El-Hayah

JURNAL BIOLOGI

Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index

e-ISSN: 2460-7207, p-ISSN: 2086-0064

Original research article

Potential Analysis of Cheese Whey as an Alternative Media Growth for *Lactobacillus casei* Group

Nur Kusmiyati[1], Maria Massora[2], Septian Tri Wicaksono[3]

[1]*Agricultural Product Technology Department, Faculty of Agricultural Technology, Universitas Brawijaya, Jl Veteran, Malang, 65145*

[2]*Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Papua, Jl Gunung Salju Amban, Manokwari, 98134*

[3]*Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Jl Veteran, Malang, 65145*

[*]*Corresponding author*

Email: kusmiy4tinur@gmail.com

DOI: 10.18860/elha.v8i4.15800

Article Info

Article history:
Received 2 February 2022
Received in revised form 15 February 2022
Accepted 7 March 2022

Key Word:
Lactobacillus casei
Media
Growth
whey cheese

Abstract

Cheese processing industry throws a lot of whey into the environment that cause pollution. Whey cheese is known to contain lots of organic matter and lactose which is the main energy source for the Lactobacillus genus. This study aims to determine the effect of using cheese whey media on bacterial cell count, total lactic acid, and total sugar in the Lactobacillus casei group consisting of Lactobacillus casei, Lactobacillus paracasei, and Lactobacillus rhamnosus bacteria. The method used in this study was bacterial culture rejuvenated and propagated on MRSB media for 24 hours. As much as 1% of bacterial culture was inoculated into cheese whey medium and incubated at 37 C for 24 hours. Furthermore, observations were made on the number of bacterial cells, total lactic acid, and total sugar in the L. casei group. The results showed that L. paracasei had the highest number of cells (4.862 CFU/ml), total lactic acid (0.264 mg/ml), and total sugar (93.805 mg/ml) and was not significantly different from L. casei and L. rhamnosus. The cheese whey medium was not significantly different from the positive control (MRSB), however, there was a difference in the growth ability of each isolate on the positive control medium. Based on the research, it was shown that cheese whey media could be used as an alternative medium for the growth of the L. casei group.

1. INTRODUCTION

Cheese whey is a clear, greenish-yellow liquid produced from cheese processing [1]. Cheese whey is considered a waste so that it is immediately dumped into the environment so that it can cause pollution [2]. According to Lestari et al. (2020), cheese whey still contains about 55% of the nutrients leftover from milk processing, in the form of macronutrients and micronutrients. Macronutrients contained in cheese whey include lactose around 4.5-5% (w/v), protein around 0.6-0.8% (w/v), fats and oils around 0.4-0.5 (b/v) and mineral salts [3]. The micronutrients contained in cheese whey include lactoperoxidase, lysozyme, immunoglobulins, iron, iodine, and several vitamins [4]. In addition, cheese whey also contains lactoferrin of about 0.02-0.2 g/L [5].

Nursiwi et al. [6] stated that the lactose content in cheese whey reached 5.43% and this amount was high enough to be utilized by microorganisms. Lactose is known to be the main carbon source for the genus *Lactobacillus* [7]. *Lactobacillus* has the enzyme lactase which can break down lactose into glucose and galactose [8]. Therefore, cheese whey can be utilized by Lactobacillus as a growth substrate

One of the most studied members of the Lactobacillus genus is the *Lactobacillus casei* group. Based on molecular research with DNA hybridization, it shows that the L. casei group consists of three species, namely *Lactobacillus casei*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus* [9]. The three bacteria are grouped into one group because they have high phenotypic and phylogenetic similarities [10]. The growth ability of the *L. casei* group on cheese whey has not been widely published. According to Soenarno et al. [4], cheese whey can increase the growth of *Lactobacillus plantarum* IIA-1A5 by 1.21 log CFU/mL. Perez et al. [11], investigated the use of cheese whey as a growth medium for *Lactobacillus helveticus*.

