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Original Research Paper

The detection of Genes Encoding Enzyme Fructosyltransferase the Exopolysaccharide (EPS) Produce Potential Probiotic Candidates for Breast Milk (ASI)

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Abstract
Bacteria is one of the microorganisms that we can easily find in nature. Bacteria can be classified into benefical bacteria and harmful bacteria. One of the bacteria classified as benefical bacteria is lactic acid bacteria (LAB). One of the bacteria included in LAB is probiotic bacteria. Probiotic bacteria can be isolated from several sources, one of which is breast milk. The functionality of probiotics is seen by the production of exopolysaccharides (EPS). EPS is produced through the expression of the fructosyltransferase (*ftf*) gene. The samples used in this study were 16 isolates of candidate probiotic bacteria from breast milk. The stages of this research included reculture of probiotic isolates, isolation of bacterial genomic DNA, amplification of isolates with 16s rRNA primers, amplification of isolates with primary lactic acid bacteria (LAB) and amplification of isolates with specific primers of *ftf* gene. The results showed that 10 of 16 probiotic bacterial isolates had the *ftf* gene indicated by the presence of DNA bands on the electrophoregram with a size 58 bp.

Keywords: breast milk; exopolysaccharide; fructosyltransferase; probiotics

1. Introduction

Bacteria is one of the microorganisms that are widely distributed in nature and even found in the human body. Bacteria can be classified into beneficial microorganisms or into harmful microorganisms. One of the bacteria included in beneficial microorganisms is lactic acid bacteria (LAB) (Detha et al., 2019). LAB is one of the microbes that still needs to be explored (Surbakti & Hasanah, 2019). Microbes that are included in the LAB category some of which are candidates for probiotic bacteria with certain conditions. Bacteria that can be classified into probiotics must have several conditions, the first is resistant to low pH conditions or resistant to acidic conditions (Seveline, 2018). Second, bacteria are resistent to bile acids and salts so that bacteria can survive as long as they are in the small intestine (Allen et al., 2011 cit. Sunaryanto et al., 2014). Third, it has the ability to produce antimicrobial substances that aim to suppress the growth of enteric pathogenic bacteria (Ahmed et al., 2010 cit. Sunaryanto et al., 2014). Finally, bacteria are able to grow and develop in the human digestive tract so that they are able to balance the microflora in the digestive tract and are safe when used as medical therapy in humans or Generally Recognized As Safe (GRAS).

Isolated probiotic bacteria can be obtained in the market or isolated from nature. In addition, candidate sources of probiotic bacteria can also be isolated from humans such as human feces. Research Yang et al., (2017) proved that it had succeeded in isolating the bacterium Lactobacillus rhamnosus from human feces. Zhang & Zhang (2018), who have succeeded in isolating Lactobacillus and Bifidobacterium spp from baby feces. In addition, isolates of probiotic bacteria can also be obtained from breast milk. Jiang et al., (2016) reported having succeeded in isolating Lactobacillus plantarum bacteria from breast milk as a source of probiotics. Reis et al., (2016) LAB isolated from breast milk does have the potential to be a probiotic and have the ability to survive in the digestive tract well.

The functionality possessed by probiotic bacteria is supported by the ability of probiotics to produce exopolysaccharides (EPS). EPS is a polymer derived from polysaccharides secreted by microbes and released out of cells (Nudyanto & Zubaidah, 2015). One of the microbes that has the ability to produce EPS is LAB (Nurhasanah et al., 2020). LAB which is able to synthesize EPS has increased exploration, because LAB's ability to synthesize EPS is considered important for health. This is because EPS has anti-tumor, anti-ulcer, anti-inflammatory, anti-infective activities and boosts the body's immune system. EPS also has other benefits besides health, namely as a natural stabilizer and thickener in yogurt (Halim & Zubaidah, 2013). The EPS synthesis process can be carried out by extracellular enzymes belonging to the Glycoside Hydrolase (GH) family, one of which is the ftf enzyme (Zannini et al., 2016). Ortiz et al., (2019) stated that the expression of this ftf gene encodes the formation of the fructosyltransferase enzyme that plays a role in catalyzing the synthesis of EPS. The ftf gene is a gene that belongs to the glycoside hydrolase 32 (GH32) family. The glycoside hydrolase has the ability to transfer some part of the fructosyl from one substrate to another. FTF is also a derivative of the enzyme group, namely invertase. In addition, ftf can also bind to the sugar side that experiences excess when hydrolyzing water. The EPS biosynthesis process does not have an active transport step and does not require energy. Harutoshi (2013), stated that the EPS synthesis process is carried out outside the cell by the expression of the ftf gene. The EPS synthesis process uses sucrose as a specific substrate and as energy during the synthesis process. During the production process, EPS does not require energy because the EPS synthesis process by the ftf gene does not involve active transport.

