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Isolation and Characterization of Pathogenic Mold Causing Potato Tuber Rot Disease

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Abstract: The potato plant (*Solanum tuberosum* L.) is a perennial crop that contains high amounts of carbohydrates, minerals, and vitamins in its tubers, making it a carbohydrate-rich alternative food to rice or corn. One of the diseases that often appear on potato plants is potato rot caused by pathogenic molds. This study aims to isolate and characterize pathogenic fungi that cause blight on potato tubers based on Koch's Postulates. Potato pathogenic molds were isolated from potato tubers that had been rotted, then the molds were grown on PDA and purified twice. The purified isolates were then inoculated onto 30 healthy potato tubers and incubated for 7 days. Healthy potato tubers experienced the same symptoms as potato tuber rot. The isolated pathogenic fungi were then characterized so that the pathogenic fungi of *Phytophthora infestans* were obtained. In this study, Koch's Postulate method was successfully applied to isolate and characterize the pathogenic fungi that cause potato tuber rot. The pathogenic fungi caused a potato tuber rot disease incidence value of 100% with a disease severity value of 60.7%. The two factors that determine the disease incidence and disease severity values are internal factors (genes and traits of the pathogen) and external factors (environment).

Keywords: Koch's postulates; Phytophthora; Potatoes; Tuber rot

Introduction

Potato plants are annual plants in the form of bushes (Spooner et al., 2014). The common potato, *Solanum tuberosum* L., is grown and consumed worldwide. It is the third most important food crop (FAO, 2013), As a food ingredient that contains high levels of carbohydrates, minerals, and vitamins, potatoes can replace carbohydrate foods originating from rice, wheat, or corn which are used to meet people's food needs (Samanhudi, 2001). According to records from the Central Statistics Agency, potato production in Indonesia will reach 1.36 million tons in 2021. Potato production has increased by 6.1% from the previous year to 1.28 million tons. Potato production experienced a decline in 2020 due to the Covid-19 pandemic. However, if we look at the previous year, potato production continues to increase until 2019. This increase is to meet the need for a substitute for staple food (rice) and as an industrial raw material, in addition to overcoming the increasingly high price of rice and reducing imports of rice food which has consumed large amounts of the country's foreign exchange.

This great opportunity can be hampered because potatoes have a fairly high level of disease infection among other types of tubers. The disease that often attacks potato plants is the fungus *Fusarium* sp. (Trabelsi et al., 2015) and *Phytopthora infestans* (Hussain et al., 2015), and the bacteria *Ralstonia solanacearum* (Karim et al., 2018). *Ceratocystis fimbriata* also found in sweet potato plants (Paul et al., 2018). The pathogenic fungus *Phytophthora infestans* causes leaf and tuber rot in potato plants (Rukmana, 1997) has caused a lot of harm to potato farmers in Wonosobo, Central Java, a priority location for potato agribusiness development in Indonesia. Potato productivity in Kedu District,

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Wonosobo Regency, Central Java Province in 2003 also experienced a sharp decline, this was caused by the humus layer being depleted, resulting in high disease and pest contamination. In the rainy season, potato seeds are susceptible to the pathogenic fungus *Phytophthora infestans*, while in storage warehouses the seeds are susceptible to pest attacks.

Potato plant leaf and tuber blight or what farmers in Wonosobo and Dieng call Lodoh is the most serious disease among the diseases and pests that attack potato plants in Indonesia (Katayama et al., 1997). Lodoh disease is caused by an attack by the vicious pathogenic fungus *Phytophthora infestans*. It can reduce potato production by up to 90% of total potato production in a very short time (Sembiring et al., 2021). Until now, the pathogenic fungus that causes stem and leaf rot disease in potato plants is still a crucial problem and there are no potato varieties that are truly resistant to this disease (Cholil et al., 1991). Mold can attack leaves, stems, and tubers in the soil. The pathogenic fungus *Phytophthora infestans* is not native to soil, but usually attacks potato plant organs in the soil and above the ground (leaves, stems, branches, roots, and tubers) (Djafaruddin, 2008). Root rot diseases remain a major global threat to the productivity of agricultural crops. Root rot disease can cause significant economic losses because it can reduce crop yields and potato quality (Williamson-Benavides et al., 2021).

