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Research Article
Utilization of Local Microorganisms as Bioactivators to Produce Organic Fertilizers and Analysis of Molecular Bacterial Diversity

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Abstract

Background and Objective: Local micro organism (LMO) is the result of the fermentation of various mixtures of organic matter. One of the organic materials used, based on the local wisdom of West Sumatra, is tapai (fermented Cassava), which is used as a bio activator in the manufacture of organic fertilizer. The research aims to produce organic fertilizers that meet national quality standards in terms of the physical and chemical quality of fertilizers as well as to determine the diversity of bacteria in bio activators through next-generation sequencing analysis. **Materials and Methods:** The organic ingredients for bio activators, cow feces as basic fertilizer ingredients, materials for analyzing bacterial diversity, LMO gDNA was extracted using ZymoBIOMICS DNA Miniprep Kit DNA and sequenced using Oxford Nanopore Technology. **Results:** On a scale of 1-3, the physical quality of organic fertilizers had an average value of 2.58 for smell, 2.83 for texture and 2.58 for color. The chemical quality of organic fertilizers is C-organic (23.56%), nitrogen (1.60%), carbon and nitrogen ratio (14.75%), phosphate (0.47%) and potassium (0.64%). The results of the analysis of bacteria on the bioactivator consisted of 7 phyla, 9 families, 45 genres and 297 species. The most common species is *Lentilactobacillus hilgardii* (62%). **Conclusion:** The organic fertilizer produced using the mole tapai bio activator complies with Indonesian national standard 19-7030-2004 based on physical and chemical parameters. The type of bacteria that dominates the bioactivator is the lactic acid bacteria group, which reaches 90%.

Key words: Bioactivation, biofertilizers, microorganisms, *Lentilactobacillus*, cassava

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bioactivator is a material that contains microorganisms that can work effectively and actively in the process of decomposing organic matter into organic fertilizer[1]. One of the activators that can be used is local microorganisms (LMO)[2]. The LMO solution is the result of fermentation from various materials available in the surrounding environment and based on the local wisdom of West Sumatra. This solution contains microorganisms that can decompose organic matter and stimulate plant growth[3]. Local microorganisms (LMO) are used as starters in the manufacture of organic fertilizers. One source of LMO that can be used is 'Tapai' (fermented cassava, a traditional food typical of West Sumatra). The taste is sweet, sour and slightly alcoholic. The sweet taste comes from the breakdown of carbohydrates into simple sugars, while the sour taste is due to the formation of organic acids by enzymes produced by a mixture of microorganisms. The smell of alcohol arises because the fermentation takes place anaerobically[4].

This study used tapai and other organic mixtures as a source of LMO for bio activators. It naturally takes a long time to create organic fertilizer and the fertilizer's quality isn't the best. Therefore, a bio activator must be added to accelerate the composting process and create high-quality organic fertilizer. Livestock waste is one of the organic materials that can be used as a raw substance to make organic fertilizer. The quantity of waste produced, such as cow dung, also rises as the livestock industry grows. Cow dung can pollute the ecosystem if it is not properly managed and used[5]. One of the ways to improve use value is to turn leftover cow manure into organic fertilizer.

This study aimed to analyze the diversity of bacteria in the bioactivation molecularly using the Next Generation Sequencing (NGS) technique and to analyze the quality of organic fertilizers based on physical and chemical parameters.

MATERIALS AND METHODS

Study area: The research was carried out at the Microbiology Research Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University and Genetic Science Laboratory, Tangerang. The research was carried out from July, 2022 to December, 2022.

Materials and Methods: The LMO fermented organic matter, cow manure and DNA from MOL samples were extracted using a DNA extraction kit ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). The DNA concentration was determined using both NanoDrop spectrophotometers and Qubit fluorometers, in Padang and Jakarta City, Indonesia.

Library preparations were conducted using Kits from Oxford Nanopore Technology and used as a template for 16S rRNA amplification. The V1-V9 hypervariable region of the 16S rRNA gene was amplified using next-generation sequencing (NGS) techniques. The fertilizer produced was analyzed for the physical environment (temperature, pH and humidity), the physical fertilizer (color, smell and texture) and the chemical environment (C-organic, nitrogen, C/N ratio, phosphate and potassium of organic fertilizers).