The growth activity of a microorganism can be seen based on several parameters. According to Safitri et al. [12], the number of bacterial cells, total lactic acid, and total sugar can be used as parameters for the growth of lactic acid bacteria. The same parameters were also used in the study of Yeni et al. [13], the results showed that the number of *Pediococcus pentosaceus* bacteria that grew was 6.4×10^7 CFU/mL, the total lactic acid produced was 0.38 mg/mL, and the amount of sugar consumed by bacteria was 49.35%. Research on the use of cheese whey as a growth medium for the *L. casei* group using these three parameters has not been carried out. Therefore, this research needs to be carried out as an effort to reduce environmental pollution and show the potential of cheese whey as an alternative growth medium for the *L. casei* group.

2. MATERIALS AND METHODS

Preparation of de Man Rogosa Sharpe Agar (MRSA) and de Man Rogosa Sharpe Broth (MRSB) media

MRSA medium was used to rejuvenate pure cultures while MRSB was used for bacterial propagation and as a medium for positive control. MRSA was made with 62 g of instant media while MRSB with 52.2 g of instant media. Each intra medium was homogenized with 1000 mL of distilled water in Erlenmayer. Furthermore, it is heated on a hot plate and homogenized with a magnetic stirrer at a temperature of 60 °C until it boils. Then sterilized using an autoclave for 15 minutes at a temperature of 121 °C [14].

Preparation cheese whey media

The cheese whey was filtered using 0.45 m filter paper to separate the dissolved solids. Then, it is pasteurized at a temperature of 63-66 °C for 30 minutes and placed in ice bath water until the temperature reaches 4 °C [15]. Furthermore, the acidity level is set to pH 6 [13]. Adjusting the acidity level of the media can use HCl to reduce pH and NaOH to increase pH [16].

Bacterial rejuvenation

Each bacterial colony of the *L. casei* group (*L. casei*, *L. paracasei*, and *L. rhamnosus*) was taken one ose from a pure culture. Furthermore, it was inoculated on MRS media so that it was tilted MRS using the streak plate method. Then it was incubated for 24 hours at 37 °C [17].

Preparation of Mc Farland standard solution

Mc Farland's standard solution was used to create a standard curve for bacterial growth. The Mc Farland series is used, starting from 0.5-10. Preparation of standard Mc Farland 0.5 solution was done by mixing 0.05 mL of 1.175% BaCl2 solution with 9.95 mL of 1% H2SO4 solution. Mc Farland 1 standard solution was prepared by mixing 0.1 mL of 1.175% BaCl2 solution with 9.9 mL of 1% H_2SO_4 solution, and so on until an Mc Farland, 10 standard solution was obtained. Then each solution was vortexed until homogeneous and the absorbance was measured using a spectrophotometer with a wave length of 625 nm [18].

Inoculation of bacteria into the media

The cheese whey medium was poured into a 10 mL test tube. Then added inoculum of bacteria *L. casei*, *L. paracasei*, and *L. rhamnosus* which had been diluted according to the turbidity of the standard Mc Farland 0.5 solution. The turbidity value of the test suspension is said to be by the standard Mc Farland 0.5 solution if the absorbance results are 0.08-0.13 [18]. Mc Farland standard solution 0.5 is equivalent to a bacterial density of 1x107 cells/mL – 1x108 cells/mL [19]. The number of bacteria inoculated was 1% (0.1 mL) [20]. Bacterial inoculum was also inoculated into MRSB media as a positive control. The suspension was then incubated for 24 hours at 37 °C [21].

Bacterial cell count analysis

Calculation of the amount that grows on cheese whey media is carried out using the spectrophotometer method by taking into account the absorbance value or optical density (OD). The incubation media containing bacteria was homogenized with a vortex and 1 mL was taken and then put into a cuvette [22]. Before use, the cuvette must be cleaned with distilled water and dried with a tissue. The cuvette that has been filled with suspension is inserted into the cell holder in the sample chamber and placed to the bottom of the cell and then closed [23]. The blank solution used was cheese whey media without bacteria. The absorbance value of the suspension was measured with a wavelength of 630 nm. The absorbance value obtained was then converted into a linear regression equation formula from Mc Farland's standard solution. The final results obtained were expressed as bacterial density values (cells/mL) [15].

