

Isolation of food waste degrading bacteria as green agent for making compost fertilizer

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ABSTRAK

Limbah sisa makanan merupakan salah masalah yang terjadi di kota Semarang. Dampak yang muncul akibat banyaknya sisa makanan adalah munculnya bau busuk, menimbulkan penyakit dan pencemaran lingkungan. Salah satu alternatif yang digunakan dalam mengurangi jumlah limbah sisa makanan adalah mengubahnya menjadi pupuk kompos. Penelitian ini bertujuan untuk mengetahui potensi bakteri hasil isolasi limbah sisa makanan yang diperoleh dari kantin dalam mendegradasi limbah sisa makanan. Media yang digunakan untuk mengkultur bakteri merupakan media sintesis YEMA (Yeast ekstrak mannitol agar). Proses isolasi bakteri menghasilkan 10 isolat bakteri yang tumbuh pada media, kemudian isolat tersebut dilakukan skrining dengan berbagai uji untuk mengetahui kemampuan dalam mendegradasi selulosa, protein, lipid dan amilum. Dari hasil penelitian diperoleh data yang menunjukkan uji skrining diperoleh 4 isolat yang memiliki potensi tertinggi yaitu isolate dengan kode FWD, FWH, FWI, dan FWJ dengan indeks selulolitik terbesar adalah 1.5, indeks proteolitik terbesar adalah 3.3, indeks lipolitik terbesar adalah 3.3, dan indeks amilolitik terbesar adalah 1.2.

Keywords:

Bacterial isolation
Food waste
Compost
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ABSTRACT

Food waste is one of the problems that occurs in the city of Semarang. The impact that arises from the large amount of food waste is the appearance of bad odors, causing disease and environmental pollution. One alternative used to reduce the amount of food waste is to turn it into compost. This research aims to determine the potential of bacteria isolated from food waste obtained from the canteen in degrading food waste. The medium used to culture bacteria is the synthetic medium YEMA (Yeast extract mannitol agar). The bacterial isolation process produced 10 bacterial isolates that grew on the media, then these isolates were screened with various tests to determine their ability to degrade cellulose, protein, lipids and starch. From the results of the research, data was obtained showing that the screening test obtained 4 isolates that had the highest potential, namely isolates with the codes FWD, FWH, FWI, and FWJ with the largest cellulolytic index of 1.5, the largest proteolytic index of 3.3, the largest lipolytic index of 3.3, and the amylolytic index. the largest is 1.2.



Introduction

Population growth is one of the main factors influencing the increase in food waste in Indonesia. With a growing population, the demand for food also increases, increasing food production. However, to meet this demand, there is often significant food wastage. Unsold or wasted foodstuffs by producers, distributors, and traders are a major contributor to the increase in food waste (Aydin & Yildirim, 2021). Food waste is waste generated from leftover production from homes, restaurants, hotels, canteens, and the food industry. Food waste can be in the form of leftover meat, fish, leftover cooked food, moldy bread, leftover bones, expired food, dairy products, fruit, and vegetables (Priyambada & Wardana, 2018). Food waste, in general, has characteristics that have a high water content of more than 74%, easily decaying, and also spreading a pungent aroma that is quite disturbing. This results from the anaerobic decomposition process that produces H₂S, NH₃, and other gaseous compounds. These gaseous materials are the cause of the greenhouse effect (Giroto et al., 2015).

In addition to the growing population, urbanization also plays an important role in increasing food waste. As more and more people move to big cities, their lifestyle and consumption habits tend to change towards a consumptive society that generates a lot of food waste. (Eckert Matzembacher et al., 2020). In cities, food is often more readily available, and people tend to buy food in bulk, which often results in food wastage. (Giroto et al., 2015).

Food waste is not only a social problem but also an economic one. Food waste means a waste of valuable economic resources. Producers, traders, and consumers suffer significant financial losses due to the disposal of food that is still edible. (Aydin & Yildirim, 2021). In addition, farmers and food producers often face falling prices due to excess food supply. Environmental impacts are also a serious concern. Unmanaged food waste ends up in landfills, where the decomposition process produces methane, a greenhouse gas very harmful to the environment. (Ropiatningsuari et al., 2018). In addition, food

production requires significant use of natural resources, such as agricultural land, water, and energy. When food is wasted, these resources are wasted (Chauhan et al., 2021).