Research related to probiotic bacteria candidates from breast milk that have the ability to produce EPS through the detection of the ftf gene encoding the fructosyltransferase enzyme has not been done much. Therefore, this study conducted an experimental study related to the presence of the ftf gene encoding the fructosyltransferase enzyme which acts as an EPS synthesizer in candidate probiotic bacteria isolated from breast milk.

2. Research Methods

The research conducted was an experimental research using the following tools, materials and methods:

2.1. Tool

The tools used for the reculture process are Laminar Air Flow (LAF) (Telstar Bio-II-A/P), long test tubes, short test tubes, vortex (Maximix I type 16700), 500 ml beaker glass, round loop, tip micropippete blue, micropippete 100-1000 l (Socorex), micropippete 5-50 µl (Socorex), analytical balance (AND GR-200) and incubator (Memmert). The tools used for DNA isolation are centrifuge, vortex, incubator, microcentrifuge, micropestle, spin column, FATG mini column, and collection tube.The tools used for 16s rRNA, LAB and ftf amplification were PCR thermocycler machine (Peqlab Primus Hain 25), hotplate stirrer (Cimarec SP88857105), blue micropippete tip, yellow micropipette tip, white micropippete tip, 100-1000 µl micropippete (Serana), micropippete 5- 50 µl (Socorex), micropippete 0.5-10 µl (Serana), micropippete 50-200 µl (Accura 825), micropippete 0.1-2 µl (Accura), microcentrifuge, centrifuge (Galaxy 7D), vortex (Nissin). The tools used to observe the amplification results were analytical balance (OHAUS), microwave, 50 ml beaker, 50 ml measuring cup, spatula, electrophoresis machine and UV transilluminator.

2.2. Material

The materials used for the reculture process of bacterial isolates were bacterial isolate, De Man Rogosa Sharpe broth (MRS broth) (Oxoid CM0359), bile salt and sterile distilled water. The material for the DNA isolation process was to use a DNA isolation kit (Favorgen) consisting of FATG1 buffer,

lysozyme, Proteinase K, RNAse A, 96% absolute ethanol, FATG2 buffer, buffer, Ellution buffer, aquabidest nuclease free water. To observe the results of DNA isolation, using agarose (Promega) and using a DNA ladder as a marker for the results of DNA isolation, which was 1kb in size.

The materials used for the 16s rRNA amplification process were aquabidestilata (nuclease free water), GoTaq green master mix (Promega) consisting of Taq DNA polymerase, dNTPs (400µM dATP, dGTP, dcTP and dTTP, MgCl2 and PCR buffer respectively. Primers). The primers used in this study were forward pA (5'-AGAGTTTGATCCTGGCTCAG-3´) and reverse pB (5´-AAGGAGGTGATCCAGCCGCA-3) primers. The DNA ladder used as a marker for the amplification of the 16s rRNA gene was 1kb (Promega). The materials used for the electrophoresis process of 16s rRNA amplification were ethidium bromide (Merck) and agarose from Promega (Madison, USA).