Total lactic acid analysis

The first step in testing total lactic acid is to make a cell-free culture. The sample in the form of a bacterial solution that has been incubated on cheese whey media was centrifuged at 4500 rpm for 20 minutes. Next, the cell-free culture in the form of the supernatant was diluted by taking 1.25 mL and then put into a 25 mL volumetric flask and then added with distilled water until it reached the 25 mL mark. Furthermore, 3 drops of PP indicator (phenolphthalein) were added and homogenized. Then titrated with 0.1 N NaOH while homogenizing. The titration process was stopped when a constant pink color was formed [24]. Calculation of lactic acid levels from the titration is shown by the following equation:

$$\text{Lactic acid} = \frac{\text{mL NaOH} \times \text{M NaOH} \times \text{M.E}}{\text{mL sample}}$$

Note:
mL NaOH: Volume of NaOH used (mL)
M NaOH: Molarity of NaOH

M.E: Lactic acid equivalent factor (90.08 g/equivalent)
mL sample: Volume of a titrated sample (mL)

Total sugar analysis

The total sugar test in cheese whey was carried out by diluting the supernatant first 100 times. Take 0.5 mL and put it into a test tube. Then 0.5 mL of 5% phenol was added and homogenized with a vortex. After being homogeneous, 2.5 mL of concentrated H_2SO_4 was added carefully through the walls of the test tube and allowed to stand for 10 minutes. The sample was placed in a water bath with a temperature of 40 °C for 20 minutes and homogenized and the absorbance was measured with a spectrophotometer using a wavelength of 490 nm. The absorbance value obtained from the test was then entered into the calculation results of the standard curve [25].

Standard curves were made from glucose solutions with concentrations of 0, 10, 20, 30, 40, 50, and 60 ppm which were treated the same as testing the total sugar in the sample. The decrease in total sugar in the media during fermentation was calculated at 24 hours. Thus, the total sugar value is obtained from the reduction between the total sugar at the beginning of the fermentation and the total sugar at the end of the fermentation [13].

Data analysis

The research data were tested for Shapiro-Wilk normality and Levene homogeneity first. Then it was analyzed descriptive statistics with Analysis of Variance (ANOVA) using SPSS IBM 23. If the results of the variance of the data showed a significant effect, the analysis was continued with Duncan's Multiple Range Test (DMRT) with a level of = 5%.

3. RESULTS

Observation of the parameters of the number of bacterial cells that grew during the 24-hour incubation period showed the growth of *Lactobacillus casei* groups on cheese whey media (Figure 1). The growth of the *L. casei* group was indicated by the higher absorbance value of cheese whey media with added inoculum compared to media without inoculum or negative control

