

Characterisation and pathogenicity of bacteria causing bacterial heart rot from Bestari Jaya, Selangor, Malaysia

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Abstract

Pineapples are a common commodity that can contribute to Malaysia's economy. However, like any crop in the agriculture and food production industry, pineapple growth is often affected by pests and diseases. Currently, the pineapple sector in Malaysia is facing a devastating disease known as Bacterial Heart Rot (BHR), caused by *Dickeya zeae* (formerly *Erwinia chrysanthemi*), which has also been observed in Bestari Jaya, Selangor. This study aims to characterise the bacteria responsible for Bacterial Heart Rot and determine its pathological effects on Malaysia's pineapple varieties. In this study, diseased fruit samples exhibiting heart rot symptoms were collected from various fields in the area. The results showed that all bacterial isolates from the diseased samples were rod-shaped, Gram-negative, and had a red/pink colour. Furthermore, during the pathogenicity test, all bacterial strains induced lesions on potato samples, and disease symptoms were also observed on healthy fruit samples that had been inoculated with the bacterial suspension. The pathogenicity test confirmed that the isolated bacteria were capable of causing symptoms similar to heart rot disease, including lesions, a foul smell, and odour. To satisfy Koch's Postulates, the isolated bacteria causal to heart rot disease were subjected to re-isolation. The results of the pathological effect on Malaysia's pineapple varieties revealed that the Crystal Honey variety exhibited the lowest percentage of disease severity compared to the MD-2 and Morris varieties. This study provides insights into the characteristics of the isolated bacteria responsible for causing heart rot in pineapples from Bestari Jaya, Selangor, and highlights their pathogenicity on Malaysia's pineapple varieties.

1. Introduction

Rice and Pineapples were by far the most prevalent commodities in amount, with global exports of around 3.3 million tons. Their request in international trade is principally driven by the fruit's exceptionally low average unit prices of exports, which contributed to their dominance in terms of quantity (Malaysian Pineapple Industry Board, 2022). Malaysia's pineapple industry is smaller than Costa Rica, Brazil, China, India, and Thailand. Despite this, the pineapple sector in Malaysia has a strong chance of expanding in the future (Sapak *et al.*, 2021). Bacterial Heart Rot (BHR) disease, caused by *Dickeya zeae* (formerly known as *Erwinia chrysanthemi*), is one of many bacterial infections that have caused disease outbreaks (Nor *et al.*, 2019). Hawaii, Indonesia, and Brazil have recorded 40% of yield losses from this bacterial heart rot (BHR) disease of pineapples (Young *et al.*, 2022).

The symptoms begin as water-soaked zones on the centremost leaves surrounding the apical meristem. Next, brown streaks begin to appear on the lamina and in the mesophyll tissues of the plant. The pineapple heart and stem can be easily separated from the components of the plant that are located below the ground parts of the plant within a few days after the initial infection of the meristem, as well as the apical and lateral buds (Kaneshiro *et al.*, 2008). Infected planting material, inadequate drainage, sanitation, and splattering of soil are some of the mechanisms through which these pathogens were spread (Nor *et al.*, 2019). There is less information from the local area, Bestari Jaya, available at this time concerning the characteristics and the pathogenicity of the pathogen towards Crystal honey, Mooris and MD-2 pineapple variety. Gaining this information is very important, especially for disease management in the future. Aside from that, the

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Malaysian pineapple varieties that are resistant to Bacterial Heart Rot (BHR) disease remains limited. To prevent the spread of Bacterial Heart Rot (BHR) disease and crop losses, the best pineapple variety must be found. Thus, this study was carried out to characterise the causal pathogen of BHR from Bestari Jaya, Selangor and to evaluate disease tolerance between Crystal Honey, Morris, and MD-2 varieties of pineapple towards the Bacterial Heart Rot (BHR) pathogen.

2. Materials and methods

2.1 Isolation of plant pathogenic bacteria

A total of ten pineapple leaf samples with probable BHR disease were from several farms of pineapple in Bestari Jaya, Selangor. Infected pineapple leaves with BHR symptoms were cut into 1 cm-long pieces from the edge. The leaf pieces were placed on top of the Nutrient Agar (NA) medium and placed in the incubator at $28\pm 2^{\circ}\text{C}$ for 24 h after being surface sterilised with 10% Clorox for 1 minute and rinsed 3 times in sterile distilled water. After 24 h, the visible bacteria were streaked over Crystal Violet Pectate (CVP) and Nutrient Agar (NA) media to get the pure pathogen culture and placed back in the incubator at $28\pm 2^{\circ}\text{C}$ for 24 h.