Root rot can spread quickly if not managed properly (Mohammed et al., 2014). Spread of pathogenic mold spores via wind, water, or insects. Spores that fall to the ground will infect the tubers, and rot can occur in the soil or storage. Cases of leaf blight usually occur in highland areas with low temperatures and high humidity (Kimbrough et al., 1996). Apart from that, the spread of spores of the pathogen *Phytophthora infestans* is triggered by the relatively humid air environment (above 80% like the environmental conditions in Wonosobo). Attacks on tubers are wet tuber rot which is gray or black. If the tubers are incubated at a temperature of 15 - 20°C, conidia will appear in large quantities, in the form of gray powder (Cholil et al., 1991).

Control that this fungus can carry out is by spraying the synthetic fungicide Sandofan MZ 10/56 WP with the recommended concentration, Benlate with the recommended concentration, and Kocide 54. The habit of farmers spraying pesticides haphazardly causes the emergence of new strains of these pathogenic fungi. This shows the resistance of the mold to certain synthetic fungicides or effective doses, synthetic fungicides can reach twice the recommended dose. The use of biological agents made from biofungicides is an appropriate alternative for controlling pathogenic microbes that cause disease in cultivated plants (Purwantisari et al., 2012). Four bacteria endofit can be capable for inhibiting pathogenic bacteria that are *Bacillus brevis*, *Bacillus latesporus*, *Virgibacillus pantothenticus*, and *Bacillus circulans* (Zulkifli et al., 2018). *Trichoderma* spp. can be an example fungi of an environmentally friendly biological agent to suppress the growth rate of pathogens (Hadi et al., 2023). The use of alternative fungicides containing mixed cultures of antagonistic fungi has never been carried out, therefore it is necessary to carry out preliminary research on the effectiveness and potential of antagonistic fungi using the Koch Postulate method.

Koch's postulates are a series of tests to prove the presence of certain microbes that cause disease. To determine whether bacteria are pathogenic or not, the bacteria must fulfill the principles of Koch's Postulates: certain microorganisms are always found associated with the disease they cause; microorganisms can be isolated and grown as pure cultures in the laboratory; pure cultures If injected into suitable plants it can cause disease; these microorganisms can be isolated again from infected plants. This research aims to isolate and identify pathogens that cause disease in potato tubers using Koch's Postulates. Kaur et al. (2014) said that better plant disease control strategies can help reduce the use of pesticides and other chemicals that can harm the environment. Therefore, it is hoped that the results of this research can provide management alternatives that are more environmentally friendly and sustainable.

Method

This research was carried out in June 2023 at the microbiology laboratory of the biology study program, Jakarta State University. This research is referring to Koch's postulates to isolate and characterize pathogenic fungi that cause root rot in potato tubers.

Making Culture Media

According to Uthayasooriyan et al. (2016), the medium is prepared by making potato stock first, then cooling. The potato extract is filtered into an Erlenmeyer to get pure potato extract. Then the broth mixture with sucrose was stirred, heated on a hot plate, and homogenized using a magnetic stirrer, and the pH was measured (± 6-7). Then, nutrient media is added until it is homogeneous. The homogenized media was then sterilized by autoclaving for 15 minutes at 121°C.

Isolation and Purification

Isolation is carried out by spraying alcohol on the surface of the potato. Potatoes that have been infected with the disease are taken a little, half infected, and half healthy using a sterile scalpel, then placed in PDA media. The culture is incubated for 3-4 days at room temperature ranging from 25 – 30°C. Microorganisms that grow on PDA media are taken back to be purified or grown again on other PDA media to obtain pure cultures. The purification results were re-incubated for 3-4 days at room temperature ranging from 25 – 30°C. Purification is carried out 2 times. The pure isolate obtained was then described morphologically and carried out simple staining to determine the shape of the microbes obtained. The preparations were then observed under a binocular microscope with 100x magnification.