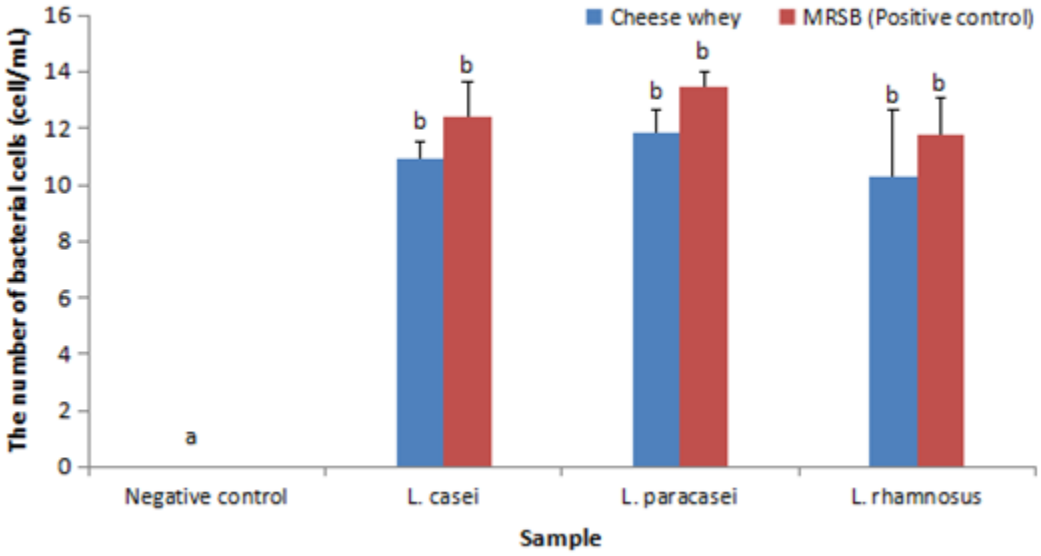

Figure 1. The number of bacterial cells

Based on statistical analysis showed that there was no difference in the average number of cells between bacteria. *Lactobacillus paracasei* has the most growth ability. When compared with MRSB media or positive control, the number of bacterial cells growing on cheese whey media was less but not significantly different. This is the same as the research of Salma et al. [26] which showed the highest average number of *Lactobacillus helveticus* MTCC 5463 cells was in MRSB media at 9.12 log CFU/mL, followed by skim milk media at 9.03 log CFU/mL, and cheese whey media at 9.02 log CFU/mL. The number of bacteria that grows on cheese whey is less because the nutritional content in it is reduced.

Lactic acid is the main metabolite product of the breakdown of carbohydrates during the fermentation process [27]. The increase in acidity in the media is indicated by the amount of organic acid formed which is followed by a decrease in pH [28] Lactic acid bacteria can live in environments with low pH because bacteria can to maintain pH conditions in their cells so that they remain more neutral than the pH in their environment [29]. The high acid content in the media indicates the presence of bacterial activity. Therefore, the calculation of total lactic acid is one of the parameters measured in this study. The results of the calculation of lactic acid in this test show different values (Figure 2).

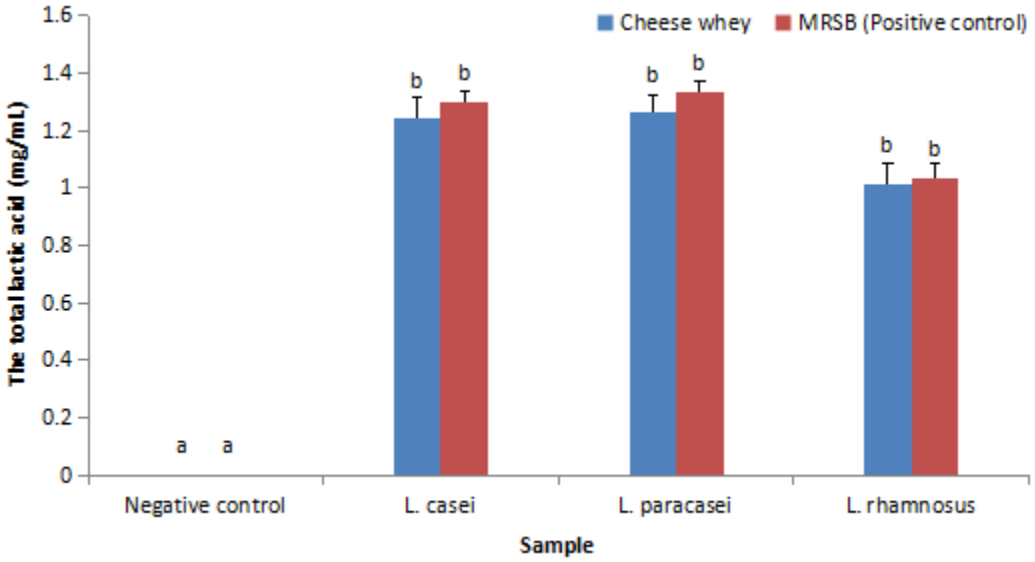

Figure 2. The total lactic acid

Based on the results of statistical analysis showed that there was no difference in the average total lactic acid between bacteria in the *L. casei* group. When compared with MRSB media or positive control, total lactic acid in cheese whey media was lower but not significantly different. The titration test on the negative control showed the absence of acid content. The negative control used was media without the addition of bacteria.