Fermentation of organic matter as a source of LMO for bio activators: The organic materials used are derived from the surrounding environment, fermented cassava which is combined with other nutritional sources such as bean sprouts, tempeh, washed rice water, crushed tofu and sugar. Homogenize all mixtures and ferment for a week[6].

Extraction of Genome DNA: Pallets were gathered after centrifuging a total of 50 mL of local microorganism (LMO) tapai samples at 3000 × g for 5 min. The CTAB/SDS technique[7] was used to recover samples' entire genomes of DNA. On 1% agarose gels, DNA quantity and purity were examined. The DNA was diluted in sterile water to 1 ng L^{-1} depending on the quantity.

Amplicon generation: The separate 16S rRNA/18S rRNA/ITS genes of regions 16S V1-V9 were amplified using designated 16S (27F-1492R) primers. Phusion® High-Fidelity PCR Master Mix was used for all PCR processes (New England Biolabs).

PCR Products quantification and qualification: Mix the PCR products with the same amount of 1X loading buffer, which contains SYBR green and run electrophoresis on a 2% agarose gel for detection. For additional experiments, samples with a bright main strip between 400 and 450 bp were selected.

PCR products mixing and purification: Equidensity ratios were used to combine the PCR results. Following that, PCR products were mixed and filtered using the Qiagen Gel Extraction Kit (Qiagen, Germany). The HiSeq2500 PE250 would examine the libraries made with the TruSeq® DNA PCR-Free Sample Preparation Kit PCR products were mixed in equidensity ratios. Then, the mixture of PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany) and quantified with Qubit and Q-PCR.

Organic fertilizer production using bio-activators LMO: The bioactivator is inoculated into the digester containing cow dung at a ratio of 1:20[6,8]. Composting is done for a month. Mixing is done every two days. Physical factors (temperature, pH and humidity) were measured at the beginning and end of the composting process. Chemical analysis of the quality of organic fertilizers include, carbon, nitrogen, the C/N ratio, phosphate and potassium. Physical analysis of organic fertilizers includes colour, taste and texture[9]. Statistical analysis using the experimental method with purposive sampling.

RESULTS AND DISCUSSION

Amplification of gDNA with primer 16S (27F-1492R): The 16S rRNA gene in the bioactivation sample from LMO tapai was amplified using primers 27F and 1492R.

On a 2% agarose gel, the fluorescent DNA rings were visible in Fig. 1 at 1000 and 3000 base pairs. This showed that the sample's 16S rRNA gene has been amplified effectively using the 1500 bp primers 27F and 1492R.

Diversity of the bacterial community bio activator LMO: The total sequence of bacteria in the mole tapai bio activator was obtained as many as 102.378.047.

The bio activator has a variety of bacteria, according to a study of alpha diversity. The category of moderate species diversity falls under the Shannon diversity index, which has a value of 2.3 and was higher than 1 and less than 3[10].

Bacterial phylum taxa on bioactivator LMO: A phylogenetic phylum analysis of bio activators. The Firmicutes group comprised up to 98% of the most common phyla, followed by proteobacteria, 1.9%, deinococcus-thermus, 0.004%, actinobacteria, 0.002%, cyanobacteria, 0.001%, nitrospirae and bacteroidetes. Generally speaking, the family Firmicutes contains facultative aerobes or some anaerobes that are Gram-positive bacteria as shown in Fig. 2.

Members of the genus *Firmicutes* are typically chemoorganotrophic, though some are also photoheterotrophic[11]. Firmicutes is a phylum of bacteria that thrives at mesophilic temperatures, with a range of 25-37°C and a growth pH of 3-8, where the pH in bioactivators varies from 3.5-4.1 and is included in the *Firmicutes* phylum growth range[12]. Photosynthetic, heterotrophic or chemolithotrophic bacteria make up proteobacteria, the second-largest family after *Firmicutes*. According to the pH and temperature of the bio activator, the phylum proteobacteria can be found in environments with a pH of 3.5-8.2 and temperatures varying from 20-37°C. These bacteria are typically Gram-negative, may or may not have cilia and can have cells that are spherical, spiraled, rod-shaped, facultatively aerobic or anaerobic[11]. Actinobacteria are also pretty abundant along with proteobacteria Gram-positive bacteria known as actinobacteria can exist in both land and aquatic environments[13]. The actinobacteria group can thrive in a pH range of 6.5 to 82 and can survive at temperatures between 25 and 55°C[14]. Since plants rely on actinobacteria's input to the soil system, they are crucial to plants. These microbes aid in the breakdown of organic materials in the soil. As Plant Growth Promoting Rhizobacteria (PGPR), several bacteria from the phyla *Firmicutes*, proteobacteria and actinobacteria can bind free nitrogen, create phytohormones like Indole acetic acid (IAA), gibberellins and ethylene and produce siderophores, chitinase and cellulase enzymes. The pH of the activator showed a decreasing trend of 3.4-5.2 during the fermentation process. The decrease in pH in the stationary phase where in this phase there is a process of formation of secondary metabolites in the form of lactic acid, resulting in an

