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Identification of Bacterial Diversity in the Magot Digestive System based on 16S rRNA Gene Analysis

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Abstract:- The use of feed ingredients has not been addressed until now, in the sense that competition between food and feed is still continuing, especially protein source feed, thus causing a dilemma for farmers.Therefore, in connection with the steps that will be taken in efforts to health livestock and fish farming that will be carried out, efforts are needed in preventing bacterial-based diseases. The purpose of this study was to determine the diversity of all bacteria, both pathogenic and non-pathogenic bacteria found in the magot digestive system using 16S rRNA gene analysis. The results of this study will be a scientific reference for the development of maggot asasafe, effective, and efficient alternative to animal feed.Based on the results of the studies obtained, it can be concluded that the bacteria contained in the digestion of maggots show a diversity of pathogenic and non-pathogenic bacteria, with the form of bacil and cocobacil. Inaddition, DNA extraction and ba cterial identification have also been carried out using the PCR method. The results of the sequencing of the seven bacterial isolates of magot digestive origin showed gooddendograms and nucleotide sequences, so the next stage was bioinformatics analysis and phylogenetic construction, to identify species and their kinship relationships between species.

Keywords:- *Maggots, PCR, Larvae, Insecta, Animal Feed.*

I. INTRODUCTION

Indonesia is a tropical country that has a diversity of biological food stuffs and a geographical location that can make it easier for every Indonesian to meet food needs (Fatmasari, 2017). Fruits, vegetables and geographical locations are used as a source of livelihood for the community, besides that they are also often used as feed as protein fulfillment for livestock. The use of feed ingredients has not been addressed until now, the competition between food and feed still continues, especially protein source feed, causing a dilemma for farmers (Djissou *et al*.,2016;Ngatung,*etal*.,2017). But along with the increase in animal feed prices, farmers need other solutions to reduce feed costs, one of which is by replacing purchased feed with home made feed. One of the suggested and widely reported feeds is the use of insecta as a source of protein (Wang *et al*., 2005; Oyegoke *et al.*, 2006; Premalatha *et al*., 2011; Wardhana, 2016). According to Bosch *et al.*(2014), *Black Soldier Fly* (BSF) larval insecta has a high protein content of 40-50% and fat ranges from 29-32%.

Black Soldier Fly (BSF) which has the Latin name *Hermetia illuciens L*, including a relative of flies (FamilyDiptera), its body resemble sawasp, is black incolor and has a length of 15-20 mm (Afkar *et al*., 2020). Maggot *H. illuciens* is one ofthe larvae that can be used as a substitute for feed in meeting protein needs, while another benefit of maggot is as an agent to decompose organic waste and as an additional feed for fish. Indarmawan (2014) stated that maggots also contain antifungi and antimicrobials so that when consumed by livestock or fish, they will form immunity to diseases caused by bacteria and fungi. This statement issupported by Amandanisa and Suryadarma (2020) who stated that magot is one of the fly larvae that has a high protein content of up to 30-45%, therefore magot can be used as an animal feed ingredient.

Asone of the sources of animal feed raw materials, insecta-based feed must also besafe from bacterial, chemical, fungal contaminants and soon. Mulyasim (2019) analyzed the safety and feasibility of insecta as a source of feed for livestock, including conventional BSF (*Hermetiaillucens*) larvae. The results of the study stated that the TPC test obtained the number of colonies that grew beyond the maximum limit of SNI microbial contamination in the feed, but it was not yet known what type of bacterial colony was produced. In addition topathogenic bacteria, in the maggot digestive system there are also reported to be several beneficial bacteria such as the Wardhana report (2016) which states that BSF larvae have antibacterial properties (*Escherichia coli* O15:H7, *Salmonella enterica* serovar Enteritidis) and antivirals. Some previous studies have also reported that bacterial identification is one of the first steps to determine the strains of pathogenic bacteria isolated from magot.