This situation illustrates the importance of finding solutions to address the food waste problem in Indonesia. Comprehensive solutions must include public education on the importance of reducing food waste, developing infrastructure for food waste collection and processing, regulations, and policies supporting waste reduction, and promoting sustainable food management practices. (Yuriandala et al., 2020). There are various methods for making compost, including mixing food waste in the form of vegetables and fruit with a starter, which is then fermented under anaerobic conditions. (Bestari & Suharjono, 2015; Elyza et al., 2016). This practical method has several drawbacks, including the fact that starters do not have specific specifications for food waste. The starter is generally used for a mixture of organic materials, while food waste has different characteristics. The characteristics of food waste include one dominant material, for example, protein, lipids, carbohydrates, and others, because there are various types of food waste. To be more effective, the waste is grouped separately according to the dominant ingredients that make it up. (Zhou et al., 2020).

The character of food waste raises other efforts to optimize it, namely specific starter cultures used according to the characteristics of the waste. In addition to the problem of waste materials, the unpleasant odor produced by processing food waste into compost is another problem that must be considered (Susilawati et al., 2015). So, to overcome this, researchers want to research the theme of isolating potential microbes in making compost from leftover food waste. (Kavitha et al., 2020).

Method

a. Tools and materials

The tools used are 50 mL conical tube, 6s ice cooler box, Ose, Yellow tip, oven, tissue, cutter, Petri dish, test tube, vortex, beaker, glass funnel, 10 mL measuring flask, measuring cup, and spatula. Materials include Ethanol 70%, Akuades, Spirtus, Yeast Extract Manitol $MgSO_4$ (Magnesium Sulfat), K_2HPO_4 , Bactoagar, $CaCO_3$, Congo red, Aquades, $MnSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, Carboxy methyl cellulose agar (CMC), Skim milk agar, Nutrien broth, Yeast extract agar, Soluble starch, CPO (Crude palm oil), Bromothymol blue, NaCl, Pepton, $NaNO_3$.

b. Research stages

This research was conducted in a laboratory (laboratory research) using the Completely Randomized Design (CRD) method. This research is included in descriptive qualitative research. The methods used include sorting household organic waste that is composted for 20 days in a composter and then taking leachate from the composting results for multilevel dilution. Cultured on YEMA (Yeast extract manitol agar) media after 48 hours, the sample is then purified. Samples are selected based on the color and shape of the unique colonies formed; the results of observations mark the petri dish with a marker for the purification process. The selected sample is taken at the tip of the nose needle and then scratched using the streak method on a new petri dish containing YEMA media. Furthermore, screening of potential isolates that are able to degrade the main ingredients to degrade household waste (food waste) is carried out, which includes cellulolytic tests, proteolytic tests, lipolytic tests, and amylolytic tests.

Results and Discussion

The stages of the research process are carried out in the following order: 1) Taking/sorting food waste samples, 2) Isolation of food waste bacterial microorganisms, and 3) Screening of potential food waste bacterial isolates. The following are the results of the research.:

a. Isolation of Microorganisms from Food Waste

Bacteria that have been cultured on YEMA media are then purified for 48 hours and produce a single isolate as follows Figure 1.

