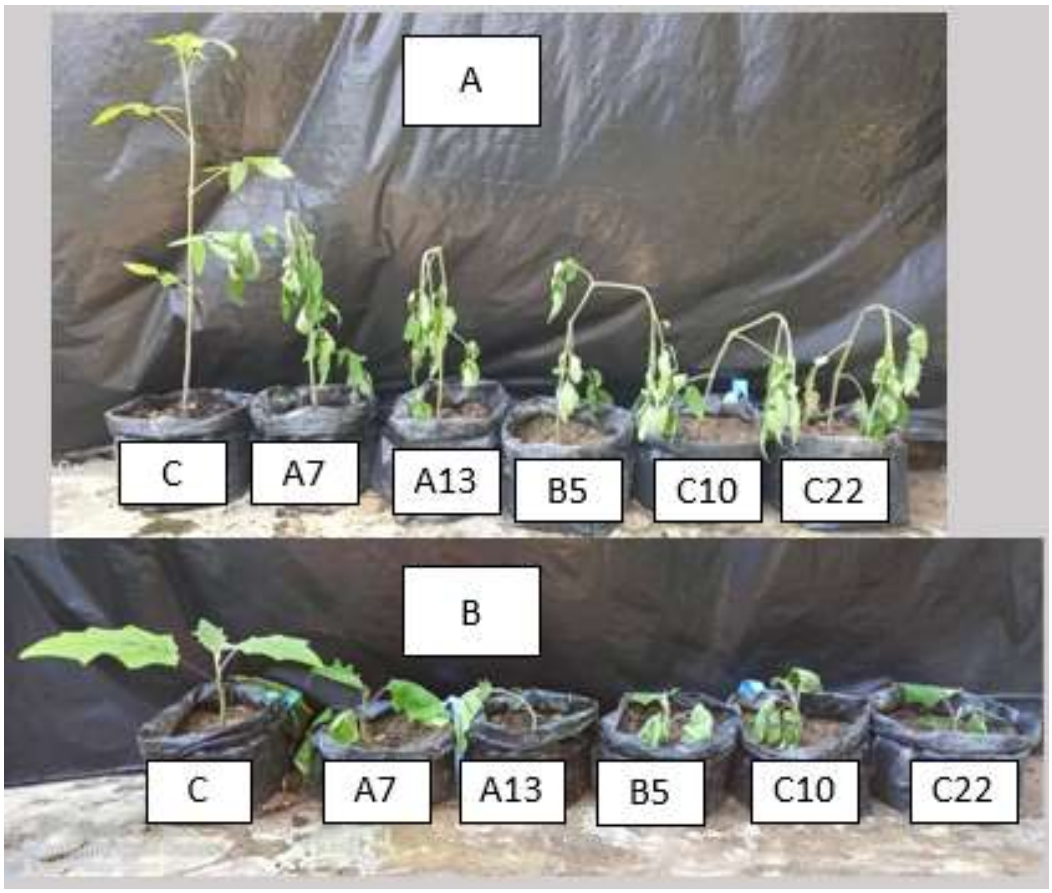

Figure 9. Pathogenicity test of 5 selected bacteria strains on tomato and terung asam plants. C is a control plant inoculated with sterile distilled water. The percentage of disease index (DI) of *R. solanacearum* infection on tomato (A) and terung asam plants (B) was based on rating scales 0 to 5, where 0 = no symptom; 1= 1 leaf partially wilted; 2= 2 or 3 leaves wilted; 3= all except the top or 3 leaves wilted; 4= all leaves wilted; 5= plant dead

Table 1. The rating scale of disease severity

Rating scale	Symptoms
0	No symptoms
1	Leaf partially wilted
2	2 or 3 leaves wilted
3	All except the top 2 or 3 leaves wilted
4	All leaves wilted
5	Dead

Table 2. Morphologies of virulent and avirulent bacteria isolates.