2.2 Morphological characterisation and pathogenicity test for bacterial heart rot disease

The morphological characteristics of the isolated pathogen were observed on Crystal Violet Pectate (CVP) medium and NA, and were subjected to Gram-staining. Then, BHR disease pathogens were identified using potato pathogenicity tests and pineapple pathogenicity tests. Healthy potatoes were used to test pathogenicity, where the first potato was injected with 1 mL of standardised fresh overnight bacterial suspension (10^8 CFU/mL) while the second was injected with 1 mL of sterile distilled water (negative control) using a sterile syringe with a needle and left at room temperature for 5 days. For the pineapple pathogenicity test, an overnight bacterial suspension was standardised to 10^8 CFU per mL. The pathogen was then inoculated onto fresh, healthy fruits. The fruits were surface-sterilised, and 8-point injections were made on each of three fruits, with an additional 8-point control on one fruit.

Table 1. Scale description of pineapple leaves' physical parameters.

Parameters Scale	Definition	Presence of Lesion	No. of leaves infected per plant
0	Resistant	No lesion on leaves	No leaves infected
1	Moderately resistant	Slight lesion on leaves (Less than 5%)	Less than 10% of leaves are infected
2	Moderately susceptible	Slight lesion on leaves (Over 5% to 10%)	10-20% of leaves are infected
3	Susceptible	Lesion on leaves (Over 10% to 20%)	20-30% of leaves are infected
4	Very susceptible	Lesion on leaves (21% and above)	40% or more leaves are infected

Source: Nor *et al.* (2019).

2.3 Disease tolerance between three different varieties of pineapple towards the bacterial heart rot pathogen

Pineapple suckers, namely a healthy MD-2, Morris, and Crystal Honey variety, were planted in 16-inch polybags using a Randomised Complete Block Design (RCBD) with three replicates in the UiTM Jasir greenhouse in Malacca. The pineapple suckers were watered every 3-5 days, and after one month, they were appropriately fertilised with NPK (15:15:15). The inoculum was prepared by comparing it to 0.5 McFarland Standards (equivalent to 1.5×10^8 cells) to create the BHR disease inoculum, as outlined in Aryal (2021). Before injecting 0.5 mL of the standardised bacterial suspension into each test plant, which included MD-2, Morris, and Crystal Honey varieties, healthy pineapple leaves were meticulously selected, following the methodology described by Young *et al.* (2022). Control treatments were executed similarly with sterile distilled water. The percentages of lesions and diseased leaves were recorded and categorised according to a disease observation scale index (Table 1), as detailed in Nor *et al.* (2019).

3. Results and discussion

3.1 Isolation and characterisation of plant pathogenic bacteria

Pineapple plants with water soaking on the white basal side of the leaves and light brown to dark brown leaf colours were randomly sampled from several pineapple farms in Bestari Jaya (Figure 1). Then, the isolated bacteria from 10 pineapple leaf samples were grown in the incubator for 24 h and had a similar creamy-white, smooth, and flat surface on Nutrient Agar (NA)



Figure 1. Pictures A and B show a sample of pineapple leaves that have symptoms of BHR disease.

medium. The bacteria on Crystal Violet Pectate (CVP) medium created cavities at various depths (Figure 2). As shown in Figure 3, the possible pathogens, the isolated bacteria were Gram-negative, red-pink, and rod-shaped after Gram-staining.

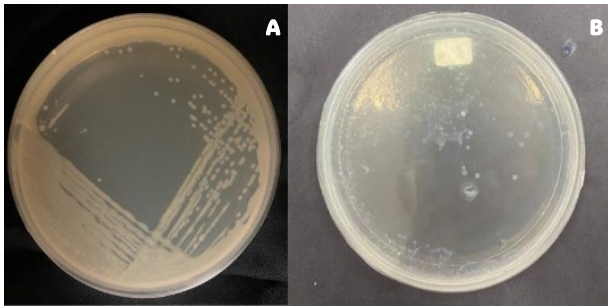


Figure 2. (A) Bacteria colony with a creamy-white, smooth, and flat surface on Nutrient Agar (NA) medium and (B) bacteria on Crystal Violet Pectate (CVP) medium, creating cavities at various depths.

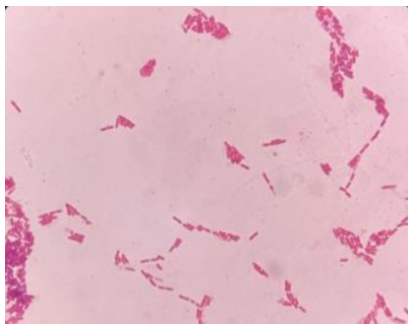


Figure 3. Gram-negative (red-pink colour), rod-shaped bacteria at 100× magnification.