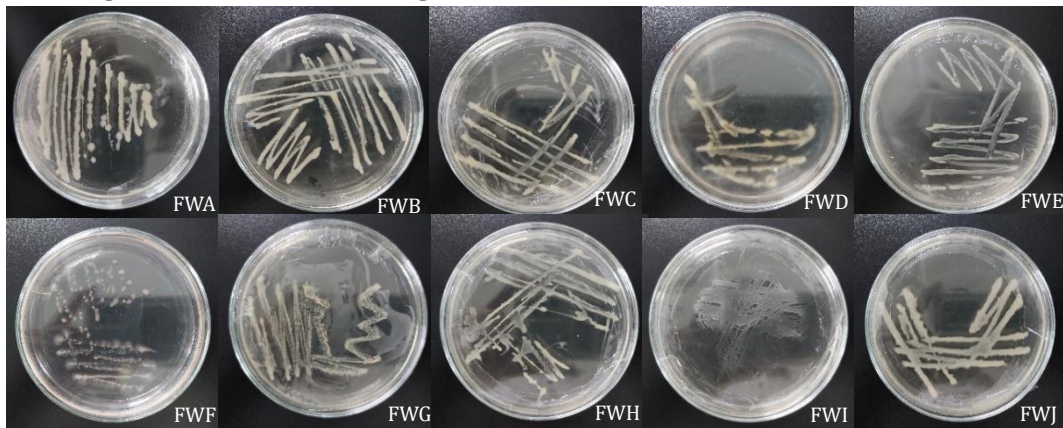


Figure 1. Purification results of isolates from food waste on YEMA media for 48 hours, Based on the purification results of all isolates, 10 single isolates were obtained which were then used for the degrading ability test.

The image above is the result of purification from 48-hour-old waste isolates. Based on the results of observations of the color and shape of the colonies, 10 isolates of bacteria from food waste were obtained. Based on image 1, it can be seen that each isolate code has a different color and colony appearance. It is pretty contrasting to compare the appearance of the FWI isolate colony, which tends to be transparent and thin compared to the FWJ isolate. Each isolate has a different character. Furthermore, the purified isolates were screened for their ability to degrade household food waste.

b. Cellulolytic enzyme production test

Bacteria produce cellulase enzymes as part of a strategy to digest cellulose, the main component of plant cell walls. Cellulase enzymes act as catalysts in the breakdown of cellulose into simple sugars such as glucose, which can be used by bacteria as an energy source. Cellulase enzyme production in bacteria is controlled by various factors, including genetics and the environment. (Teuku Athallah et al., 2021). Cellulolytic bacteria, such as *Clostridium* and *Bacillus*, naturally produce cellulase enzymes. This production process begins with the bacteria detecting the presence of cellulose in the environment. When cellulose is found, the bacteria activate genes that code for cellulase enzymes. These enzymes are then produced and released into the bacteria's environment to digest the cellulose and convert it into sugar. The production of cellulase enzymes is important in the natural cycle, as they help break down dead plant matter, recycle nutrients, and influence diverse microenvironments (Kim et al., 2021).

Humans can utilize the production of cellulase enzymes by bacteria to degrade food waste. Cellulolytic bacteria produce cellulase enzymes that can break down cellulose in food waste, such as vegetables and fruit peels, into simple sugars. This process is called cellulose fermentation. To start the production of cellulase enzymes, bacteria need a cellulose-rich environment. When food waste includes cellulose components, such as fiber, bacteria will detect it and trigger the production of cellulase enzymes as needed. This enzyme helps break down food waste that is difficult to decompose into more easily degraded compounds (Arifin et al., 2019). This study tested the ability of cellulase enzyme production to decompose rice, vegetables, and other fibrous foods. The results of the study can be seen in the Figure 2.