No.	Sample of isolates	Morphology of colony observed on TZC media	Type of bacterial isolates
1	A3	Round shape with a reddish color	Avirulent
2	A7	Round shape with pink-centered cream colonies	Virulent
3	A8	Round shape with pink-centered cream colonies	Virulent
4	A13	Small round shape with a reddish color	Avirulent
5	A16	Round shape with pink-centered cream colonies	Virulent
6	B1	Round shape with pink-centered cream colonies	Virulent
7	B3	Round shape with cream colony	Avirulent
8	B4	Round shape with pink-centered cream colonies	Virulent
9	B5	Round shape with reddish color	Avirulent
10	B8	Round shape with pink-centered cream colonies	Virulent
11	B14	Small round shape with reddish color	Avirulent
12	B16	Round shape with reddish color	Avirulent
13	B18	Round shape with a reddish color	Avirulent
14	B19	Pink centered with bonder colonies	Virulent
15	B20	Pink centered with bonder colonies	Virulent
16	B21	Pink centered with bonder colonies	Virulent
17	B22	Pink centered with bonder colonies	Virulent
18	B23	Pink centered with bonder colonies	Virulent
19	B24	Pink centered with bonder colonies	Virulent
20	B25	Pink centered with bonder colonies	Virulent
21	B26	Pink centered with bonder colonies	Virulent
22	B27	Pink centered with bonder colonies	Virulent
23	B28	Pink centered with bonder colonies	Virulent
24	B32	Round cream with cream colony	Avirulent
25	C10	Round shape with reddish color	Avirulent
26	C11	Round shape with pink-centered cream colonies	Virulent

27	C21	Round shape with pink-centered cream colonies	Virulent
28	C22	Round shape with pink-centered cream colonies	Virulent
29	C23	Round cream with cream colony	Avirulent
30	C32	Round cream with cream colony	Avirulent

Table 3: Differentiation of *R. solanacearum* strains into biovars based on their ability to utilize disaccharides and oxidize hexose alcohols producing acid when positive (+).

	Biochemical test of biovar determination					
	Utilization of:			**Oxidation of:**		
Sample	**Sucrose**	**Lactose**	**Maltose**	**Dulcitol**	**Sorbitol**	**Mannitol**
A3	+	+	+	+	+	+
A7	-	-	-	-	-	-
A8	+	+	+	+	+	+
A13	+	+	+	+	+	+
A16	+	+	+	+	+	+
B1	-	-	-	-	-	-
B3	-	-	-	-	-	-
B4	-	-	-	-	-	-
B5	+	+	+	+	+	+
B8	-	-	-	-	-	-
B14	-	-	-	-	-	-
B16	-	-	-	-	-	-

B18	+	+	+	+	+	+
B19	+	+	+	+	+	+
B20	+	+	+	+	+	+
B21	+	+	+	+	+	+
B22	+	+	+	+	+	+
B23	+	+	+	+	+	+
B24	+	+	+	+	+	+
B25	+	+	+	+	+	+
B26	+	+	+	+	+	+
B27	+	+	+	+	+	+
B28	+	+	+	+	+	+
B32	-	-	-	-	-	-
C10	+	+	+	+	+	+
C11	-	-	-	-	-	-
C21	-	-	-	-	-	-
C22	+	+	+	+	+	+
C23	-	-	-	-	-	-
C32	-	-	-	-	-	-

Table 4. BlastN results of isolated bacteria

No	BlastN NCBI Results

	Isolated Bacteria	Match in GeneBank	GenBank Accession
1	B24	*Burkholderia solanacearum*	KJ018985.1
2	B27	*Burkholderia solanacearum*	KJ018985.2
3	B23	*Burkholderia solanacearum*	KJ018985.3
4	B20	*Burkholderia solanacearum*	KJ018985.4
5	B19	*Burkholderia solanacearum*	KJ018985.5
6	B21	*Burkholderia sp.*	JQ912592.1
7	B22	*Burkholderia sp.*	JQ912592.1
8	B25	*Burkholderia sp.*	JQ912592.1
9	B26	*Burkholderia sp.*	JQ912592.1
10	B28	*Burkholderia sp.*	JQ912592.1
11	B5	*Ralstonia solanacearum*	KY594789
12	A7	*Ralstonia solanacearum*	KY594789
13	A13	*Ralstonia solanacearum*	KY594789
14	C10	*Ralstonia solanacearum*	KY594789
15	A8	*Ralstonia solanacearum*	KY594789
16	A16	*Ralstonia solanacearum*	KY594789
17	B18	*Ralstonia solanacearum*	KY594789
18	C22	*Ralstonia solanacearum*	KY594789

Table 5. Vigor Index (VI) of seed germination of tomato and terung asam seedlings

Plant	Sum of plant height	Germination rate%	Vigor Index (VI)
Tomato	869.5	83%	1047.59
Terung asam	379	83%	456.63

Figure 6: Percentage of disease index (%DI) of tomato and terung asam plants

Plants	Bacteria strain	%DI
Tomato	A7	56%

	A13	64%
	B5	80%
	C10	80%
	C22	80%
Terung asam	A7	56%
	A13	80%
	B5	80%
	C10	90%
	C22	64%

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