Total sugar is the amount of reducing and non-reducing sugars resulting from carbohydrate hydrolysis [30]. The total sugar test showed different results (Figure 3).

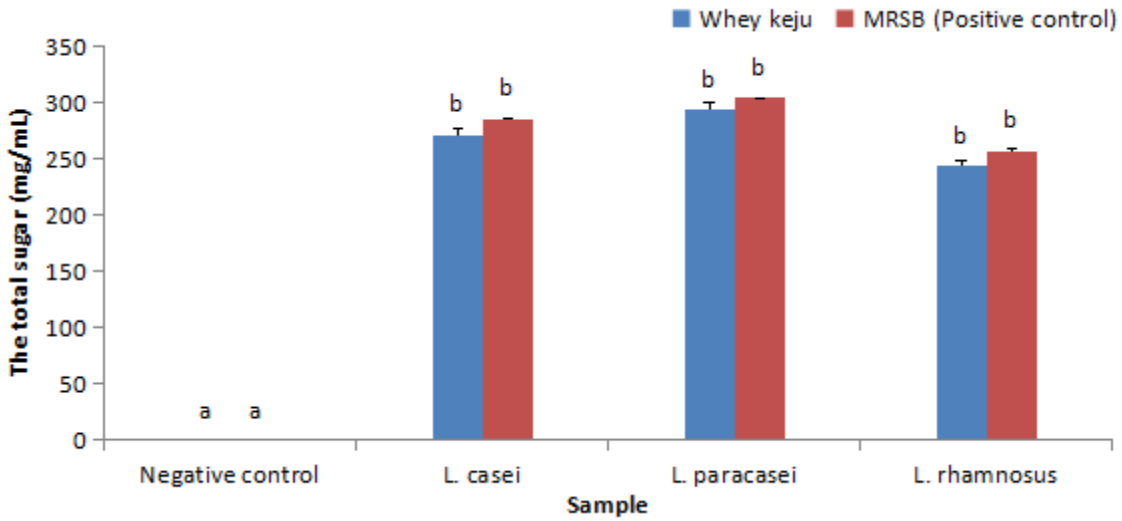

Figure 3. The total sugar

The increase in the number of bacteria was inversely proportional to the total sugar in the medium. The more bacteria that grow, the less total sugar. This happens because during the fermentation process bacteria will consume sugar in the media for growth. The sugars contained in the media are utilized by bacteria for cell maintenance, biomass growth, and the formation of by-products in the form of organic acids.

4. DISCUSSION

de Man Rogosa Sharpe media as a commercial medium for lactic acid bacteria contains various components, including casein, meat extract, yeast extract, D(+) glucose, di-potassium hydrogen phosphate, tween, di-ammonium hydrogen citrate, sodium acetate, magnesium sulfate, and magnesium sulfate. Manganese sulfate. According to Safitri et al. [12], the nutritional content of MRSB media has been measured and adjusted to the needs of the bacteria so that the number of bacteria that grow on this medium is more than the bacteria grown in cheese whey media. The addition of nutritional sources such as ingredients for MRS media may need to be done to enrich the content of cheese whey media. This is supported by the statement of Azhari et al. [31], that the availability of nutrient content of growth substrate is an important factor that must be considered to increase the growth rate. Soenarno et al. [4] conducted a study on the use of a mixture of cheese whey and skim milk as a growth medium for *L. plantarum* IIA-1A5, the results showed that the number of bacteria growing on a mixture of cheese whey and skim milk (1.49 ± 0.09a) was higher than in cheese whey medium without supplementation (1.21 ± 0.01c).