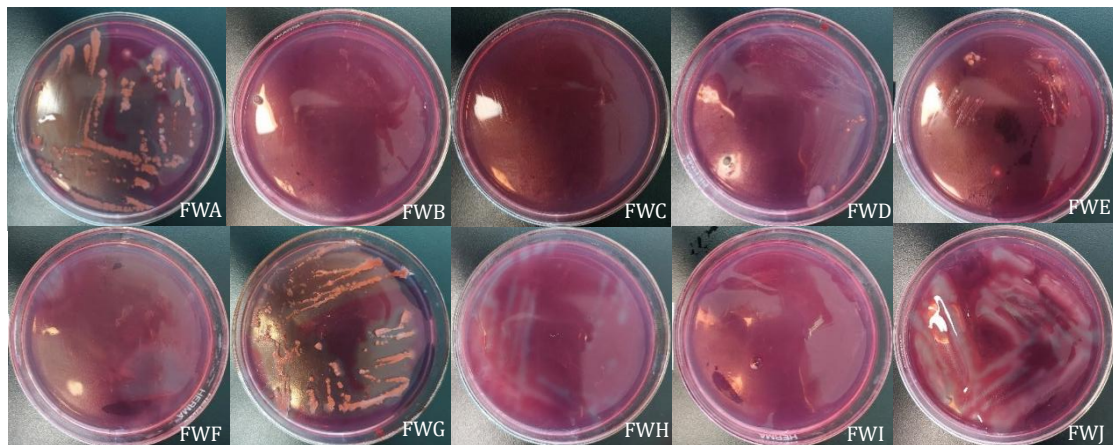


Figure 2. Test results of cellulose degrader isolate from food waste. Positive results of the cellulose degradation test can be identified based on the presence of a clear zone around the bacterial isolate growing on the media.

Based on Figure 2 above, several isolates can degrade cellulose, which is indicated by the presence of a clear zone formed around the isolate after adding 1% congo red and rinsed using NaCl solution. Each isolate that has a clear zone is measured using a caliper. (Arifin et al., 2019), Of the ten isolates, two were able to degrade cellulose after screening tests with the clearest and widest results, seven were able to produce a clear zone but very thin, and one isolate was unable to produce a clear zone at all. The clear zone can be measured using a caliper. Then, the value between the diameter of the medium clear zone and the diameter of the bacterial colony is calculated, which is expressed as the Cellulolytic Index (IS) with the formula:

$$\text{Cellulolytic Index} = \frac{\text{Clear Zone Diameter} - \text{Colony Diameter}}{\text{Colony Diameter}}$$

3 isolates have very thin criteria producing clear zones, including isolates coded FWB, FWD, and FWG with cellulolytic indices of 0.18, 0.41, and 0.06, respectively. Four food waste isolates have thin cellulose-degrading ability criteria, namely isolates with codes FWA, FWC, FWF, and FWH with cellulolytic indices of 0.41, 0.50, 0.33, and 1.16, respectively. Isolates with codes FWI and FWJ are isolates with the best cellulose-degrading ability. Among them, FWI has a colony diameter of 22. In contrast, the clear zone formed has a diameter of 55mm, so the cellulolytic index that can be calculated using the formula is 1.5. isolate code FWJ has a colony diameter of 24mm, the clear zone formed

from the results of degrading cellulose is 60mm so that the cellulolytic index formed is 1.5mm (Nugraha et al., 2014).

c. Proteolytic enzyme production test

The production of protease enzymes by bacteria is an important mechanism in the breakdown of proteins in the microbiological environment. The following are the results of testing the content of proteolytic enzymes:

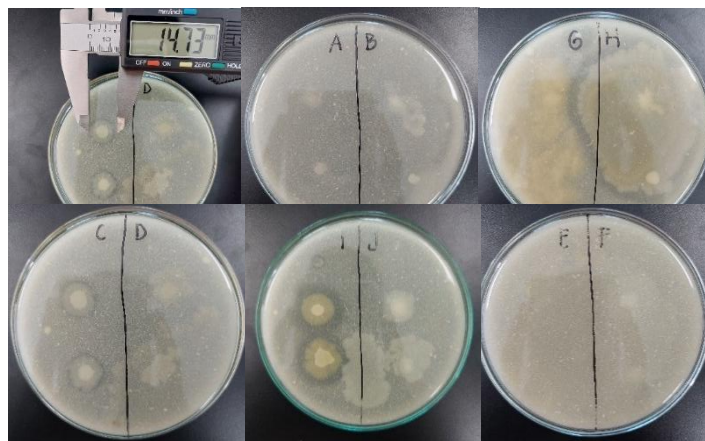


Figure 3. Results of the test of protein degradation isolates from food waste. Positive results of the protein degradation test can be identified based on The presence of a circular clear zone around the bacterial isolate.

