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Analysis of the Density of Soil Bacteria Causing Root Rot of Red Kulai Chili (*Capsicum annuum* L. var. Kulai) and Its Resistance to Various Combinations of Growth Media

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ABSTRACT

This study analyzed the density of soil bacteria that cause root rot of red chili pepper (Capsicum annuum L. var. Kulai) and tested a combination of growth media to improve plant resistance. Contaminated soil samples from organic fields were inoculated into sand and organic soil media, then treated with rice bran and rice water. Isolation of bacteria using the spread plate method resulted in 16 isolates with various morphological characteristics. Biolog Ecoplate™ analysis showed an increase in microbial activity, functional diversity, and community evenness in the combination of rice bran and water. This treatment increased the activity of Azospirillum sp. bacteria that inhibited the pathogen Ralstonia solanacearum while increasing the content of soil micronutrients. The results of statistical tests confirmed a significant increase in microbial activity parameters ($p<0.05$) and the Shannon-Wiener diversity index. The combination of rice bran and water proved effective in increasing soil resistance to pathogenic bacteria that cause root rot through the mechanism of microbial antagonism and improving soil fertility. These findings offer an organic-based solution for environmentally friendly plant disease control.

Keywords: Kulai Chili, Pathogenic, Ralstonia solanacearum, Azospirillum.

INTRODUCTION

Chili (*Capsicum annuum* L. var kulai) is a horticultural plant from the Solanaceae family that contains a spicy chemical compound, capsaicin, and has a very high antioxidant content (Najihah et al., 2023). Kulai chili is a chili that is quite popular in Malaysia as a food seasoning and medicine since ancient times, this chili has a very high economic value. Malaysian society needs around 50,000 tons per year just to meet the needs of its people (Aziz et al., 2025). The production of kulai chili (*Capsicum annuum* L. var kulai) in Malaysia is still very low, this encourages Malaysians to import kulai chili to meet their market (Mohidin et al., 2022).
One of the problems of declining chili cultivation in Malaysia is also caused by root rot disease. Root rot disease is caused by the bacteria Ralstonia solanacearum (Thakur et al., 2021). Ralstonia solanacearum bacteria enter the plant through the roots in contaminated soil, then spread to the xylem tissue of the plant (Xue et al., 2020). Symptoms of root rot are characterized by leaves that begin to turn yellow, roots and base of the stem rot, plants will wilt and die (Shao et al., 2024).

Soil is a very common growing medium used for plants, soil contains important nutrients that can retain water and provide good aeration (Harefa et al., 2024). Sand can be a substitute for planting media; sand has very good permability so that it can increase drainage and aeration in plant roots. In addition to having nutrient content, soil contains microbes that can increase soil fertility and play an important role in maintaining the soil ecosystem.

One of the microbes in the soil is *Azospirillum* sp which plays an important role in binding nitrogen in the soil by changing it into a form that can be absorbed by plants (Yasuda et al 2022). In addition to functioning to maintain soil fertility, Azospirillum sp bacteria can inhibit the growth of pathogenic microbes such as *Fusarium oxysporum* which causes fusarium wilt disease (Hadiwiyono, et al., 2023). According to Rahmayani (2018) giving a mixture of rice bran and water can increase the growth of *Azospirillum* sp bacteria. Rice water itself has a high content of N, P, and K elements so it is very good for microbes that bind N elements (Nabayi et al., 2022). According to Suryatmana et al., (2022) bran can increase the effectiveness of N-fixing bacterial incoluna.

MATERIALS & METHODS

This study was conducted by taking random soil samples (Simple Random Sampling) that had been contaminated with root rot disease in the organic fields of the Malaysian Agriculture Research and Development Institute (MARDI) located at 2 ° 59'31.4 "N north latitude and 101 ° 41'43.8" E east longitude. The soil samples were inoculated into sand and organic soil media so that the soil was contaminated and then treated with various media with a combination of bran and rice water. Then incubated after treatment for 1 week using the following levels:

T1: Organic soil control,
T2: Sand control,
C1: Organic soil with a combination of rice bran & water,
C2: Sand with a combination of rice bran & water.