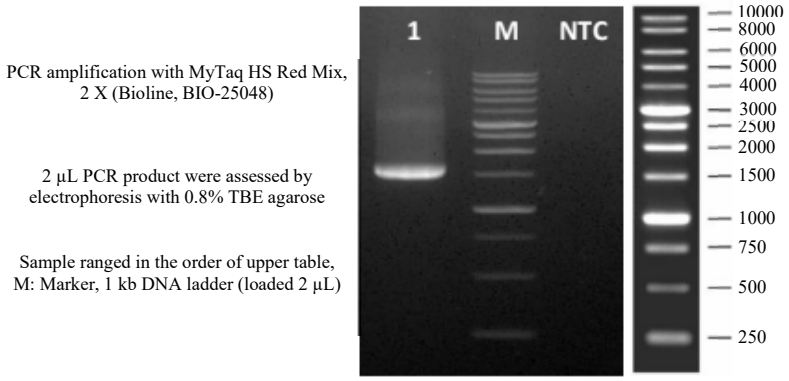

Fig. 1: Electrophoretic results of 16S rRNA gene amplification using primers 27F and 1492R on 2% agarose gel
M: Marker 1 kb ladder

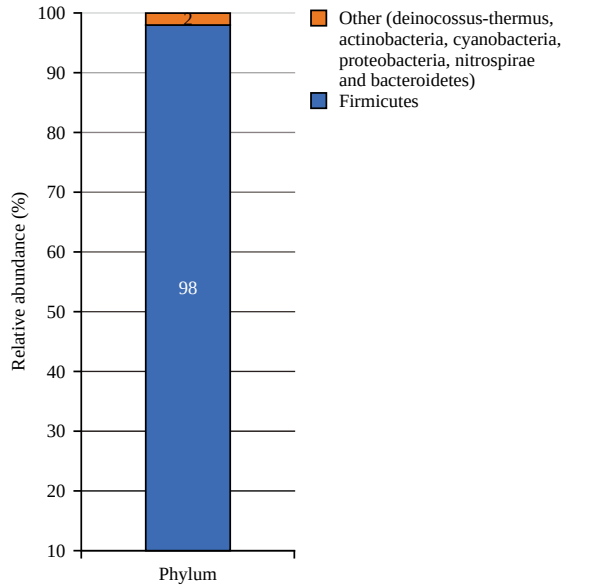

Fig. 2: Abundance of bioactivation bacterial phylum

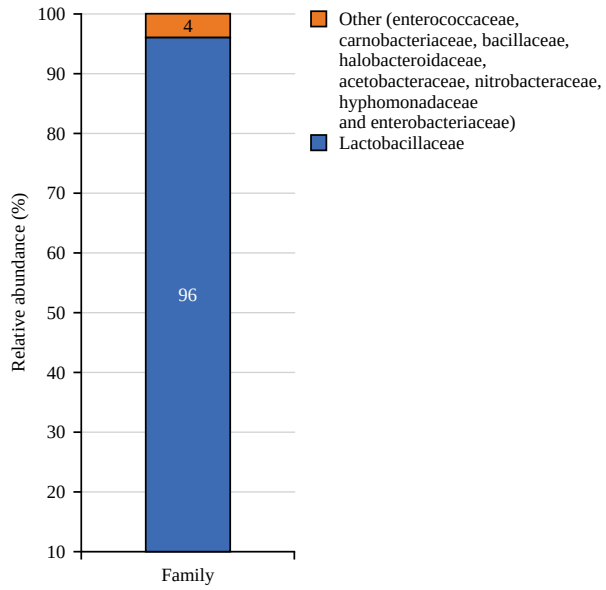

Fig. 3: Abundance of the bioactivator bacterial family

acidic pH. Acidity caused by the decomposition of organic matter by microbes will produce CO_2 gas. This CO_2 gas will form Carbonic acid (H_2CO_3) which easily decomposes into H^+ and HCO_3 ions. These H^+ ions will make the acidity of the LMO solution decrease (acidity increases)[15].