The type of cheese used in this study was mozzarella cheese. The type of cheese can affect the potential of whey as a medium for bacterial growth. This is by Macwan et al. [32], who examined the potential of cheddar cheese whey with Paneer cheese whey as a growth medium for *L. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides*, and *Lactococcus lactis* bacteria, the results showed that the four bacteria were able to grow at an average growth rate of 8.32 log CFU/mL, 9.16 log CFU/mL, 8.72 log CFU/mL and 9.82 log CFU/mL on cheddar cheese whey and 8.25 log CFU/mL, 9.19 log CFU /mL, 8.66 log CFU/mL and 9.80 log CFU/mL in paneer cheese whey.

Several factors that can affect the growth of bacteria include the condition of the

bacteria, nutrients, pH, temperature, and oxygen. In addition, bacterial growth can also be affected by the time [33]. In this study, the incubation time used was 24 hours. This is based on the research of Salma et al. [26], that the bacteria *L. helveticus* MTCC 5463 grown on cheese whey media with incubation intervals of 24-120 hours showed the highest growth, namely at a 24-hour incubation period of 9.73 log CFU/g and the rate of increase in the number of bacteria was very high. Later after an incubation period of 24 hours.

Mulyani et al. [34], stated that the longer the fermentation time, the more nutrients remodeled by lactic acid bacteria for growth. So there is a possibility that the number of bacteria in cheese whey media can still increase if it is incubated for a longer time. This statement is supported by the research of Manzano et al. [35], which showed that lactic acid bacteria are grown on fresh cheese whey medium initially were 4.86 log CFU/mL then increased to 7.63 log CFU/mL after 60 hours of incubation until finally decreased to 5.82 log CFU. /mL after incubation for 120 hours. The use of incubation time must also pay attention to the adequacy of nutrients in the media so that bacteria can still grow and do not reach the statistical phase or death phase when harvested [4].

Incubation time is a variable related to the growth phase of bacteria during the incubation process [33]. The length of the growth phase of each bacterium varies according to different species and environmental conditions. Therefore, it is necessary to observe the test parameters at certain time intervals to determine the optimum time for the growth of each bacterium [36].

Factors that affect the difference in total lactic acid in the experimental unit are thought to be related to factors that affect the number of bacterial cells because the calculation results of the two parameters are directly proportional. This is by the statement of Safitri et al. [12] that the amount of acid formed will be proportional to the number of bacteria that grow.

The nutrient content in the media can affect the total lactic acid produced. Therefore, it is necessary to adjust the nutritional content of cheese whey so that the total acid formed becomes more. Adjustment of the cheese whey media content can be done by adding supplements that can support bacterial growth. This is following the research of Perez et al. [11], regarding the addition of yeast extract into cheese whey media as a growth medium for *L. helveticus*, the results showed that the total lactic acid produced by bacteria in mixed media with cheese whey and yeast extract (3.7g/1 h) was more than in whey media. Cheese without the addition of yeast extract (3.1 g/1 h).

Incubation time can also affect the total lactic acid. According to Wulandari et al. [37], the longer the incubation time, the more carbohydrates are reshuffled, carbohydrate reshuffle will produce glucose and will eventually form lactic acid. This statement is supported by Mulyani et al. [34], that the longer the fermentation time, the more lactic acid bacteria will grow and the more lactic acid will be produced from their metabolic processes. This statement is supported by research by Mulyani et al. [38], the results showed that the lactic acid levels of beverages fermented for 48 hours (15,4550 ppm) were higher than drinks fermented for 24 hours (14,9169 ppm).

Another factor that is often associated with total lactic acid is the type of lactic acid fermentation. Based on the type of lactic acid fermentation, lactic acid bacteria can be divided into homofermentative and heterofermentative bacteria. Homofermentative bacteria will remodel almost all nutrients into lactic acid so that the amount of lactic acid produced by these bacteria tends to be much higher [28]. The *L. casei* group includes homofermentative bacteria so that each bacterium produces

lactic acid levels that are not much different [39].