Spread Plate

The research method for analyzing the density of soil pathogenic bacteria using the spread plate method was carried out using a laboratory experiment approach. Soil samples were taken aseptically from several locations at a certain depth, then taken to the laboratory for analysis. A 1 gram soil sample was dissolved in 9 ml of sterile distilled water to make an initial dilution of 10^{-1}, then a multi-level dilution was carried out to the desired concentration. From each dilution level, 0.1 ml of solution was taken and planted on the surface of the agar medium in a sterile petri dish using the spread plate technique with a sterile spreader so that the liquid was evenly distributed. After that, the petri dish was incubated at a temperature of 30–37°C for 24–48 hours. After incubation, the growing bacterial colonies were counted, especially in dishes with the number of colonies between 30 and 300. This spread plate method is an effective and commonly used technique in microbiology to determine the density of pathogenic bacteria in soil samples.

Isolation & Characterization of Bacteria

After the bacterial colonies have grown well separated on the petri dish using the spread plate method, the next step is to take the colonies aseptically using a sterile loop. The colonies taken are then transferred to new agar media using the streak plate technique to obtain pure cultures. These pure cultures are incubated at an optimal temperature, usually between 30–37°C for 24–48 hours, until the colonies grow well again. After the pure culture is obtained, macroscopic characterization is carried out by observing various colony characteristics such as shape, size, color, edges, texture, elevation, and transparency on the agar media. Observations are made visually and the results are documented to distinguish the types of bacteria based on colony morphology. The entire isolation and characterization process takes place aseptically to prevent contamination, and colonies are taken from petri dishes that show even and separate colony growth according to the principles of the spread plate method.

Analysis of Microbial Functional Diversity

Microbial functional diversity was assessed by measuring microbial activity and community-level physiological profiles using the Biolog Ecoplate™ System. This instrument contains 31 types of carbon sources relevant to ecosystem conditions, producing a metabolic fingerprint through the interaction between the microbial community and the carbon substrate (Garland, 1997). The procedure involved preparing a soil suspension at a ratio of 100 g of soil per liter of distilled water, which was stirred for 30 minutes, then filtered through a 0.45 µm pore membrane. A 100 µl of the suspension was inoculated into the Ecoplate™ and incubated at 27°C. Microbial respiration during carbon metabolism reduces the tetrazolium dye to a purple compound, which is measured as optical density (OD) at 590 nm. OD measurements were performed at 48 hours of incubation to ensure a pure metabolic response to the carbon source, avoiding interference from dissolved organic carbon in the rhizosphere (Gomez et al., 2006).

Microbial Activity

The average microbial activity results in a color increase that can be measured by OD after 72 hours of incubation. The average color development can be calculated (Chong et al., 2021).

$$\mathrm{AWCD} = \sum \mathrm{OD}_i / 31$$

Where, ODi is the optical density value of Ecoplate.

Community Level Physiological Profile

To evaluate the physiological profile of the community at the community level, several important parameters were used, namely richness (R), Shannon diversity index (H), and Shannon evenness (E) according to the method described by Garland (1997). These values were calculated based on the Optical Density (OD) values of the BIOLOG EcoPlate™ wells incubated for 48 hours. Richness was determined as the total amount of carbon substrate oxidized, with a threshold of OD ≥ 0.25 as a positive response to carbon substrate oxidation. The Shannon diversity index was calculated using the following formula:

$$H = \sum p_i (\text{In } p_i)$$

where pi is the ratio of activity on each substrate (ODi) to the total amount of activity on all substrates (ΣODi) in the BIOLOG EcoPlate™ well, and ln is the natural logarithm. Furthermore, Shannon evenness was calculated based on the H and R values (Zak et al., 1994) with the formula:

$$E = H / \text{In } R$$

Utilization of Carbon Sources

Measurement of carbon source utilization by microbes was carried out by observing the Optical Density (OD) value in the Ecoplate well after 48 hours of incubation. The carbon sources contained in the Ecoplate were then classified into five groups (guilds) according to the categories proposed

Soil Analysis

The soil testing procedure begins by weighing 10 grams of soil sample and 10 grams of clean sand as a control (blank). The soil sample is then placed in a tube whose bottom has been covered with cotton. Next, 100 mL of ammonium acetate solution (CH_3COONH_4) is added to the tube and incubated overnight. After incubation, the volume of the incubation solution is replenished to 100 mL with ammonium acetate to prepare for the analysis of Ca, Mg, K, and Na cations. This solution is then further analyzed using the Inductively Coupled Plasma (ICP) method to determine the concentration of these cations.