The materials used in the LAB amplification process are aquabidestilata (nuclease free water), GoTaq green master mix (Promega) which consists of Taq DNA polymerase, dNTPs (400µM dATP, dGTP, dcTP and dTTP, MgCl2 and PCR buffer respectively. used are forward primers LABfw (5'-AGAGTTTGATYDTGGCTCAG-3') and LABRev (5'CACCGCTACACATGGG-3') (Malik et al., 2010). The DNA ladder used as a marker for the results of LAB amplification was 100 bp (Promega) .The material for electrophoresis as a result of LAB amplification was ethidium bromide (Merck), while agarose was from Promega (Madison, USA).

The materials used in the ftf amplification process were aquabidestilata (nuclease free water), GoTaq green master mix (Promega) consisting of Taq DNA polymerase, dNTPs (400µM dATP, dGTP, dcTP and dTTP, MgCl2 and PCR buffer respectively. used is a modified primer Malik et al. (2009), namely the forward primer5FTF (5-GAYGTNTGGGAYWSNTGGGCC-3) and the reverse primer used is FTFrev (5-GATTGAACCTGCATTGCG-3). The DNA ladder used as a marker for amplification of the *ftf* gene is 50 bp in size from Bioline.

2.3. Method

2.3.1. Bacterial Isolate Reculture

Bacterial reculture Isolate process used the method of Safitri et al., (2017) modified. The reculture process begins with the manufacture of MRS broth media which is poured into test tubes, each tube containing 5 ml of MRS broth media. The composition of the media used in 75ml MRS broth was 3.93G MRS broth and supplemented with 0.112G bile salt. The media was put into an erlenmeyer and 75 ml of distilled water was added for 15 test tubes to be used for the reculture process. The media is then heated using a hotplate until dissolved. After the media temperature is the same as room temperature, 5 ml is poured into test tubes for each test tube. The media was then sterilized using an autoclave at 121°C for 15 minutes. Then 200 l of isolate was taken to be recultured and inoculated in the growth medium. Media that had been inoculated with 200 l isolates, incubated at 37°C for 24 hours. This reculture process was repeated 3 times.

2.3.2. Molecular Detection of the Presence of the ftf Gene

2.3.2.1. DNA isolation

The DNA isolation procedure followed the protocol on a modified DNA isolation kit (Favorgen, 2018). 1 ml of culture isolate was put into a sterile microtube for centrifugation at a speed of 18.000X for 2 minutes at a temperature of 4°C. The results of centrifugation showed that the layers separated as supernatant and pellet, part of the supernatant was removed and the pellet was taken as material for further preparation of DNA isolation. Next, add 200µl of FATG1 buffer, 50µl of lysozyme 10mg/ml and homogenize using a micropestle. Incubate the mixture at 37°C for 1 hour. Then 20µl proteinase K (10mg/ml) was added and homogenized using a vortex and incubated at 60°C until tissue lysis for 2 hours with optimized inversion every 30 minutes. Furthermore, 8µl 100mg/ml RNAse A was added,

then homogenized using a vortex and incubated at room temperature for 2 minutes. Then, 200µl of FATG2 buffer was added, homogenized by vortexing and incubated at 70°C for 10 minutes in a dry bath. The supernatant formed was taken to be transferred to a new microtube and 200µl absolute 96% ethanol was added and homogenized using a vortex. The mixed solution with Ethanol was then transferred to the spin column FATG mini column (filter in the collection tube) carefully so that the pellets were not included. Furthermore, the separation was carried out by centrifugation at a speed of 18.000X for 1 minute. The liquid collected in the collection tube with the FATG mini column was discarded and 400 µl of buffer was added to the FATG mini column for washing out. Then centrifuged again at a speed of 18.000X for 1 minute, the liquid collected in the collection tube and washed out by adding 750 l of wash buffer into the FATG mini column, centrifuged at a speed of 18.000X for 1 minute and the liquid in the collection tube. After centrifugation, the mini column was transferred to a sterile 1.5 ml microcentrifuge and centrifuged again at 18.000X speed for 3 minutes. Then 100µl of Ellution buffer which had been preheated at 70°C was carefully added to the center of the membrane filter. Next, centrifuged again at 18,000 g for 3 min, mini column was taken and centrifuged again at 18.000X speed for 2 minutes. Pellets were stored in microtubes, and dissolved using aquabidest free water nuclease and stored at -20°C.