The cheese whey and MRSB media before incubation had high sugar content and the values were almost the same, namely 314.58 mg/mL on cheese whey and 316.25 mg/mL on MRSB. After incubation, the total sugar remaining in the cheese whey medium was more than MRSB but not significantly different. Based on these results, it can be seen that the Lactobacillus casei group was easier to use sugar in MRSB media than cheese whey media. According to Lusi et al. [40], media viscosity can affect bacterial growth. Viscosity can be affected by the number of solids dissolved in the medium. The higher the amount of solids in the fermented liquid causes a decrease in the solubility of oxygen in the media, while bacteria need oxygen for growth. Therefore, media with low dissolved oxygen content can reduce the metabolic activity of bacteria [41].

The bacteria that used the most sugar were *L. paracasei*, which was 93.805 mg/mL on cheese whey media and 304.417 mg/mL on MRSB media. The results of this parameter calculation are by the results of the calculation of the number of bacterial cells and total lactic acid parameters, where the bacteria that showed the most growth were *L. paracasei* bacteria. Based on this, it can be seen that the factors that affect the total sugar are related to the factors that affect the number of bacterial cells and total lactic acid. According to Rama et al. [3], in addition to environmental factors, the ability of bacteria to grow on a medium can be influenced by the physiological and morphological conditions of the bacteria itself and the bacterial strain.

The high growth rate of *L. paracasei* compared to *L. casei* and *L. rhamnosus* could be due to the L. paracasei strain used which a better ability to utilize the nutrients had contained in cheese whey and MRSB media. This is by the statement of Kurniawati et al. [42], that different strains can affect the ability of bacteria to degrade substrates. This statement is supported by the research of Bottari et al. [10] (2017) about 14 strains of the *L. casei* group grown on milk media, the results showed that each bacterial strain gave different test results. The bacterial strains that had the best ability to ferment milk were *L. paracasei* strain 2461 (30.91 ± 0.06^{d}), while the worst were *L. paracasei* strain 2247 (23.01 ± 0.17^{a}) and 4202 (21. 70 ± 0.30^{a}). While the bacteria *L. casei* (strain 1247 (28.09 ± 0.56^{bc}), strain 2046 (29.51 ± 0.10^{cd}), and strains 2138 (29.30 ± 0.30cd) and *L. rhamnosus* strain 1216 (26.69 ± 0.42^{b}), strain 2075 (28.14 ± 0.18^{bc}), strain 2167 (29.17 ± 0.28^{cd}), and strain 2233 (29.88 ± 0.06^{cd}) showed insignificant results.

5. CONCLUSION

Based on the parameters used, the L. casei group (*L. casei*, *L. paracasei* and *L. paracasei*) did not have a significant difference. Cheese whey media had a poor ability to grow *L. casei* group compared to MRSB (positive control), but the results of statistical analysis showed no significant difference. This shows that cheese whey media can be used as an alternative medium for the growth of the *L. casei* group.

6. REFERENCES

1. Hudha, I., Dewi, K., Fitri, J., & Ayu, N. 2020. Potensi Limbah Keju (Whey) sebagai Bahan Pembuatan Plastik Pengemas yang Ramah Lingkungan. Jurnal Teknik: Media Pengembangan Ilmu dan Aplikasi Teknik. 19(1):46-52.

2. Martini, S., Bonazzi, M., Malorgio, I., Pizzamiglio, V., Tagliazucchi, D., & Solieri, L. 2021. Characterization of Yeasts Isolated from Parmigiano Reggiano Cheese Natural Whey Starter: from Spoilage Agents to Potential Cell Factories for Whey Valorization. Preprints.

3. Rama, G. R., Kuhn, D., Beux, S., Maciel, M. J., & Souza, C. F. V. D. 2019. Potential Applications of Dairy Whey for the

Production of Lactic Acid Bacteria Cultures. International Dairy Journal. 98:25-37.