STATISTICAL ANALYSIS

Data analysis in this study was carried out by data exploration, Analysis of variance (ANOVA) and significant difference test (LSD) were used for the interpretation of AWCD, R, H, E, and culturable microorganisms. The amount of change in AWCD, R, H, E, and culturable microorganisms was calculated using the percentage change. Principal component analysis was performed for the substrate in Ecoplate™ (Hassan et al., 2020).

RESULT

Spread Plate

Table 1. Serial Dillucination

Samples	Serial Dillucination (cfu/ml)-							
Sampel 1	TMTC	TMTC	TMTC	49	32	31	20	17
Sampel 2	65	58	43	35	32	27	23	15
Sampel 3	TMTC	77	65	58	41	33	21	14
Sampel 4	TMTC	62	54	51	47	32	28	12
Sampel 5	79	66	61	54	44	42	33	21

Isolation & Characterization of Bacteria

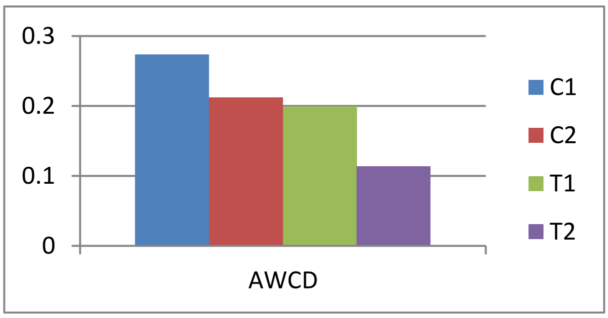
Isolation of pathogenic bacteria from soil has obtained 16 isolates from different soil points. The results of the table show the diversity of different bacteria from Whole, elevation, margin and color.

Table 2. Characterization of bacteria

Sample	WHOLE	ELEVATION	MARGINS	COLOR
SL1 sp1	Circular	Raised	Lobate	White
SL1 sp2	Circular	Raised	Entire	white
SL1 sp3	Circular	Convex	Eros	white
SL2 sp1	Circular	Convex	Eros	cream
SL2 sp2	Irregular	Flat	Lobate	cream
SL2 sp3	Circular	Flat	Entire	cream
SL2 sp4	Filamentous	Flat	Filamentous	cream
SL3 sp1	Irregular	Raised	Lobate	cream
SL3 sp2	Circular	Raised	educate	White
SL3 sp3	Irregular	Flat	Lobate	White
SL3 sp4	Filamentous	Flat	Filamentous	White
SL4 sp1	Circular	Flat	educate	White
SL4 sp2	Irregular	Convex	Lobate	cream
SL5 sp1	Circular	Convex	erosion	cream
SL5 sp2	Irregular	Raised	Lobate	cream
SL5 sp3	Filamentous	Convex	Filamentous	cream