Inoculation

The isolate obtained was inoculated into 30 healthy potatoes to prove that the isolated pathogen was the disease-causing pathogen using Koch's postulates. According to Koch, the four postulates must be fulfilled to determine a causal relationship between parasites and disease, namely that the organism is always associated with its host in all disease incidents, the organism (pathogen) can be isolated and bred into a pure culture, the isolation results when inoculated on healthy plants will produce the same disease symptoms as plants affected by the disease (Semangun, 2006). Healthy fruit is made injured. The isolate in the media was taken with a sterilized straw and then inoculated into the wound on the fruit. The wound is then covered with sterile tissue moistened with sterile distilled water and then closed tightly over the wound area. Then scoring was carried out after incubation for 3 days, 5 days, and 7 days (on the seventh day the sterile tissue was opened).

Scoring

Scoring is determined by removing the wound cover on the potato fruit and then observing the wound on each potato from the total number of infected potatoes. Then sort the potatoes based on the disease symptoms visible on the skin. Disease scores refer to Driscoll et al. (2009) can be seen in Table 1. Data obtained from determining the potato score is then used to calculate the intensity of disease attacks. The calculation of disease intensity refers to Wiguna et al. (2015) which consists of calculating disease incidence or disease incidence (IP) and disease severity (KP).

Table 1. Assessment Scores Used to Classify the General Severity of Potato Tubers

Score	Lesion (%)	Presence of lesions on the surface
0	0	0
1	1-10	0
2	11-20	0
3	21-50	1-5
4	51-80	6-25
5	80-100	>25

Measurement of disease incidence (IP) can be done using the formula:

$$IP = \frac{n}{N} \times 100\% \quad (1)$$

n: Number of plants attacked by the pathogen;
N: Total plants observed regardless of disease severity.

Measurement of disease severity (KP) can be done using the formula:

$$KP = \frac{\Sigma n \times v}{N \times V} \times 100\% \quad (2)$$

n: Number of plants attacked in the i^{th} score category
v: Score in each i-th attack category
N: Total plants observed
V: Score for highest attack refers to scoring assignment (Table 1)

Result and Discussion

Based on the Koch postulate method, data obtained from observations are isolates and purification results of microbes that are thought to be associated with rot symptoms in potatoes. Then the scoring results are also obtained from potatoes that have been inoculated with previously purified microbes so that the occurrence and severity of disease can be calculated.

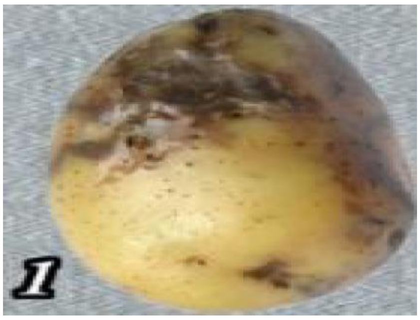

Figure 1. Example of a potato experiencing potato blight symptoms (Korchagin et al., 2021).

Symptoms of rot in potatoes (potato blight) (Figure 1) are usually characterized by the growth of microbes (in this context fungi) in the inoculation area followed by color changes in the surrounding area or all parts of the potato. On the affected tubers, gray or brown (depending on the variety and color of the skin), slightly depressed hard spots are formed, extending inward in an uneven shape (Korchagin et al., 2021).

To determine the symptoms of rot in potatoes and the microbes associated with causing symptoms by Koch's postulates, the potatoes are first rotted for around 7 days, then the microbes in the rotten potatoes are isolated and grown on potato media (PDA) for around 3-5 days. The isolate was then transferred to another PDA medium for microbial purification and incubated for around 3-5 days until the microbes grew. The use of PDA media was chosen because it has similarities to the original host (potato).

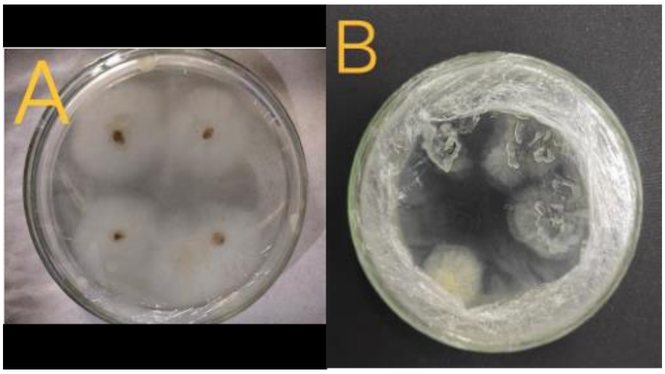

Figure 2. Results of microbial isolation from rotten potatoes (A) and results of microbial purification (B). From a rough observation, it can be seen that the colonies formed in the two images are a group of molds because hyphal fibers are formed.