Bacterial family composition in bioactivation: The profusion of bacterial groups was presents in the bioactivator. With a 96% relative frequency, the Lactobacillaceae family is the most numerous, followed by the Bacillaceae (1.9%), Acetobacteraceae (1.8%), Enterococcaceae and Carnobacteriaceae (0.03%), Enterobacteriaceae (0.002%), Halobacteroidaceae, Nitrobacteraceae and Hyphomonadaceae (0.001%). A genus of lactic acid-producing bacteria is known as Lactobacillaceae as shown in Fig. 3.

The Lactobacillaceae family is homofermentative, belongs to the Gram-positive group, is mesophilic and cannot produce spores. It corresponds to the class of probiotic bacteria and the ideal environment for its growth is a pH of 5.5 and a temperature of 37°C, with lactic acid as the main byproduct of carbohydrate fermentation[16]. The family Bacillaceae contains facultative anaerobic and aerobic bacteria that are highly sensitive to environmental factors like temperature, pH and salinity and can ferment carbohydrates. Additionally, the genus of probiotic bacteria known as Bacillaceae is included[17]. A genus of acidic bacteria called acetobacteraceae can oxidize sugar and alcohol, most notably turning ethanol into acetic acid. Mesophyll bacteria, which thrive at temperatures between 25 and 400°C and have a pH range of 3-4.5, are members of this family. Typically, foods from this genus are fermented. The substrate's nutrient composition includes its water, protein, fat, carbohydrates and some elements. A Gram-negative, rod-shaped, strictly aerobic group makes up the entire family of acetic acid bacteria known as Acetobacteraceae found in environments that are sugary, sour and drunken[18].

Composition of the genus of bacteria in the bioactivator: The bioactivator contains bacteria from 45 different genera, with the six most abundant species being shown in Fig. 4. The top taxa are *Lacticaseibacillus* (34%), *Schleiferilactobacillus* (9%), *Lactobacillus* (6%), *Bacillus* (2%), *Acetobacter* (2%) and others (1%). *Lentilactobacillus* (46%), *Lacticaseibacillus* (34%), *Lactobacillus* (6%) and others (1%). A genus of bacteria known as *Lentilactobacillus* utilizes carbohydrates, proteins and fats as sources. It is a member of the lactic acid bacteria family. The optimal growth temperature for the *Lentilactobacillus* genus is 300°C and its growth pH varies from 3.5-7 depending on the pH of its substrate, bioactivator 2 (which ranges from 3.5 to 4.1)[19].

The *Lentilactobacillus* species can be found in a variety of fermented foods, including tapai, kefir and curd. Tapai is also used to make bioactivators. Gram-positive and heterofermentative bacteria are members of this family. A family of lactic acid bacteria called *Lacticaseibacillus* contains facultative anaerobic or microaerophilic bacteria that can withstand acid but do not produce spores[20]. Tapai mole is prepared using cassava yeast. Bacteria from the genera

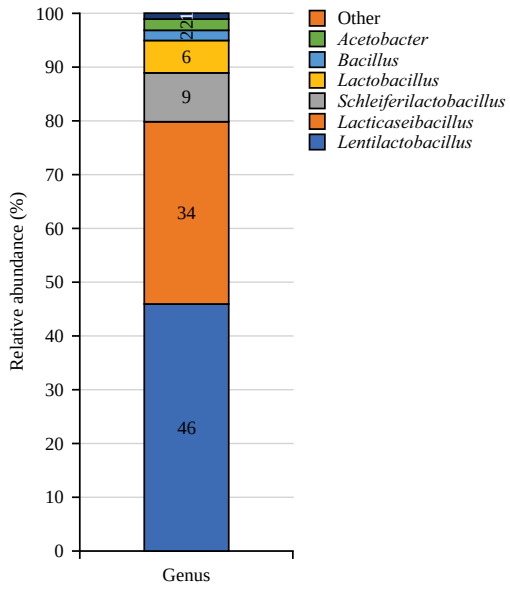

Fig. 4: Abundance of the genus of bioactivator bacteria

Pediococcus and *Bacillus* are frequently detected in cassava yeast. The genus *Pediococcus* and *Bacillus* is one of the bacteria with the lowest abundance, this was due to the composition of the yeast, which includes a small amount, making the genus have a low abundance[21].