Microbial Activity

Table 3. Average Well Color Devlopment



Community Level Physiological Profile

Table 4. Shannon Weaver Index

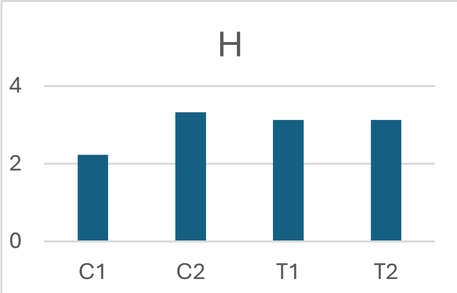


Table 5. Species Richnes

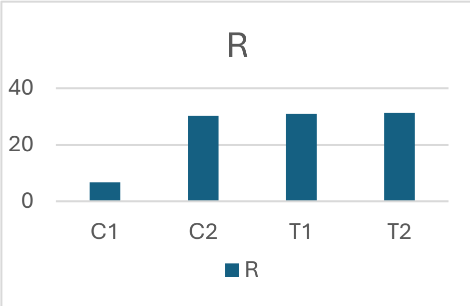
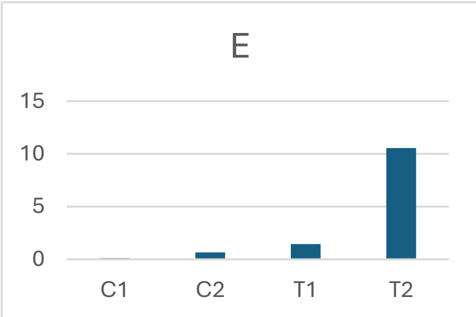


Table 6. Shannon Evennes



Soil Analysis

Based on the results of the analysis of soil that has been incubated with a combination of bran and rice water, there has been an increase in the micro elements contained in the soil, this can be explained in the following table 7.

Table 7. Soil Analysis

Sample	Fe	K	Mg	M N	There	P	S	Zn
C1	0.00531	1.34852	3.46322	0.18255	-3.41436	0.96714	1.88829	0.01200
C2	0.01050	138.97939	36.57444	0.37852	14.53784	2.65409	55.54789	0.04911
T1	1.27589	10,80368	17.53412	0.78654	71,6547	0.76198	2,26708	0.17630
T2	0.29520	55.62315	24,70629	0.84046	68.91250	2.31805	19,56593	0.03997

DISCUSSION

Spread Plate

Based on table 1, it is found at low dilutions (10^0 to 10^{-2}), as evidenced by the TMTC value and colony count above 60. for calculations (30–300 colonies/plate) generally found at dilutions of 10^{-3} to 10^{-6}, according to the rules of the spread plate method. Variations between samples indicate the heterogeneity of the bacterial population in the soil associated with root rot in Kulai red chilies. The spread plate method is very effective for calculating the density of soil bacteria because it is able to produce colonies that are evenly distributed and easy to count, especially after serial dilution (Hadayati el al., 2024). The use of the right dilution range (until 30–300 colonies/plate are obtained) is very important to obtain valid data. The high density of pathogenic bacteria in the tested soil indicates a high potential for root rot in Kulai red chilies, especially if there is no control treatment. Combination treatment of growing media and the use of antagonistic bacteria (as recommended by national and international journals) can be an effective strategy in suppressing pathogen populations and increasing the resistance of red chili plants to root rot, this study is in line with Mannai et al., (2018) that the density of pathogenic soil bacteria such as Rhizoctonia and Phytophthora greatly affects the severity of root rot in Capsicum annuum. This study also emphasizes the effectiveness of biocontrol using antagonistic bacteria (e.g., Pseudomonas, Bacillus) which are able to suppress pathogen growth by up to 12% and increase plant resistance to root diseases.

Isolation & Characterization of Bacteria

This variation indicates the diversity of bacterial species that were successfully isolated from the research samples. Circular and irregular shapes dominate, while filamentous shapes also appear in several isolates. Margin variations such as lobate, entire, erose, and filamentous show differences in the structure of the colony edge which are important in the initial identification of bacteria. Macroscopic characterization such as shape, elevation, margin, and color of the colony is a crucial initial step in the bacterial identification process before continuing with microscopic and biochemical tests. These characters provide initial clues about the possible genus or group of bacteria found. Similar studies in journals also highlight the importance of macroscopic characteristics in bacterial identification. For example, a study of the isolation of lactic acid bacteria from clove kombucha found variations in shape (circular, irregular), elevation (raised, flat), margin (entire, curled), and color (white) (Sanggor et al., 2024). This is in line with the results of the table you attached, where variations in colony morphology are the main indicators of the diversity of bacterial isolates from the environment.