Identification is generally conventionally enforced biochemically and physiologically (Gulaydin *et al.* , 2019) namely by isolating organisms and studying them phenotypically through Gram staining and culture (Suardana, 2014), but conventional methods have many short comings and along with the development of science, methods in a molecular-based way have proven to be very accurate and can be used as a reference in knowing the potential of a bacterium from its gene sequence (Suardana, 2014;Osman *et al.*,

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2020;Masykur, 2021). The *Polymerase Chain Reaction* (PCR) method is a method of DNA amplification by *in vitro* (Yusuf, 2010). A widely recommended molecular-based bacterial identification method is genotype analysis using 16SrRNA gene sequencing with PCR techniques. This method is often used to identify bacteria because its ideal base length is 1540 nucleotides (Madigan *et al.,* 1997). In connection with livestock health efforts and fish farming that will be carried out, efforts are needed in preventing bacterial-based diseases. The purpose of this study was to determine the diversity of all bacteria, both pathogenic and non-pathogenic bacteria found in the magot digestive system using 16S rRNA gene analysis. The results of this study will be a scientific reference for the development of maggot as a safe, effective, and efficient alternative to animal feed.

II. MATERIALS AND METHOD

A. Isolation and Purification of Bacteria from the Magot Digestive System

A total of 25 g of magot samples that had been sterilized 17y stem 17n17 and had been finely refined were then put in sterile aqueducts and carried out radiant dilution from 10-1 to 10-6. Theampel was then grown on 0.1 ml of NA solid media with two repeats using 17 a spreading saucer stem and sub merged at a temperature of 37C° for 24 hours. After obtaining several different types of colonies, then proceed with purification or purification 17of ystem 17 using the quadrant scratch cup technique. The sample dishwas then subjugated in an inverted position at a temperature of 37 □C for 24 hours.

B. Purification of Bacteria of Magot Origin

The isolated acts that have grow non NA media form a single culture of bacteria or have been pure, then Gram staining is carried out using the bacterial Gram staining procedure, to determine gram positive or negative. Pure isolates are used for subsequent tests, then isolates are also stored in test tubes containing na-tilted media and stored at 4°C. Bacteria are smeared on the glass of the object and fixed on the fire. The spread of bacteria is inundated with violet crystals for 1 minute and inundated with17 ystem17 with lugol for 1 minute. The smear is washed with 95% 17 ystem 17 for 30 seconds and rinsed with running water. Smear the bacteria are inundated with sapphire dye for 30 seconds so that the dye dissolves, then rinsed using running water. The spread of bacteria is dried using filter paper, then dripped with imersi oil and observed under a microscope with magnification 1000x.

C. Isolation of Bacterial

DNA in the Magot Digestive System Bacterial isolates that have been obtained from the results of isolation, then prepared by growing in NB media. Total extraction of the bacterial DNA genome using *the Presto™ Mini gDNA Bacteria Kit (Geneaid),* which was modified. A total of 1.5 mL of bacterial culture culture was added to the lysis solution and heated for five minutes. Aliquot from the lysate is used for the next stage. During the incubation time, the tube is turned over once every 2-3 minutes. Furthermore, the lysis stage is to add 200 µL 17ystem GB into the micro tube and shake for 5 seconds. The sample solution and the elusion 17 ystem were then incubated simultaneously at 70 °C for 10 min. During incubation, the tube is shaken every3 minutes.

Furthermore, the DNA binding stage, namely the sample solution, is added 200 µL of absolute ethanol and shaken until a precipitate is visible on the microtube. Next the sample is transferred into a GD column that has been paired with a collective tube, then centrifuged using a centrifugator (*Mini spin*, Eppendorf) for 2 minutes at a speed of 10000 rpm. The centrifugation result supernatant on the collective tube is discharged. The DNA isolation process is continued with the washing stage, where the sample solution is added 400 µL 17ystem W1, and inserted into the GD column, and centrifuged for 2 minutes at a speed of 13000 rpm. The supernatant contained in the collective tube is disposed of. Next17 the ystem was added 600 µL of purifying 17 ystem and centrifuged for 5 minutes at a speed of 13000 rpm, then centrifuged 17 ystem17 for 3 minutes. The DNA elusion stage begins with a GD column containing17 the ystem paired with a sterile micro-tube and a 50 µL 17 ystem elusion is added to the column matrix, then allowed to stand for 15 minutes. Next the solution is centrifuged for 2 minutes at a speed of 12000 rpm. The last stage is the result of electrophoresis DNA isolation on 1% 17 ystem gel 17 containing *fluoro save DNA stain* for 45 minutes at 80 V with the ratio of samples inserted into electrophoresis wells (1 µL *loading dye*: 5 µL samples). 17 Ystem gels 17 were viewed above *transilluminator* UV light exposure, and documented using *Geldoc* 1000 (BIORAD). The success of DNA isolation is characterized by the presence of a thick and intact (non-fragmented) DNA band.