Composition of bacterial types in bioactivator: Molecular analysis using the NGS method found 297 species in the bioactivator. The most dominant species can be seen in Fig. 5. Namely, *Lentilactobacillus hilgardii* as much as 62%, *Lacticaseibacillus paracasei* as much as 12%, *Schleiferilactobacillus perolens* as much as 7%, *Lentilactobacillus parafarraginis* 5%, *Lentilactobacillus farraginis* 2%, *Lactobacillus* 2% and other types 10%. Based on research by Manullang and dan Daryono[21], the microorganisms in snail moles, banana weevils and fruit consist of *Clavibacter*, *Agrobacterium*, *Clostridium* and *Pseudomonas fluorescens*, whereas, according to research on banana weevil moles, there are bacteria *Bacillus* sp. and *Enterobacter* sp.[22]. After analyzing samples of berenuk LMO solution, coconut water LMO solution and kitchen waste, it was found that the berenuk LMO solution contained *Bacillus* sp., *Saccharomyces* sp., *Azospirillum* sp. and *Azotobacter*. Species *Lentilactobacillus hilgardii*, *Lentilactobacillus parafarraginis* and *Lentilactobacillus farraginis* belong to t he genus *Lentilactobacillus*. *Lentilactobacillus hilgardii* is a Gram-positive bacterium that lives at a mesophilic temperature of 30-35°C. Tolerant of acids and ethanol content up to 20%. Optimal growth occurs at pH 4.5-5.5[23], this is by the pH of the activator being analyzed. Usually found in fermented drinks or foods that produce alcohol, such as tapai[23].

Lacticaseibacillus paracasei belongs to the genus *Lacticaseibacillus*. Such bacteria include those that are Gram-positive, rod-shaped, non-sporulating (non-spore-forming), non-motile and anaerobic and are often used to ferment foods such as cheese and yogurt[23]. Strains belonging to this species can be isolated from milk, dairy products and vegetables, but they are also present in the human reproductive and digestive tracts, where they are widely used as probiotics[24].

Schleiferilactobacillus perolens is heterofermentative, rod-shaped and thrives best at mesophilic temperatures. Typically present in alcoholic fermented drinks and fermented vegetables[25].

Number of bacterial groups from domain to species (Sankey diagram): Bacterial groups from the domain level to the species level as shown in Fig. 6.

A total of 68,000 bacteria, spread among 66,700 phyla, with phylum Firmicutes dominating the list were displayed in Fig. 6. There are 65,200 members of the family Lactobacillaceae and there are 30,900 members of the *Lentilactobacillus*. With 25,100 and 16,100, respectively, *Lentilactobacillus hilgardii* and *Lacticaseibacillus paracasei* topped the species level.

Physical analysis of organic fertilizer (temperature, pH and humidity): Physical analysis of the organic fertilizer (temperature, pH and humidity) can be seen in Table 1.

According to the national standard for Indonesia, which varies from 28 to 30°C, Table 2 organic fertilizer temperature after composting was 30.33°C. The weather has stabilized at this point. Reduced organic matter that can be broken down by microbes causes the temperature to start to fall, a sign that the compost is beginning to mature. The temperature can be used to determine when organic nutrients are mature. The action of microorganisms that break down organic matter into organic fertilizers can be detected by temperature. The ultimate composting temperature is in the range of 28-300°C, according to research[26].

After composting, the pH of organic fertilizer varies from 6.86 to 7.49, which was within the range of the national standard for Indonesia, 6.80 to 7.00. In the first week of composting, the pH value drops as microorganism's labor to degrade organic matter. The creation of simple organic acids causes the pH of the compost to become acidic. Organic waste is broken down into organic acids by a variety of microorganisms during the composting process. The CO_2 gas