The collected samples showed similar symptoms of Bacterial Heart Rot (BHR) disease as described by Cano-Reinoso *et al.* (2021), Sapak *et al.* (2021), Husin and Sapak (2022), which are the presence of water soaking on the white basal side of the leaves, bad odour and appearances of light-dark brown colours on the leaves. The isolated Bacterial Heart Rot pathogen had similar characteristics to the findings by Aeny *et al.* (2020). The author mentioned that *Dickeya zae* colony was shaped like a circle and had a milky white and cream colouration (Aeny *et al.*, 2020). Similar results were obtained with Hélias *et al.* (2012), whereby the authors stated that *Dickeya* spp. produce cavities at various depths on Crystal Violet Pectate (CVP) media. Similar results were achieved with Boluk *et al.* (2021) for the Gram-staining, whereby the author reported that *Dickeya zae* was Gram-negative and rod-shaped based on the results above.

3.2 Pathogenicity test for bacterial heart rot disease

For the pathogenicity test on potato, the isolated bacteria produced lesions on the potato compared to sterile distilled water (negative control) (Figure 4). This result is aligned with the pathogenicity test on pineapple, where the isolated bacteria suspension produces lesions and a bad odour on the fresh and healthy pineapple

(Figure 5).



Figure 4. Pathogenicity test on the potato. (A) No visible symptoms when the potato was injected with sterile distilled water (control), (B) A lesion was observed on the potato that was injected with standardised bacterial suspension.



Figure 5. Pathogenicity test on pineapple (A) Pineapple injected with sterile distilled water, (B) Pineapple injected with the standardised bacterial suspension has lesion symptoms and a stinky smell.

The pathogenicity test showed results consistent with those documented in the journal by Keneshiro *et al.* (2008). After 7 days of conducting the test, symptoms were observed resembling water-soaked lesions, as described in the study by Aeny *et al.* (2020). These symptoms included browning or anthocyanescence (reddish or purplish colouration) prior to curling and eventual death. This symptomatology aligns with the findings presented in the journal by Green and Nelson (2015) regarding bacterial heart rot symptoms following a pathogenicity test.

3.3 Disease tolerance between three different varieties of pineapple towards the bacterial heart rot pathogen

Table 2 demonstrates that the pineapple variety of Crystal Honey had the lowest disease observation scale index (0.6667) compared to Morris and MD-2. MD-2 pineapple has the highest mean disease scale index of 1.5000. Morris pineapple variety has a middle scale between Crystal Honey and MD-2, 1.000.

Based on the data above, the pineapple varieties of Crystal Honey and Morris show moderate resistance towards the pathogen that causes Bacterial Heart Rot (BHR) disease outbreak. Crystal Honey shows the lowest mean disease observation scale index, which is 0.6667 when compared with the Morris variety of pineapple, which got 1.0000. It is supported by a statement from

Nor et al. (2019) who reported that the Crystal Honey variety was moderately resistant to the Bacterial Heart Rot disease. However, for the MD-2 variety of pineapple, MD-2 shows the highest mean disease observation scale index, which is 1.5000 which indicating moderately susceptible to Bacterial Heart Rot (BHR) disease compared to Crystal Honey and Morris varieties of pineapple. MD-2 variety of pineapple is also being reported as susceptible to pineapple heart rot disease compared to the other varieties in Uganda (Oculi et al., 2020).

Table 2. Mean disease scale index for 3 different varieties of pineapple

Pineapple variety	Mean disease observation scale index	Definition of disease observation scale index
Morris	1.0000	Moderately Resistant
MD-2	1.5000	Moderately Susceptible
Crystal Honey	0.6667	Moderately Resistant

4. Conclusion

In conclusion, the isolated bacterial suspension exhibited characteristics consistent with *Dickeya zae* as determined through morphological characterisation and pathogenicity tests. This bacterium was identified as the primary causative agent responsible for bacterial heart rot disease in pineapples from Bestari Jaya, Selangor. Gram staining confirmed that all isolated pathogens were gram-negative and with a random arrangement. The pathogenicity of the bacterial suspension was substantiated using Koch's postulate, which resulted in the development of lesions on both potato and pineapple samples within two days post-inoculation. Notable symptoms included the presence of odour, sores, brown fluid, and leaf decay. These findings were in line with previous studies on BHR causative agents affecting MD-2 pineapples, indicating the potential diversity in the origins of these isolates and emphasising the need for molecular confirmation. Next, compared to Morris and MD-2, the Malaysian pineapple variety Crystal Honey has the lowest disease observation scale index, indicating considerable resistance to Bacterial Heart Rot (BHR) disease. Since Morris and MD-2 were exported pineapple varieties, pineapple researchers must develop a new variety that is resistant to Bacterial Heart Rot (BHR) disease and has the best traits of Morris and MD-2. Further studies can be conducted on the types of soil if this plays an important role in the growth of pathogens for BHR disease.