D. Identification of Bacteria in the Magot Digestive System Using Polymerase Chain Reaction (PCR)

Bacterial isolates will be identified based on specific primary sequences ofthe 16S rRNA encoding gene. To amplifythe gene, a pair ofprimers specifically designed to amplify the 16SrRNA gene was used. The primers are 63*f* (5'-CAGGCCTAACACATGCAAGTC- 3') and 1387*r* (5'-GGGCGGWGTGTACAAGGC-3') (Marchesi *et* al.1998).

The PCR reaction (25 µL) contained a PCR mix of 12.5 µL, a primer of 63*f* 1 µL, a primer of 1387*r* 1 µL, and a genome DNA of 3 µL, and ddH_2O 7.5 □L. PCR conditions used were 1 pre- denaturation cycle (94 ºC, 5 min), denaturation at (94 ^{oO} C, 1 minute), *annealing* (55 ºC, 1 minute), (72ºC,1minute),and*post*-elongation (72ºC, 10 minutes). The PCR process is carried out as many as 30 cycles.

E. Specific 16S rRNA Gene Sequencing, Bioinformatics Analysis, and Phylogenetic Tree Construction

DNA sequencing was carried out at the sequencing service company *First Base Co.* , Malaysia, in accordance with18 the ystem18n standards of DNA sequencers (ABI PRISM 3100).Nucleotide sequence results compared to *The Gen Bank database* through the *Basic Local Alignment Search Tool Nucleotide* (BLAST. N) contained in NCBI (http://www.ncbi.nlm.nih.gov). For nucleotide alignment and phylogenetic tree construction of the 16SrRNA gene with MEGAX software (Tamura *et al.* 2011) based on *neighbor-*

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joining tree (NJT) (Saitou and Nei 1987), with *a bootstrap* value of1000x.

F. Data Analysis

Data analysis was carried out descriptively, namely identifying the diversity of bacteria inthe18 magot digestive system based on molecular analysis and knowing the relationship between bacterial kinship based on the phylogenetictree, a swell as identifying the types of beneficial bacteria and system bacteria in the magot digestive stem.

III. RESULTS AND DISCUSSION

A. Magot in Banda Aceh Sample Collection

Magot has been successfully bred for approximately two months on fecal waste media and organic waste. Figure 1 shows the process of taking and collecting magot samples.

Fig 1: Magot Sampling and Collection Process

B. Bacterial Isolation from Magot Digestion, Bacterial Purification

Purification is the purification or separation of bacterial colonies in order to obtain pure bacteria (Pamaya *et al.*,2018). Purification of bacteria from the maggot digestive tract cultured on organic waste was carried out on *Nutrient Agar* (NA) media for 24 hours with a temperature of 37°C. Based on the results of the purification of maggot samples cultured on organic waste media as many as 35 pure colonies were each given codes ranging from codes ranging from codes M1-1 to M1-35 and maggot samples that cultured on fecal waste mediaobtained as many as 52 pure colonies. From the entirety of maggot samples cultured using organic waste media k and feces obtained seven different types of colonies based on their morphology (Figure 2).