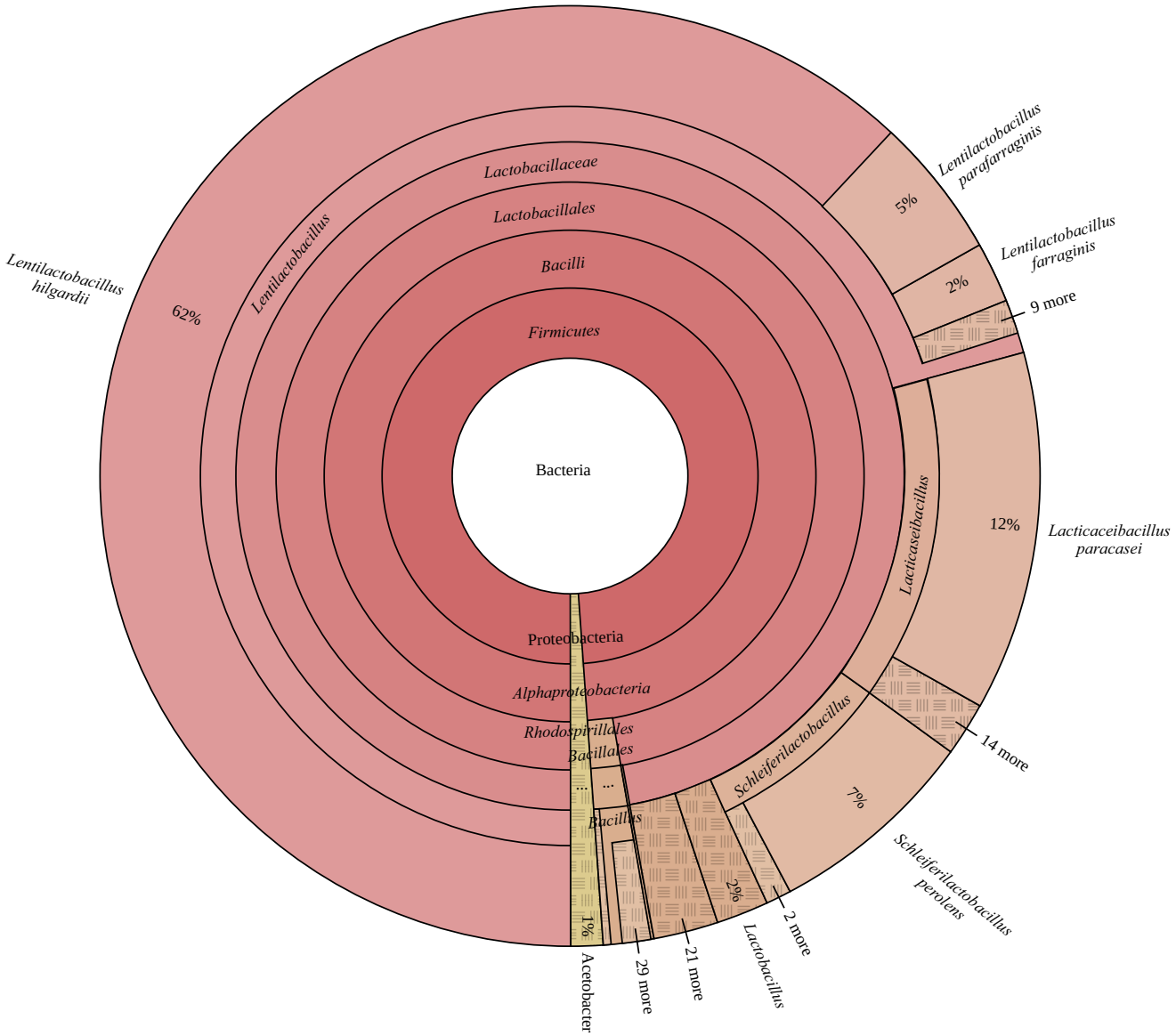

Fig. 5: Abundance of bioactivator bacterial communities

Table 1: Value of temperature, pH and humidity of organic fertilizers

Parameter	Value (%)	SNI 19-7030-2004
Temperature (°C)	30.33	28-30°C
pH	6.86	6.80-7.49
Humidity (%)	30.43	<50%

Table 2: Value of chemical content of organic fertilizers

Parameter	Value (%)	SNI No. 19-7030-2004
C-organic	23.56	9.8-32%
Nitrogen	1.60	>0.40%
C/N	14.75	10-20
Phosphate	0.47	>0.10%
Potassium contents	0.64	>0.20%