2.3.2.2. Amplification Using 16s rRNA Primer

The amplification process used universal 16s rRNA primer. The primer used in this study refers to the research primer Serrano-Nino et al. (2016), namely pA and pB primers with a target sequence of 1500 bp. The primers used in the 16s rRNA amplification process were pA (5´-AGAG TTTGATCCTGGCTCAG-3´) and pB (5´-AAGGAGGTGATCCAGCCGCA-3). Visualization of 16s rRNA amplification using 1.5% agarose gel with a molecular marker of 1kb DNA ladder. 16s rRNA gene amplification was carried out using the modified Chun and Goodfellow (1995) method. The amplification of the PCR program is as follows: 1) Initial denaturation at 95°C for 3 minutes, 2) Denaturation at 95°C for 30 seconds, 3) Annealing at 54°C for 30 seconds, 4) Extension at 72° C for 2 minutes with 35 cycles of running, 5) final extension 72°C for 2 minutes and 6) hold at 4°C temperature.

2.3.2.3. Amplification Using LAB-Specific Primer

The amplification of specific LAB genes in this study used the method of Malik et al., (2010). The primers used were LABFw and LABRv primers with a target sequence of 700 bp. The LAB specific primers used were LABFw (5'-AGAGTTTGATYDTGGCTCAG-3') and LABrv (5'CACCGCTACACATG GG-3'). Visualization of LAB specific amplification results was carried out using 1.5% agarose gel with 100 bp DNA ladder molecular markers. The amplification of the PCR program was as follows: 1) Initial denaturation at 95°C for 5 minutes, 2) Denaturation at 94°C for 30 seconds, 3) Annealing at 58°C for 30 seconds, 4) Extension at 72° C for 45 seconds with 35 running cycles, 5) Final extension 72°C for 5 minutes and 6) Hold at 4°C.

2.3.2.4. Screening and Amplification Using ftf Specific Primers

The last step of molecular detection was amplification with specific primer ftf. The amplification of the *ftf* gene in this study used the method of Malik et al., (2009) with modifications. The primer used to amplifygen ftf is 5FTF and FTFrev with a target sequence size of 58 bp. The forward primer used was 5FTF (5'-GAYGTNTGGGAYWSNTGGGCC-'3) and the reverse primer used was FTFrev (5'-GATTGAACCTGCATTGCG-'3). Visualization of the amplification results was carried out using 2% agarose gel with a 50bp DNA ladder molecular marker. The PCR program used was 1) Initial denaturation at 95°C for 5 minutes, 2) Denaturation at 95°C for 30 seconds, 3) Annealing at 58°C for

30 seconds, 4) Extension at 72°C for 45 seconds with 35 running cycles, 5) Final extension 72°C for 3 minutes and 6) Hold at 4°C.

3. Results and Discussion

3.1. Bacterial Isolate Reculture

The initial stage is the reculture process of LAB which has been isolated in previous studies. The isolate cultures used in this study were 16 isolates derived from the results of previous studies (Anindita et al., 2018). This reculture process aimed to regrow bacteria that had just experienced a period of dormancy. The reculture process was carried out by inoculating on the growth medium MRS broth and 0.15% bile salt. The 0.15% bile salt supplementation on MRS Broth growth medium was aimed at confirming that the cultured bacterial isolates were probiotic bacteria isolates.