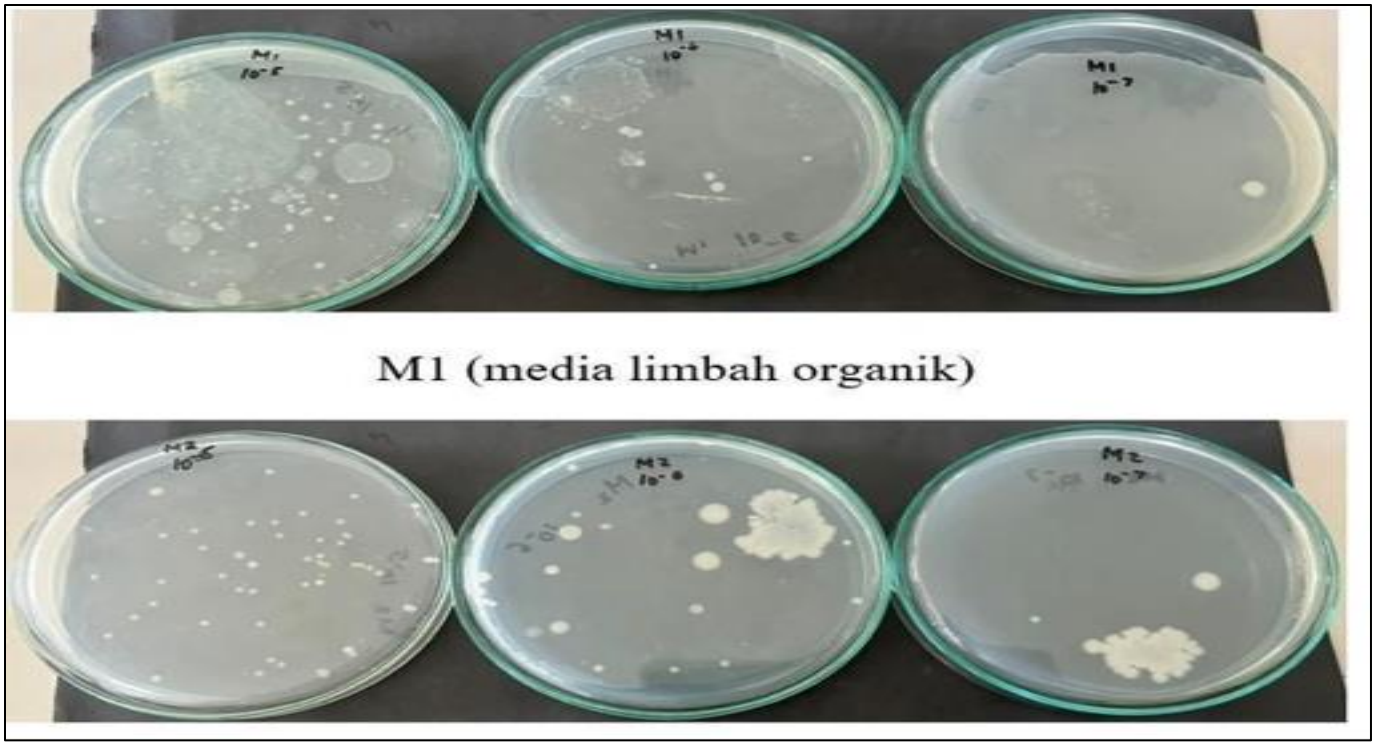

Fig 2: The Result of the Isolation of Bacteria from the Digestion of Maggot on NA Media, at a Temperature of 37°C

C. Hemolysis Ability of Bacteria of Magot Digestive Origin

A total of 87 bacterial isolates of maggot digestive origin have been successfully tested for hemolysis. The results of the hemolysistest showed the diversity of pathogenic and non- pathogenic bacteria (Figure 3).

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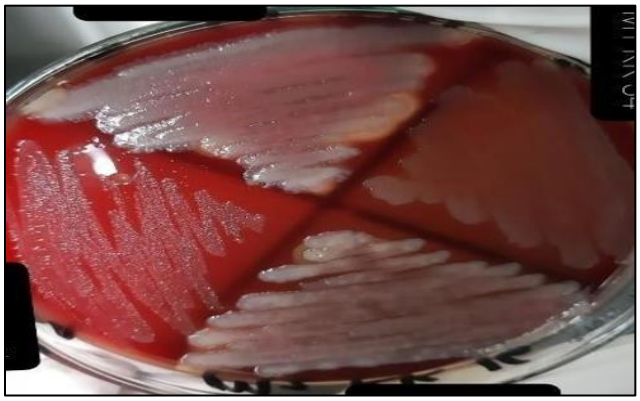

Fig 3: Hemolysis Test Results on Blood Media Agar, for 24 Hours, Incubation at 37°C

Bacterial inoculation of blood media *to* serve to group bacteria based on the nature of blood hemolysis. The nature of bacterial hemolysis is three fold,namely : α-hemolysis,bacteria that indicate a decrease in hemoglobin of red blood cells around the colony, so that the surrounding bacteria will appear green or brown in the medium. While β-hemolysis, bacteria show perfect lysis with a transparent color display around the bacteria in the medium, and -hemolysis, bacteria point to the lack of signs of hemolysis present in the media (Rebecca, 2005).