SNI: Indonesian National Standard

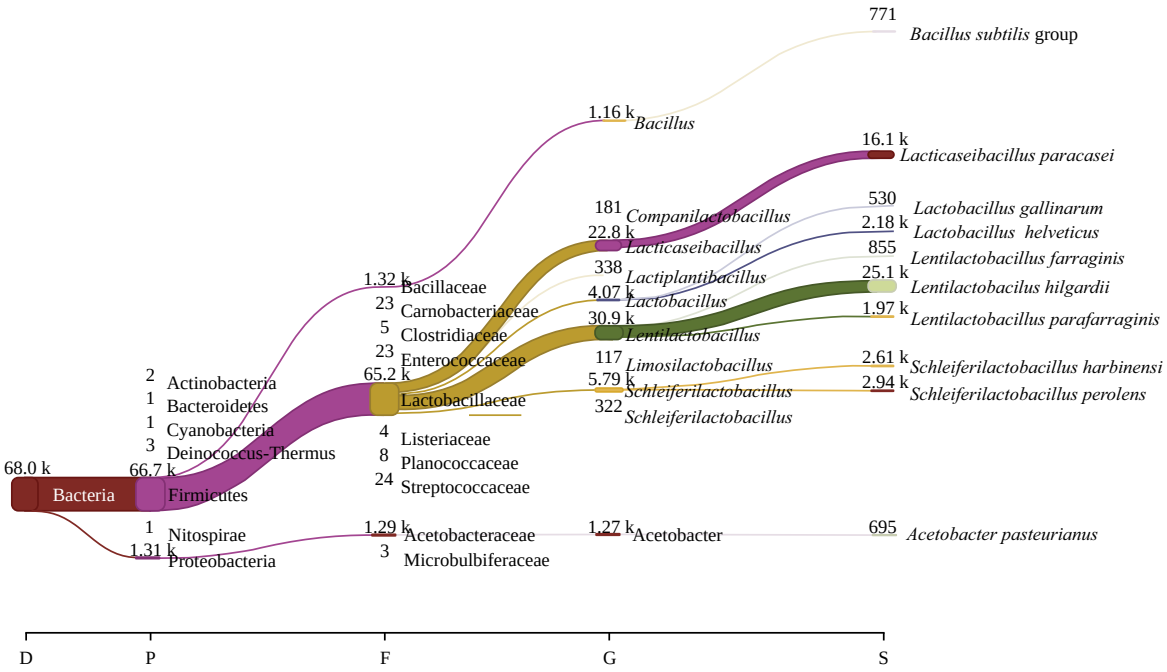

Fig. 6: Flow of dominating bacterial groups from domain to species

will also be produced due to the acidity brought on by microbial activity in decaying organic materials. This CO_2 gas will eventually turn into Carbonic acid (H_2CO_3), which breaks down into H^+ and HCO^-_3 ions with ease. Because of the impact of these H^+ ions on acidity, organic nutrients' pH will drop (and their acidity will rise)[15]. According to research by Agustina and Sriharti[27], the pH varies from 6.3 to 7.8 after composting.

The humidity level is 30.43%, which is within the acceptable range of the 50% national norm for Indonesia. During the composting process, the compost's moisture content dropped. The humidity value ranged from 20 to 30% at the end of the third week of composting after starting between 60 and 70%, declining in the first week and remaining consistent. The water concentration in the compost material evaporates as a result of heat, stirring and the consumption of microbes that transform protein into nutrients required by plants during aerobic composting[28]. According to a study[29], organic fertilizers have final moisture values ranging from 65.6%.

Analyzing organic fertilizer physically (color, smell and texture): Analysis of the organic fertilizer's color, scent and texture following SNI 19-7030-2004, which specifies that the organic fertilizer is a crumbs-like substance with a blackish-brown color and a smell similar to that of soil. Examining organic manure physically (Fig. 7). The hedonic test has a value range of 1 to 3 and the results indicated that the average value for smell, texture and color were 2, 5 and 2, respectively.

According to physical assessments of the grade of organic fertilizers, a starter made from tapai mole has an organic fertilizer texture that is fine or crumbly. This was a result of the Lactobacillaceae family of microbes breaking down organic compounds like cellulose, hemicellulose and lignin, which are components of organic matter. The fertilizer will have a smooth texture if these organic substances are broken down. The nitrifying bacteria in the bioactivation, which can decompose ammonia into nitrate and produce nitrogen that can be absorbed by plants, are what give compost its oily smell after the addition of mole tapai[30].

Results of chemical analysis of organic fertilizers (C-organic, nitrogen, C/N ratio, phosphate and potassium): Based on the chemical analysis of organic fertilizers with fermented cassava added, the findings meet Indonesian National Standard (SNI) requirements and can be applied to the production of organic fertilizers. This claimed that adding microorganisms from fermented cassava produced chemical results that were superior to the control.