Conflict of interest

The authors declare no conflict of interest.

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References

- Aeny, T.N., Suharjo, R., Ginting, C., Hapsoro, D.W.I. and Niswati, A. (2020). Characterisation and host range assessment of *Dickeya zae* associated with pineapple soft rot disease in east Lampung, Indonesia. *Biodiversitas*, 21(2), 587–595. <https://doi.org/10.13057/biodiv/d210221>
- Aryal, S. (2021). McFarland Standards: Principle, Preparation, Uses, Limitations. Retrieved from Microbe Notes website: <https://microbenotes.com/mcfarland-standards/>
- Boluk, G., Arizala, D., Dobhal, S., Zhang, J., Hu, J., Alvarez, A.M. and Arif, M. (2021). Genomic and Phenotypic Biology of Novel Strains of *Dickeya zae* Isolated From Pineapple and Taro in Hawaii: Insights Into Genome Plasticity, Pathogenicity, and Virulence Determinants. *Frontiers in Plant Science*, 12, 663851. <https://doi.org/10.3389/fpls.2021.663851>
- Cano-Reinoso, D.M., Soesanto, L., Kharisun, and Wibowo, C. (2021). Fruit collapse and heart rot disease in pineapple: Pathogen characterisation, ultrastructure infections of plant and cell mechanism resistance. *Biodiversitas*, 22(5), 2477–2488. <https://doi.org/10.13057/biodiv/d220504>
- Green, J. and Nelson, S. (2015). Heart and Root Rots of Pineapple. Retrieved from website: <https://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-106.pdf>
- Hélias, V., Hamon, P., Huchet, E., Wolf, J.V.D. and Andrivon, D. (2012). Two new effective semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61(2), 339–345. <https://doi.org/10.1111/j.1365-3059.2011.02508.x>
- Husin, N. and Sapak, Z. (2022). *Bacillus cereus* for Controlling Bacterial Heart Rot in Pineapple var. MD2. *Tropical Life Sciences Research*, 33(1), 77–89. <https://doi.org/10.21315/tlsr2022.33.1.5>
- Kaneshiro, W.S., Burger, M., Vine, B.G., De Silva, A.S. and Alvarez, A.M. (2008). Characterisation of *Erwinia chrysanthemi* from a bacterial heart rot of pineapple outbreak in Hawaii. *Plant Disease*, 92(10), 1444–1450. <https://doi.org/10.1094/PDIS-92-10-1444>

- Malaysian Pineapple Industry Board. (2022). Malaysian Pineapple Industry Board Annual Report 2021. Retrieved from website: https://www.mpib.gov.my/en/publication/#12Mf99h9YfcHI7JdFJz3cz94PyWO5_bmE
- Nor, A.A.M., Zainol, R., Abdullah, R., Jaffar, N.S., Rasid, M.Z.A., Laboh, R., Shafawi, N.A. and Aziz, N.B.A. (2019). Dissemination pattern of bacterial heart rot (BHR) disease and screening of the disease resistance among commercial pineapple varieties in Malaysia. *Malaysian Journal of Microbiology*, 15 (4), 246–250.
- Oculi, J., Bua, B. and Ocwa, A. (2020). Reactions of pineapple cultivars to pineapple heart rot disease in central Uganda. *Crop Protection*, 135, 105213. <https://doi.org/10.1016/j.cropro.2020.105213>
- Sapak, Z., Mohd Faisal Mahadeven, A.N., Nurul Farhana, M.H., Norsahira, S. and Mohd Zafri, A.W. (2021). A review of common diseases of pineapple: the causal pathogens, disease symptoms, and available control measures. *Food Research*, 5(S4), 1–14. [https://doi.org/10.26656/fr.2017.5\(S4\).004](https://doi.org/10.26656/fr.2017.5(S4).004)
- Young, A.J., Pathania, N., Manners, A. and Kenneth G.P. (2022) Heart rot of Australian pineapples caused by *Dickeya zea*. *Australasian Plant Pathology*, 51, 525–533. <https://doi.org/10.1007/s13313-022-00880-x>