This reculture process was carried out repeatedly with the aim that the bacteria to be isolated could be completely regenerated. Bacteria that were successfully regenerated during the reculture process were characterized by the presence of white biomass at the bottom of the tube, hereinafter referred to as bacterial biomass. Observations of reculture results were carried out every 24 hours after the incubation process at 37ºC. In the results of the first reculture of bacterial isolates (Figure 1), some bacteria have already produced biomass which was indicated by the formation of a white precipitate. The bacterial biomass formed indicates the presence of bacterial activity after a period of dormancy. Biomass in the first reculture there were several tubes that had not formed biomass. That was because the previously bacterial isolates were stored in a refrigerator with a sufficiently frozen temperature so that their activity decreased. Bilang et al. (2018) stated that the decrease in LAB activity was due to the absence of encapsulation in LAB, resulting in direct contact with the frozen environment and causing the permeability of bacterial cell membranes to decrease and even damage. In addition, the presence of bile salt during culture had an influence on the activity of cells that had just experienced dormancy, because bacterial cells that had decreased permeability and had not had normal cell activity couldn't tolerate the toxic properties of bile salt. In addition, Mardalena (2016), Bacteria that had just experienced a dormancy phase needed time to adapt to the new media used as a place for bacteria to live.

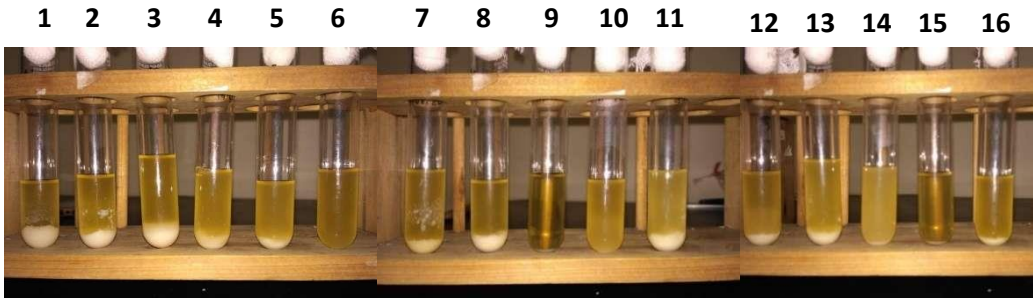

Figure 1. Results of the first reculture of LAB isolates

The first reculture several tubes that had produced bacterial biomass were still mixed with milk biomass. Milk biomass was formed because the isolates used were stored in media containing skim milk and glycerol. In line with the research of Susilawati & Purnomo (2016), which states that the use of skim milk as a cryoprotectant had been widely carried out. This was because skim milk was superior in maintaining cell viability. Skimmed milk was most widely used as a cryopreserve for LAB members.

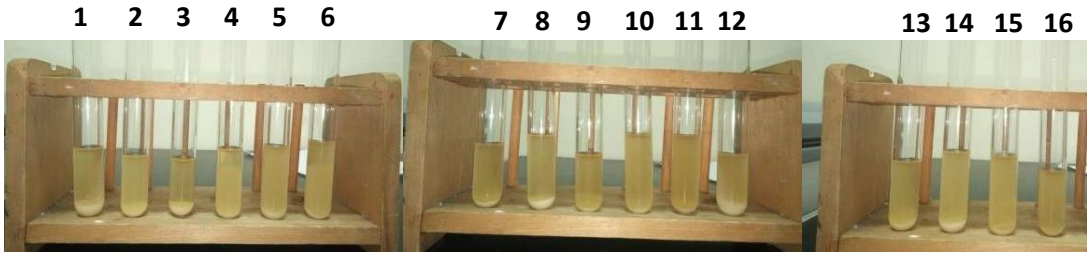

Figure 2. The results of reculture of the two LAB isolates from breast milk

In the second reculture (Figure 2), the tube which in the first reculture stage had not yet formed biomass, in the second stage had already formed biomass. It can be said that the bacteria were still able to carry out the replication process. Several tubes, which in the first stage had already formed biomass, also experienced an increase in the amount of biomass. The increase in biomass was due to the bacteria undergoing the reculture process that was able to adapt to the new growth medium, so that the bacteria underwent a fairly fast and stable replication process.