Food waste bacterial isolates were tested for their proteolytic activity using selective growth media Skim milk agar (SMA) during bacterial culture in an incubator for 24-48 hours with an incubation temperature of 25-30°C. Indication of favorable results in producing protease enzymes is marked by forming a clear zone (halo zone) around the growth of bacterial colonies (Paskandani et al., 2014). Based on Figure 3, it can be seen that the measurement uses a digital caliper to measure the diameter of the colony and the diameter of the clear zone, where the clear zone is assumed to be a medium that has been successfully degraded by the enzyme produced by the isolate from the isolation of food waste. Based on the documentation photos, several variations in the clear zone and the color of the isolate produced by the isolate. This is because the character of the isolate used has different properties. The formula for proteolytic bacterial activity is known by the following proteolytic index (IP) (Asril & Leksikowati, 2019). The formula for proteolytic bacterial activity is known by the following proteolytic index (IP):

$$\text{Proteolytic index} = (\text{Clear Zone Diameter} - \text{Colony Diameter}) / (\text{Colony Diameter})$$

Based on the measurements, it can be seen that out of a total of ten successfully isolated isolates, only one isolate with the FWE code was unable to grow in skim milk agar media. Meanwhile, the isolate with the FWG code has a proteolytic index of 0 because the colony diameter is 11mm. However, no clear zone can be observed from the edge of the colony isolate. Six isolates have a thin proteolytic index criterion, namely an index with a size of no more than 1.5, including isolates with the FWA, FWB, FWC, FWF, FWH codes, and FWJ isolates with proteolytic indices in sequence 1.12, 1.06, 1.52, 1.01, and 1.52. FWD isolate is an isolate that has an isolate diameter of 23 and an apparent zone diameter formed of 75mm so that the resulting proteolytic index of 3.26 is the highest index of the 10 isolates followed by the isolate with the FWI code with a colony diameter

of 32mm and a clear zone formed of 92mm so that the proteolytic index formed is 2.83 with the criteria of perfect clarity in degrading skim milk as an indicator of food degradation from protein.

d. Lipolytic enzyme production test

Lipid-degrading bacteria play an important role in the process of decomposing food waste. Lipids are one of the main components in food, and they are found in oil, fat, and fatty food waste. The following are the results of the lipid-degrading ability test can be see in Figure 4.

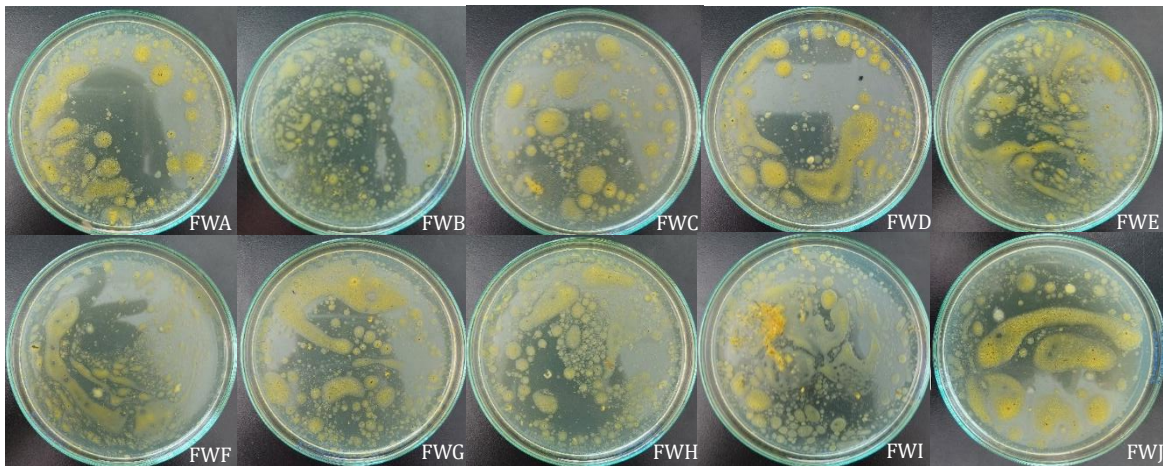


Figure 4. Results of lipid degrading tests on isolates from food waste. Positive results from the lipid degradation test can be identified based on the presence of a yellow media section that is reduced because it is used by bacteria as nutrition.