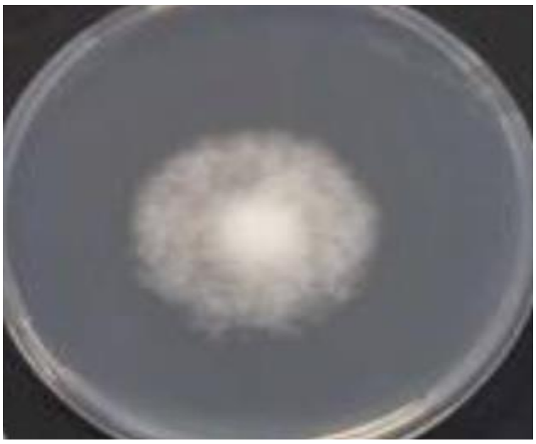

Figure 3. Macroscopic colonies of *Phytophthora infestans* on PDA media (Mideros et al., 2018)

Based on Figure 2, it can be assumed that rotten symptoms in potatoes are caused by mold. The mold isolate forms white colonies (both viewed from above and below) with a smooth texture and radial growth of around 1.5 - 2.5 cm. If referring to Mideros et al. (2018) (Figure 3), and from a macroscopic point of view, the mold that causes rot symptoms in potatoes is a mold from the *Phytophthora* group. In culture, the mycelium is white and smooth; Colonies grow rather slowly. Growth rates can vary dramatically among isolates, but fast-growing isolates can cover a 9 cm plate within 7-10 days and some isolates produce a lumpy appearance (Stamps, 1985). To confirm this, the isolate needs to be further identified using a microscope.

In microscopic observation, the identified mold has characteristics similar to the *Phytophthora infestans* group of molds. According to Stamps (1985), P. *infestans* is a coenocytic oomycete with rare cross walls. Asexual reproduction is via sporangia which are ellipsoid to lemon shaped with small pedicels. Based on the results of 100x microscope magnification and referring to the statement above, the characteristics of these microbes are per the characteristics of *Phytophthora infestans*. At microscope magnification (Figure 4), the spores that are formed are lemon-shaped, marked by a black arrow and there is enlarged tissue (blue arrow) which matches the literature photo of *Phytophthora infestans* next to it.

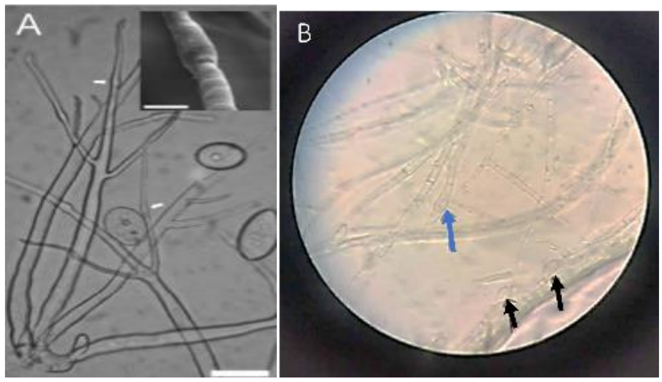

Figure 4. A: *Phytophthora infestans* (Thines, 2009). B: Results of observations of microbes through a microscope with 100x magnification. It can be compared with the literature, microbes have spores with the same shape as the literature, namely lemon-shaped (black arrow) and there is enlarged tissue on the hyphae (blue arrow)

Even though it is known that *Phytophthora infestans* is a microbe that causes rotten symptoms in potatoes, referring to Koch's postulate method, this microbe must be inoculated on healthy potatoes and produce the same symptoms as previously sick/rotten potatoes to confirm the association between the microbe and the disease or symptoms caused. Therefore, *Phytophthora infestans* was inoculated on around 30 potatoes to observe the symptoms and determine the score value (after incubation for around 7 days) to calculate the occurrence and severity of the disease caused.