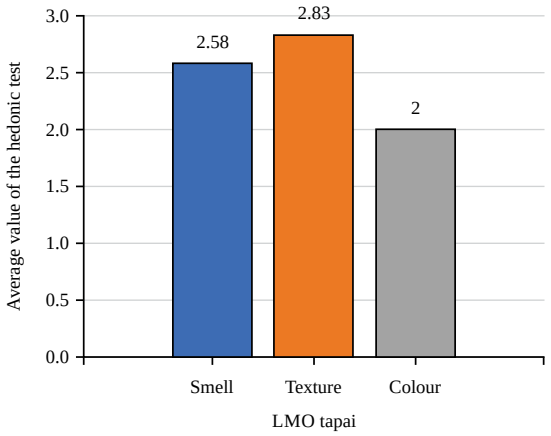

Fig. 7: Physical test of organic fertilizer

The end findings of the C-organic analysis on the five treatments with organic fertilizer was mentioned in Table 2. Its value of 23.56% was within the range of 9.8-32%, which is the national norm for Indonesia. Microorganisms require carbon as an energy source, so the carbon source in organic matter will continue to be used by microbes, resulting in a decline in this carbon source during composting[31]. According to research[29], the final C-organic content of compost was in the region of 29.58%. The final nitrogen content of organic fertilizer is 1.60%, which is in line with the >40 national normal for Indonesia. This was a result of a combination of fundamental ingredients that are rich in nitrogen, particularly cow manure, which contains more nitrogen than other types of livestock manure. In the compost pile and during the decomposition process, element N is often kept. According to a study[32], nitrogen content varies between 13.11 and 17.19%. The ultimate C/N ratio value for organic fertilizers comes out to be between 14.75 and 15. According to SNI 19-7030-2004, the C/N ratio was 10-20. The pace of decomposition and nitrogen mineralization are both influenced by the C/N ratio. The abundance of carbon and nitrogen is what causes organic matter to decay, the C/N ratio is used to measure this degradation as well as to determine the age and composition of compost. The value of the C/N ratio was about 9.21, per study[29].

The ultimate phosphate value of organic fertilizer is 0.47%, exceeding the >0.10% threshold set by SNI 19-7030-2004. The large quantity of phosphorus present in the raw materials used and the large number of microorganisms engaged in the composting process account for the compost's high or low P-total content. The weathering of composted organic waste is what causes the element P's high concentration. The finished potassium content of organic fertilizer was 0.64%, which is greater than 0.20% and with SNI 19-7030-2004. The phosphate value after composting ranges from 1.04 to 1.84%, according to the study by Cáceres *et al.*[31]. Because potassium serves as a catalyst for microorganisms during the composting process, the potassium content tends to rise, the existence of bacteria and the activity of bacteria will influence the rise in potassium content[27]. The value of potassium is 1.68-1.73%, per study[25]. The implication of this study was to provide information on bioactivator formulations from LMO cassava fermented to produce organic fertilizer of the best quality so that it can be used by farmers to produce good agricultural results. The application of the results of this study is to try directly with vegetable crops to increase the yield of vegetables good, the recommendations from this study are good for annual plants and you can use this LMO for other organic waste and the limitations of this research are research funds and time needed to analyze molecularly.

Future recommendations suggestions for further research are to do experiments on laboratory scale plants.

CONCLUSION

The number of phyla in the mole tapai bio activator was 7, with the highest number being phylum Firmicutes (98%), the number of families was 9, with the highest number being the Lactobacillaceae family (96%), the number of genera obtained was 45, with the highest number being *Lentilactobacillus* (46%) and the number of species obtained was 298 with the highest type, namely *Lentilactobacillus hilgardii*, at 62%. According to SNI 19-7030-2004, the physical characteristics of the environment (temperature, pH and humidity), the physical characteristics of the produced organic fertilizer (black-brown colour, earthy scent and fine texture) and the chemical characteristics of organic fertilizers all affect the quality of the fertilizer.

SIGNIFICANCE STATEMENT

This research was carried out because the use of inorganic fertilizers was increasing which resulted in damage to soil structure, it was necessary to substitute organic fertilizers, organic fertilizers needed bio activators to produce good quality fertilizers, therefore, the manufacture of bioactivation in this study could be used to produce good quality organic fertilizers that the basic ingredients come from the surrounding environment.

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