Microbial Activity

Based on research data showing AWCD values of 0.1; 0.2; and 0.3 with treatments T1 (combination of bran + rice water in sand media), T2 (combination of bran + rice water in soil media), and controls C1 (sand media) and C2 (soil media), an in-depth analysis can be carried out on the response of the microbial community to organic nutrient treatments in various substrate media. Rice bran contains complex carbohydrates, proteins, fats, and minerals that are very useful for the growth of microorganisms (Daeng et al., 2023) Rice bran contains glucose and starch needed by bacteria as a source of energy, as well as vitamin B complex that plays a role in microbial metabolism. The cellulose and hemicellulose content in rice bran can be degraded by microbial enzymes into simple sugars that are easy to utilize. Rice washing water contains nitrogen, phosphorus, potassium, and vitamin B1 which can increase the growth of microorganisms. The content of carbohydrates, proteins, and vitamin B1 in rice water plays a role in metabolism and can be converted into energy for microbial activity. Research shows that rice washing water can be used as a growth medium for *Azospirillum* sp. bacteria and increase soil microbial activity.

Community Level Physiological Profile

Based on the analysis of available data from the biology research attachment, the data shows an experimental design with four groups consisting of two control groups (C1, C2) and two treatment groups (T1, T2). The two main variables measured were H and R, which showed different response patterns between the control and treatment groups. Visualization of research data biology show comparison mark variables H and R in the group control (C1, C2) and treatment (T1, T2). The research data shows significant variations between the control and treatment groups . The control group (C1, C2) showed an average H value of 3.00 and R of 2.50, while the treatment group (T1, T2) showed an average H value of 20.00 and R of 12.50. This significant difference indicates a substantive treatment effect on both measured variables. Data shows high variability, especially in variable H which has standard deviation 16.52 and range value 40. Variability This show that treatment provides heterogeneous effects on subject's research. The R variable shows more variability low with standard deviation 5.59 and range value 15. Treatment group (T1, T2) shows substantial improvement in both measured variables, with variable H increases from an average of 3.00 to 20.00, and the R variable increased from 2.50 to 12.50. Although there was variation between individuals within the treatment group, a consistent pattern of improvement was observed in both variables, indicating the reliability of the treatment. The magnitude of the observed changes (a 566% increase in variable H and a 400% increase in variable R) indicates a biologically significant and practically relevant effect. Experimental design with group clear control and treatment give good internal validity (Decena et al., 2024). The use of two groups control and two treatment groups increase the reliability of the results study

Soil Analysis

Rice bran is a rich source of nutrition with a crude protein content of 11.3-14.4%, fat 15.0-19.7%, crude fiber 7.0-11.4%, carbohydrates 34.1-52.3% and ash 6.6-9.9%. These components contribute significantly to increasing the availability of nutrients in the soil. Rice washing water contains vitamin B1 (thiamine), riboflavin, pyridoxine, and minerals such as Ca, Mg and Fe. Nutritional content of rice washing water. Most nutrients are optimally available at pH 6.0-7.0. In acidic conditions (pH < 5.5), the solubility of toxic metals such as aluminum (Al) and manganese (Mn) increases, which can be detrimental to plant health. Conversely, in alkaline soils (pH > 7.5), the availability of micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) decreases due to the formation of insoluble hydroxides and carbonates. This is in line with the research of Lalla et al., (2018) Explaining the high content of vitamin B complex and minerals and its benefits in reducing the use of chemical fertilizers and increasing plant weight.

CONCLUSION

This study shows that the provision of a combination of rice bran and rice washing water in the growth media (organic soil and sand) that has been contaminated with pathogenic bacteria that cause root rot in red chili (Ralstonia solanacearum) can increase the activity of beneficial bacteria, especially Azospirillum sp., which plays a role in inhibiting the growth of pathogenic bacteria. This treatment also increases the content of microelements in the soil, which has the potential to improve soil fertility and health and reduce the incidence of root rot disease in red chili plants. Thus, the use of rice bran and rice washing water as soil amendments can be an environmentally friendly strategy to control root rot disease and increase the productivity of red chili

Declaration by Authors

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