From Figure 4., it can be seen that CPO cannot thoroughly blend with the agar medium, which is indicated by the appearance of yellowish spots on the petri dish, which are difficult to blend even after being vortexed (Murtiyarningsih et al., 2017). However, the poured agar media has a shiny color, a typical characteristic of the fat content in the agar media. After being isolated, the bacteria could grow, and after 48 hours, a clear zone could be formed, which, if observed, would continue to expand. The diameter of the clear zone and the diameter of the colony were then measured using a caliper. The measurement results were calculated using a formula to determine the lipolytic index. (Elyza et al., 2016). Measurement of the lipolytic index of the isolate can be done using the following formula:

$$\text{lipolytic index} = (\text{Clear Zone Diameter} - \text{Colony Diameter}) / (\text{Colony Diameter})$$

There are three isolates with codes FWB, FWG, and FWH that successfully grew on the prepared media, but after 48 hours, the isolate did not experience any colony growth at all. This means that even though it has successfully grown to a diameter of 11mm, this isolate then stops growing and does not produce a clear zone at all. There are four isolates with codes FWA, FWC, FWD, and FWF that grow and produce a clear zone even though it is pretty thin. The clear zone formed has the smallest FWD index with a lipolytic index of 1.04. The most extensive lipolytic index with a thin scale is produced by the FWF isolate of 1.15. The isolate with the FWI code has a relatively sizeable lipolytic index, namely the FWI isolate of 1.62. The isolate with the FWJ code has the most extensive lipolytic index

with an isolate diameter of 23mm and a clear zone produced of 78mm, so the lipolytic index formed is 3.3mm with the category of a perfect clear isolate in degrading lipids in the growth media.

e. Amylolytic enzyme production test

Starch is a type of complex carbohydrate found in many foods such as rice, potatoes, bread, and pasta.

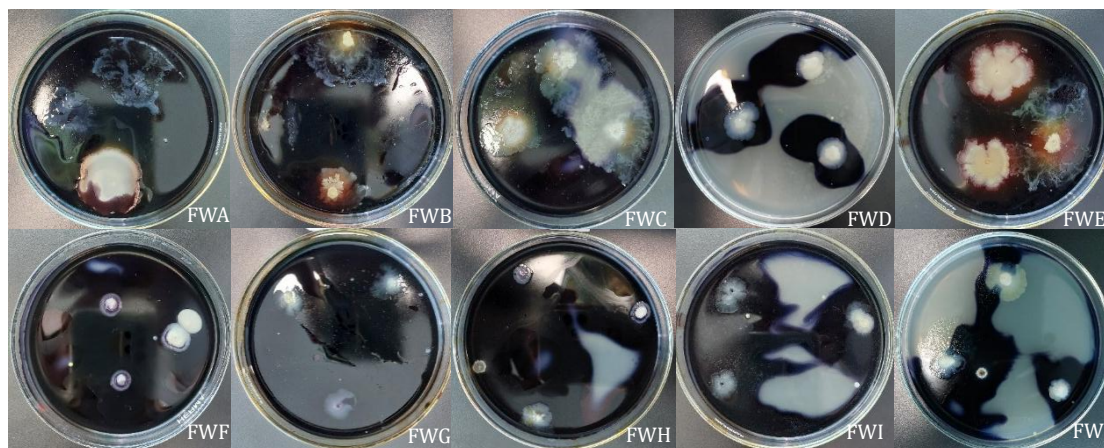


Figure 5. Results of the starch degradation test isolated from waste. Positive results of the starch degradation test can be seen from the white zone in the bacterial inoculation. The part that does not contain bacteria will remain black.