D. Gram Coloring Results

Pure culture isolates that have been successfully calcified are then carried out Gram staining to determine the characteristics of bacteria of maggot digestive origin microscopically. Overall, isolates M1-1 to M1-35 showed the results of gram negative bacteria characterized by pink bacteria with the shape of basil (Figure 4). The pink color is formed because the cell wall structure has a thin peptidoglycan layer and outer membrane so that it fails to retain the violet crystal dye and successfully absorbs the sapphire dye. This is in accordance with research conducted by Kresnawaty *et al* (2018), regarding the amyllase activity of amiolytic bacteria from *black soldier fly* larvae.

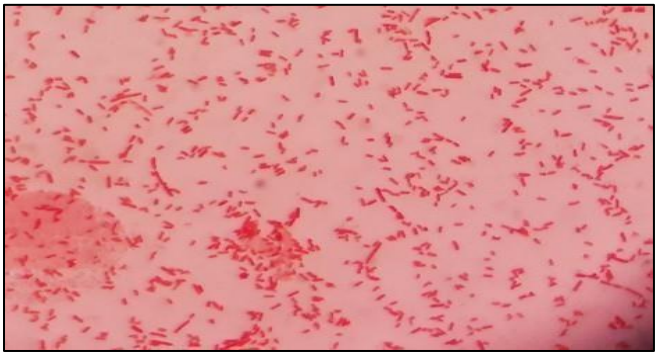

Fig 4: Gram Staining Results of Maggot Bacterial Isolates at 1000 X Microscope Magnification

E. Bacterial DNA Extraction

Results **on Magot Samples** A total of seven isolates of pathogenic and non-pathogenic bacteria from maggot digestive samples have been successfully performed DNA extraction.DNA isolation using *the Presto™Mini Gdna Bacteria Kit (Geneaid)* which has several stages,namely cell isolation,cell lysis, solution extraction, purification and precipitation .The DNA isolation procedure that has been carried out in accordance with the protokol, then an electrophoresis visualization test of agarosa 1% gel is carried out for DNA quality testing.The success of DNA extraction is characterized by the appearance of thick and intact DNA bands above the marker 3000 pb.

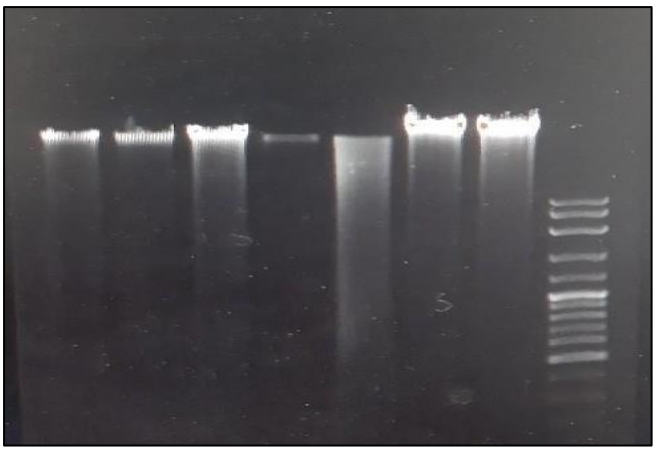

Fig 5: Gram Staining Results of Maggot Bacterial Isolatesat 1000 X Microscope Magnification

F. Amplification of Gene 16S rRNA Bacteria of Magot Digestive Origin

The DNA extraction results of the seven bacterial isolates of maggot digestive origin have been successfully amplified properly using PCR. It is characterized by the appearance of specific DNA bands or single bands according to the size of the 16S rRNA gene, that is,it ranges from 1300-1500 pb (Figure 6). The principle ofthe PCR technique is to multiply specific parts with the enzyme DNA polymerase which has been initiated primary attachment by combining deoxy ribonucleotide triphosphate (RavenandJohnson, 2002). After electrophoresis the result is visible a single band parallel to the gel agarose 2% equivalent to the marker. This indicates that the bacterial DNA fragments in the maggot have been successfully applied.