Figure 3. The results of the reculture of the three LAB isolates from breast milk

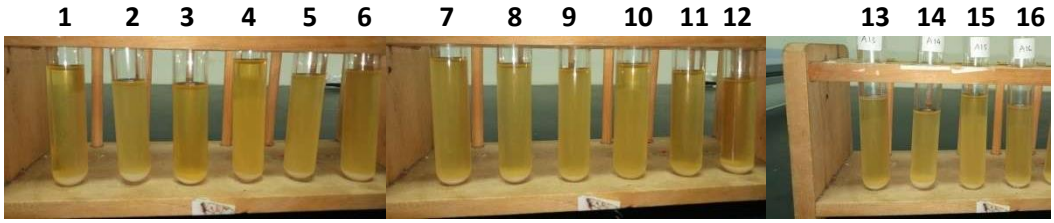

Figure 4. Results of reculture of the four LAB isolates from breast milk

In the third reculture (Figure 3), all the tubes had formed biomass. However, some tubes had experienced a decrease in the amount of biomass formed. Thus, in the fourth reculture process (Figure 4), almost all of the tubes experienced a decrease in the amount of biomass formed. The low bacterial biomass was due to a decrease in activity during the reculture process. The factor that causes a decrease in the amount of biomass formed is because the energy reserves for bacteria to grow are getting depleted. In line with the opinion of Mardalena (2016), which stated that the reduced bacterial biomass was caused by two factors, namely because the available nutrient media was running low and the energy reserves in bacteria for replication were running low.

3.2. DNA isolation

The stage after the reculture process was to carry out molecular detection of the presence of the ftf gene. The first stage in the ftf gene detection process was the DNA isolation process. DNA isolation aimed to separate DNA from other cell components so as to obtain pure DNA. Muhammad et al. (2020) stated that the principle of DNA isolation was to separate DNA from components other than DNA.

Based on the results of reculture, there were 16 isolates for DNA isolation and followed by qualitative observations using the electrophoresis method on 1% agarose gel and visualized using a UV transilluminator. In the DNA isolation process, the bacteria used were the result of the first stage of reculture. This was because in the first stage of reculture, bacteria had re-metabolized after the dormancy phase as indicated by the formation of a white precipitate in (Figure 1). Qualitative visualization was carried out to determine the quality of the DNA isolation results from 16 bacterial isolates that had been sequenced in the research of Anindita et al., (2018). Electrophorogram bacterial DNA isolation can be seen in Figure 5.

Figure 5. Electrophorogram of DNA isolation on 1% agarose gel. M: Marker (1 kb); PCR product of probiotic isolate from breast milk, *Lactobacillus casei* (A1, A8, A10, A12); *Lactobacillus paracasei* (A2, A3, A4, A14, A15); *Weisella confusa* (A5); *Lactobacillus plantarum* (A6, A11); *Pediococcus acidilactici* (A7); *Pediococcus pentosaceus* (A9, A13, A16)

Based on 1% agarose gel electrophorogram with 1Kb marker from DNA isolates, it showed that the DNA of all isolates of probiotic bacteria had been isolated. Electrophoresis results on DNA isolation were above 10,000 bp. In line with the research of Nurwati & Nawfa (2015), which obtained electrophoresis results on DNA isolation with sizes above 10,000 bp. The results of electrophoresis contained a smear on the agarose gel. Smear on DNA isolation electrophorogram caused by contamination with impurities. In addition, the thickness of the DNA bands formed is also different. Restu et al. (2015) cit. Hermansyah et al. (2018) stated that the difference in the thickness of the DNA bands formed was due to one of them, namely the concentration of DNA produced during the isolation process was too low.

3.3. 16s rRNA amplification using Universal Primer

The isolated DNA was then amplified using PCR. The process of amplification of the 16s rRNA gene was carried out using a primerpA & pB with the aim of confirming the isolates used were bacteria. Sixteen DNA of bacterial isolates that had been amplified, then electrophoresed on 1.5% agarose gel with a 1Kb marker and visualized using a UV transilluminator. The results of the 16s rRNA gene amplicon can be seen in (Figure 6).