After being inoculated and incubated for about 7 days, the potatoes experienced the same symptoms as early rotten potatoes. Potatoes that were initially firm and did not have any color change began to rot. Significant levels of rot and tuber damage appear to be the main symptoms after inoculation with *Phytophthora infestans* (Kirk et al., 2013). The rot begins with a blackish-brown discoloration in the inoculation area/wound area and visible threads of mycelium forming in the area. This wound is made so that microbes can enter easily so it doesn't take a long time to see symptoms. The color changes in these 30 potatoes varied in the context of the affected area, some potatoes only had discoloration in a small area of <10% and some potatoes experienced discoloration of up to >50%. Apart from color changes, rot also causes the inside of the potato to change its texture, becoming soft and watery (in some potatoes) and giving off an unpleasant odor. Spots of infected plants have a characteristic odor. Rotten potato tubers, showing the characteristic reddish-brown 'marbling' of the infected flesh. Infected potato tubers show both wet and dry rot (CABI, 2022).

Figure 5. Results of inoculation of pathogenic microbes on 30 healthy potatoes for 7 days according to Koch's postulate method. It can be seen that 30 potatoes experienced the same symptoms with different severity

Table 2. IP Calculation Results (Disease Incidence) or Disease Occurrence in Observed Potatoes

Potato condition	Number of potatoes	Score IP (%)
Lesions appear	0	0
No lesions appeared	30	100

Table 3. Results of KP Calculations (Disease Severity) on Observation Potatoes

Score (v)	Number of potatoes (n)	n x v	N x Z	KP (%)
0	0	0		
1	5	5		
2	8	16	150	60.7
3	5	15		
4	5	20		
5	7	35		

After knowing the symptoms that appear and the microbes associated with the symptoms/disease, a score value is determined for the 30 potatoes to determine the occurrence and severity of the disease that occurs. (Figure 5) Disease occurrence can be calculated by recording the number of potatoes attacked by pathogens or showing symptoms divided by the total number of potatoes observed. The results of calculating the occurrence of disease were found to be in the range of 100%, which means that all the potatoes observed were attacked by pathogens or showed symptoms of potato rot. This is reinforced by Yuen's statement (2021), that potatoes are the main food crop that can be infected by *Phytophthora infestans*. So it is normal for potatoes to have a disease incidence value (IP) of up to 100% because basically, pathogens can only attack compatible plants (Table 2).

Despite having a disease incidence value of 100% (Figure 5), the disease severity observed in potatoes was around 60.7% (Table 3). This means that many factors influence a pathogen to infect its host. Apart from internal factors, namely the genetics of the pathogen and the host itself and the compatibility between the pathogen host which was discussed previously, several external or environmental factors also influence the IP and KP values. According to Damiri (2011), physical environmental factors that influence plant diseases as previously mentioned include temperature, sunlight, humidity, irrigation, and wind. Each of these factors individually or together influences the development of a disease.

Conclusion

Observation results show signs of rot in potatoes (potato blight) characterized by a change in the color of the potato to blackish brown or reddish and can cause the potato to become soft and watery. Based on Koch's postulate method, *Phytophthora infestans* is associated with causing rot symptoms in potatoes. Potatoes inoculated with cultured pathogens have rot symptoms similar to those of early potatoes. The incidence of potato rot disease (IP) is 100% because potatoes are the main host of *Phytophthora infestans*, while the disease severity (KP) is around 60.7%. The factors that determine IP and KP are internal factors (genes and characteristics of the pathogen/host) and external factors (environment).

Author Contributions

Conceptualization, RKA and RI; methodology, RKA, RI, and POP; validation, RKA and RI; formal analysis, RKA; investigation, RKA, AA, EDR, RRT, HAEP; resources, RKA, POP and NAW; data curation, RKA and RI: writing—original draft preparation, RKA, AA, EDR, RRT, HAEP; writing—review and editing, RKA, RI, POP and NAW: visualization, RKA and POP. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

No conflict interest.

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