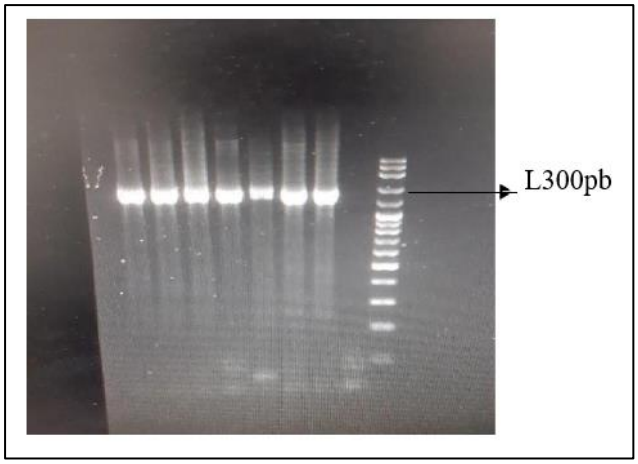

Fig 6: Results of DNA Amplification of the16S rRNA Gene of Bacterial Origin of Magot Digestion, on the Gel Agarose 1%

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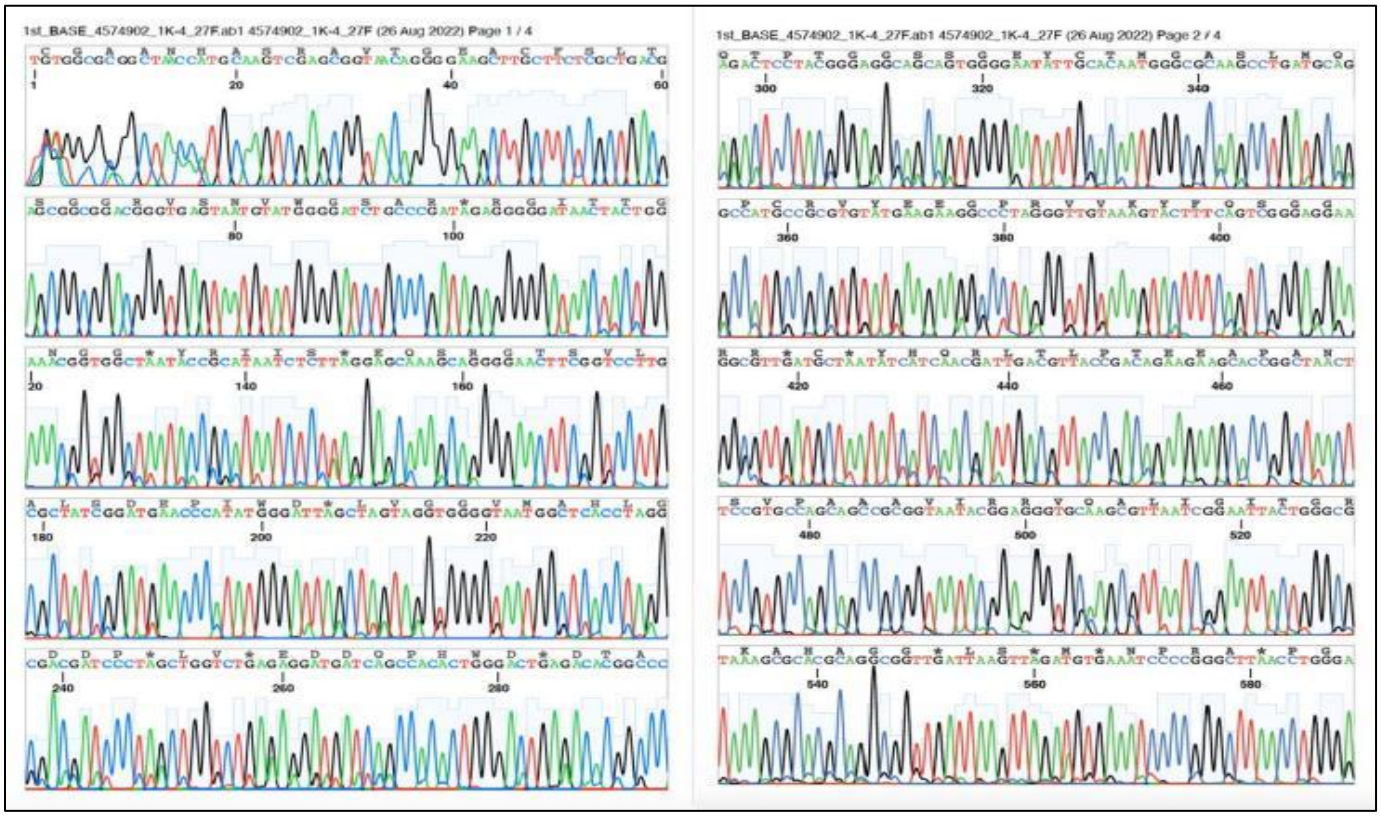
G. Nucleotide Sequence Resulting from Gene Sequencing 16S rRNA Bacteria of Magot Digestive Origin