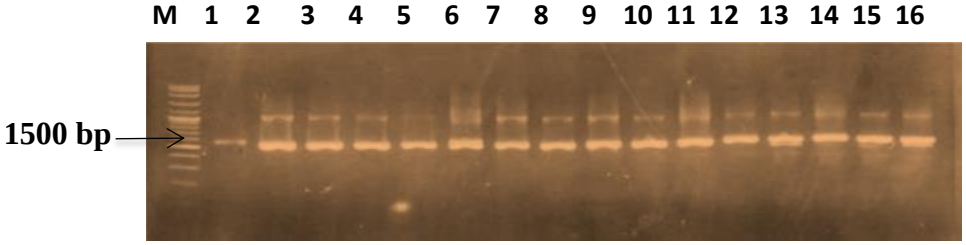

Figure 6. Electrophorogram of 16s rRNA amplification on 1.5% agarose gel. M: Marker (1 kb); PCR product isolate probiotic from breast milk, *Lactobacillus casei* (A1, A8, A10, A12); *Lactobacillus paracasei* (A2, A3, A4, A14, A15); *Weisella confusa* (A5); *Lactobacillus plantarum* (A6, A11); *Pediococcus acidilactici* (A7); *Pediococcus pentosaceus* (A9, A13, A16)

Based on the electrophorogram, the results of the 16s rRNA gene amplicons showed that the base length obtained by using universal primers pA and pB was 1500 bp. The results obtained were in line with the results in the study of Serrano-Nino et al., (2016) with pA and pB primers obtained results of 1500 bp. Karamoy & Kepel (2017), performed 16s rRNA gene amplification using universal primers, namely Bact-FI and Uni-B1 and obtained a 16s rRNA gene amplicon of 1500 bp.

The use of universal primers in 16s RNA amplification played an important role in protein synthesis (Singh et al., 2012). In addition, the use of universal primers in the 16s rRNA gene amplification process was due to the 16s rRNA gene having conserved regions. The 16s rRNA gene had a conserved area because this gene had an important role in cell function and had an important influence in reducing mutations, especially in genes encoding enzymes, especially enzymes needed for the use of lactose. The use of the 16s rRNA gene for identification in addition to having conserved areas also had other advantages (Rinanda, 2011). The 16s rRNA gene was used as a molecular marker in the bacterial identification process because it was stable and specific (Singh et al., 2012) and is ubiquitous (found in all living things) (Noor et al., 2014).

3.4. 16s rRNA amplification using LAB specific primers

DNA amplification of bacterial isolates using specific primers LABfw and LABrev which aimed to confirm that the test isolates used were of the LAB group. The results of the amplification were then carried out by electrophoresis on 1.5% agarose gel using a 100 bp DNA ladder. The visualization results showed that the DNA band (Figure 7) was at a length of 700 bp. Anindita et al. (2018), had performed sequencing of these isolates so that they were identified in several LAB genera, namely Pediococcus sp, Lactobacilus sp and Weisella sp.

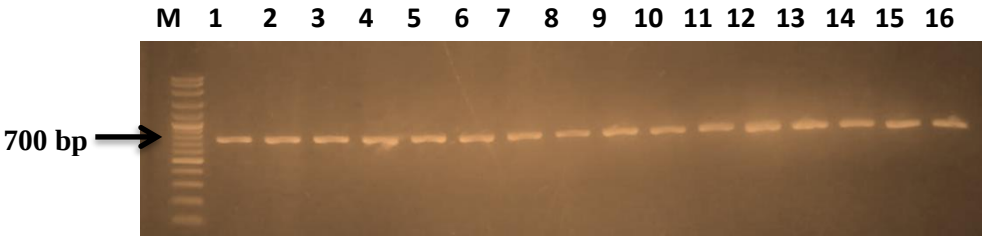

Figure 7. Electrophoregram of LAB-specific primer amplification on 1% agarose gel. M: Marker (1 kb); PCR product isolate probiotic from breast milk, *Lactobacillus casei* (A1, A8, A10, A12); *Lactobacillus paracasei* (A2, A3, A4, A14, A15); *Weisella confusa* (A5); *Lactobacillus plantarum* (A6, A11); Pediococcus acidilactici (A7); *Pediococcus pentosaceus* (A9, A13, A16)

Based on the results of PCR amplification using LABFw and LABrev primers, all tested isolates were confirmed to be LAB. Shehata et al. (2016) in their research stated that the yield of PCR products for specific LAB genes was 600 bp. Based on the obtained amplicons, LAB from breast milk were LAB group bacteria and had been tested to have potential as probiotic candidates.