The amyolytic bacteria index is a parameter used to measure the activity of microorganisms in degrading or digesting starch levels in food waste or other organic substrates. Amylolytic bacteria are microorganisms that have the ability to decompose or digest starch, which is included in food waste. The amyolytic bacteria index refers to the level of bacterial activity in decomposing starch in food waste. (Zulaika & Khofifah, 2021). Based on Table 1 below, it can be seen that the results of the amylase enzyme production test in breaking down the starch content from the soluble starch media as an indicator of degrading food waste containing sources of ingredients from foods containing high carbohydrates such as rice, potatoes, noodles and also bread. Isolates with the FWE code did not grow on the prepared media, namely agar media that had been mixed with soluble starch media sources. It is possible that the FWE isolate does not have the ability to metabolize starch, so this isolate cannot survive. (Triza et al., 2021). The resulting clear zone is then measured using the amyolytic index formula, and a comparative table is obtained between other food waste isolates as in the following table:

$$\text{amyolytic index} = (\text{Clear Zone Diameter} - \text{Colony Diameter}) / (\text{Colony Diameter})$$

There were three isolates with codes FWB, FWD, and FWG that successfully grew on the prepared media, but after 48 hours, the isolates did not experience significant apparent zone growth, with a skinny clear zone category of 0.18mm, 0.27mm, and 0.06mm respectively. There were three isolates with codes FWA, FWC, and FWF that grew and produced clear zones with thin categories of 0.34mm, 0.47mm, and 0.27mm, respectively. The clear zone formed had the smallest index, namely FWG, with a lipolytic index of 0.06mm. The lipolytic index with a perfect clear category was three isolates, namely FWH, FWI, and FWJ, with amyolytic indices of 1.16, 1.2, and 1.13, respectively.

The isolate with the FWI code had the largest lipolytic index of 1.2mm. With a perfect clear isolate category for degrading lipids in the growth media.

Table 1. Comparison of Observations of Food Waste Degradation Tests

No	Isolate Code	Cellulolytic Index	Proteolytic index	Lipolytic index	Amylolytic Index	Criteria
1	FWA	0.41	1.12	1.11	0.34	Weak
2	FWB	0.18	1.06	1	0.18	Weak
3	FWC	0.50	1.52	1.06	0.47	Weak
4	FWD	0.41	3.26	1.04	0.27	Potential
5	FWE	0	-	-	-	not survive
6	FWF	0.33	1.01	1.15	0.27	Weak
7	FWG	0.06	0	1	0.06	Weak
8	FWH	1.16	1.52	1	1.16	Potential
9	FWI	1.5	2.83	1.62	1.2	Potential
10	FWJ	1.5	1.21	3.3	1.13	Potential

Based on Table 1, several bacterial isolates show high potential in degrading food waste based on their enzymatic activity tests. Isolates FWD, FWI, and FWJ are categorized as potential bacteria because they have relatively high enzyme indices compared to other isolates. Isolate FWD showed very high proteolytic activity (3.26), although other enzyme indices were not too prominent. Meanwhile, isolates FWI and FWJ had higher cellulolytic indices than other isolates, each 1.5, with proteolytic and lipolytic indices that were also significant, indicating a more comprehensive degradation ability. In addition, the amylytic index in these two isolates was also higher than the others, indicating their ability to decompose starch. Thus, isolates FWI and FWJ are the best candidates as biological agents in the food waste degradation process because of their broad enzymatic activity. Determination of the isolate that has the best potential is based on the width of the clear zone produced in each test. Based on research (Arifin et al., 2019), the wider the clear zone, the more productive the bacteria are in producing degrading enzymes used to decompose food waste.

Conclusion

Based on a series of activities in the research program, it was concluded that the results of the isolation of indigenous bacteria from food waste were obtained from as many as 10 isolates. After the screening process was carried out to determine the ability of the isolates to produce degrading enzymes, four potential isolates were obtained, including isolates FWD, FWH, FWI, and FWJ. Isolates FWD, FWH, FWI, and FWJ had the most significant ability to degrade cellulose, protein, fat, and starch with the largest cellulolytic index of 1.5, the most extensive proteolytic index of 3.3, the largest lipolytic index of 3.3, and the most extensive amylytic index of 1.2.

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