PCR products that have been successfully amplified are then sequencing to obtain the nucleotide sequence. Nucleotide sequences that show good sequencing results are characterized by a single ornon-stacked pick dendogram (Figure 7), it may indicate that bacterial isolates are the extracted is a pure culture and can be identified species appropriately.



>1st_BASE_4574902_1K-4_27F TGTGGCGCGGCTAACCATGCAAGTCGAGCGG
TAACAGGGGAAGCTTGCTTCTCGCTGACGAG CGGCGGACGGGTGAGTAATGTATGGGGATCT
GCCCGATAGAG GGGGATAACTACTGGAAACGGTGGCTAATAC
CGCATAATCTCTTAGGAGCAAAG CAGGGGAACTTCGGTCCTTGCGCTATCGGAT
GAACCCATATGGGATTAGCTAGTA GGTGGGGTAATGGCTCACCTAGGCGACGATC
CCTAGCTGGTCTGAGAGGATGAT CAGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTGGG GAATATTGCACAATGGGCGCAAGCCTGATGC
AGCCATGCCGCGTGTATGAAGAA GGCCCTAGGGTTGTAAAGTACTTTCAGTCGGG
AGGAAGGCGTTGATGCTAATATC ATCAACGATTGACGTTACCGACAGAAGAAGC
ACCGGCTAACTCCGTGCCAGCAG CCGCGGTAATACGGAGGGTGCAAGCGTTAAT
CGGAATTACTGGGCGTAAAGCGC ACGCAGGCGGTTGATTAAGTTAGATGTGAAA
TCCCCGGGCTTAACCTGGGAATG GCATCTAAGACTGGTCAGCTAGAGTCTTGTA
GAGGGGGGTAGAATTCCATGTGT AGCGGTGAAATGCGTAGAGATGTGGAGGAA
TACCGGTGGCGAAGGCGGCCCCCT GGACAAAGACTGACGCTCTGGTGCGAAAGC
GTGGGGAGCAAACAGGAGTAGAT ACCCTGGTAGTCCACGCTGTAAACAATGTCG
ATTTGGAGGTAGTGCCCTTGAGGC GTGGCTTCCGCAGCTAACACGATTAATACAA
CACNCTGGTGAGTACAGCCTCCCC GTTAAAACTCGGGTGAATTGACTGTGGGCCG
GCCCACCCGCGGAGCTTGTGTTTG AGTCAATTCACGCGAAGAACGT

Fig 7: Dendo Gram and Nucleotide Sequence Resulting from 16S rRNA Gene Sequencing in a Sample of 27 F Bacterial Isolates of Maggot Digestive Origin

IV. CONCLUSIONS

The interim conclusion ofthis study is that the results of the isolation of bacteria from magot digestion, bacterial purification, and gram staining that have been carried out, it can be concluded that the bacteria contained in the digestion of maggots show a diversity of pathogenic and non-pathogenic bacteria, with bacil and cocobacil shapes. In addition, DNA ration and bacterial identification have also been carried out using the PCR method. The results of the sequencing of the seven isolates showed a good dendogram and nucleotide sequence, so then extstage is bioinformatics analysis and phylogenetic construction, to identify species and its kinships between species.

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