Halim & Zubaidah (2013), stated that LAB species had the ability to synthesize EPS which was a polysaccharide polymer secreted by microbes. EPS was the product of the synthesis of LAB which had the ability to stick to the small intestinal mucosa, so that it could increase the ability to suppress the growth of pathogenic bacteria. Malik et al. (2010) added that for the EPS synthesis process from sucrose, LAB used large extracellular enzymes, one of which was ftf. So in this study, the detection of the presence of the ftf gene in LAB through ftf-specific primers was carried out.

3.5. Detection and Amplification Using Specific Primers ftf

After 16 test isolates were confirmed as a genus in the LAB group, further detection was carried out for the presence of the ftf gene that plays a role in the process of synthesizing EPS from sucrose. The use of confirmed genus LAB for the detection of the presence of the ftf gene was related to the statement of Malik et al. (2010) that several types of LAB could produce EPS by using large extracellular enzymes, one of which was fructosyltransferase which was encoded by the ftf gene. Detection of the presence of the ftf gene in this study used the amplification method with specific primers for ftf which was carried out in the study of Malik et al., (2009) with modifications. The primers used in this study were 5FTF as a forward primer and FTFrev as a reverse primer. The results of ftf gene detection in 3 genera showed the length of the amplicon (Figure 8) was less than 100 bp, which was 58 bp.

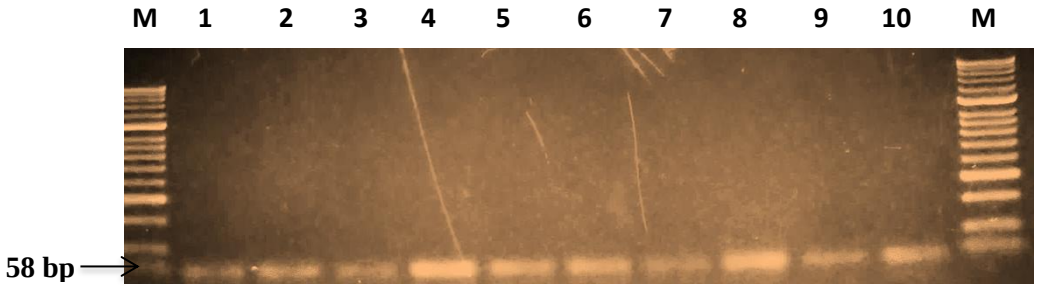

Figure 8. Electrophorogram of ftf-specific primary amplification on 2% agarose gel. M: Marker (1 kb); PCR product isolate probiotic from breast milk, *Lactobacillus casei* (A1, A8, A10, A12); *Lactobacillus paracasei* (A2, A3, A4); *Weisella confusa* (A5); *Lactobacillus plantarum* (A6); *Pediococcus acidilactici* (A7); *Pediococcus pentosaceus* (A9)

Based on the results of the detection of the ftf gene through amplification with specific primers for ftf, namely 5FTF and FTFrev, all isolates contained the ftf gene. Observations of the amplification results using 2% agraose with a 50 bp DNA ladder can be seen in Figure 8. The product size of the ftf gene amplicon obtained in this study was 58 bp. The ftf gene obtained was different from the type of ftf gene confirmed on GeneBank. This was because the primer used was the result of modification from previous

4. Conclusion

Based on the results of research related to the detection of the ftf gene in probiotic bacterial candidates from breast milk, it can be concluded that of the 16 probiotic candidates from breast milk, there are 10 probiotic candidates which have the ftf gene as a fructosyltransferase enzyme that acts as an EPS synthesizer by using ftf specific primers.

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