4. Soenarno, M. S., Sumantri, C., Taufik, E., Nuraida, L., & Arief, I. I. 2019. Lactobacillus plantarum IIA-1A5 Fermentation Patterns by Using Whey, Buttermilk, and Whey Enriched by Skimmed Milk as Growth Media. Pakistan Journal of Nutrition. 18(3):288-295.

5. Hirsch, D. B., Alvarez, L. M. M., Urtasun, N., Baieli, M. F., Martinez, J. M. L., Glisoni, R. J., Miranda, M. V., Cascone, O., & Wolman, F. J. 2020. Lactoferrin Purification and Whey Protein Isolate Recovery from Cheese Whey Using Chitosan Mini-spheres. International Dairy Journal. 109.

6. Nursiwi, A., Utami, R., Andriani, M., & Sari, A. P. 2015. Fermentasi Whey Limbah Keju untuk Produksi Kefiran Oleh Kefir Grains. Jurnal Teknologi Hasil Pertanian. 3(1).

7. Nurminabari, I. S., Sumartini, & Arifin, D. P. P. 2018. Kajian Penambahan Skim dan Santan Terhadap Karakteristik Yoghurt dari Whey. Pasundan Food Technology Journal. 5(1).

8. Sitepu, G. A., Putri, E. R. M., & Inayah. 2020. Isolasi Enzim Laktase untuk Mengurangi Kadar Laktosa Susu bagi Penderita Intoleransi Laktosa. Prosiding The 11th Industrial Research Workshop and National Seminar.

9. Surono, I. S. 2016. Probiotik, Mikrobiome dan Pangan Fungsional. Yogyakarta: Deepublish.

10. Bottari, B., Felis, G. E., Salvetti, E., Castioni, A., Campedell, I., Torriani, S., Bernini, V., & Gatti, M. 2017. Effective Identification of Lactobacillus casei Group Species: Genome-Based Selection of The Gene MutL as the Target of a Novel Multiplex PCR Assay. Microbiology. 163: 950-960.

11. Perez, S. S., Velez, L. F., Davila, P. A., Cruz, G. C., & Arriaga, S. 2012. Production of Lactic Acid from Cheese Whey by Batch Cultures of Lactobacillus helveticus. Ann Microbiol. 62:313-317.

12. Safitri, N., Sunarti, T. C., & Meryandini, A. 2016. Formula Media Pertumbuhan Bakteri Asam Laktat Pediococcus pentosaceus Menggunakan Substrat Whey Tahu. Jurnal Sumberdaya Hayati. 2(2):31-38.

13. Yeni, Meryandini, A., & Sunarti, T. C. 2016. Penggunaan Substrat Whey Tahu Untuk Produksi Biomassa Oleh Pediococcus Pentosaceus E.1222. Jurnal Teknologi Industri Pertanian. 26(3):284-293.

14. Setyowulan, I. A., Nurlaili, E. P., Nurdyansyah, F., & Hasbullah, U. H. A. 2018. Pengaruh Konsentrasi Substrat Tepung Kulit Pisang Kepok dan Kecepatan Pengadukan Terhadap Pertumbuhan Lactobacillus acidophilus. Jurnal Teknologi Pertanian Andalas. 22(2).

15. Mukti, P. R., Feliatra, & Effendi, I. 2020. Growth of Bacteria Bacillus cereus in Liquid Media with Different Protein Sources. Journal of Coastal and Ocean Sciences. 1(1):35-40.

16. Suhartati, R., Sulistiani, & Nuraini, A. 2018. Pemanfaatan Serbuk Kacang Kedelai (Glycine max) Sebagai Bahan Pembuatan Media Manitol Salt Agar (MSA) Untuk Pertumbuhan Bakteri Staphylococcus. Prosiding Seminar Nasional dan Diseminasi Penelitian Kesehatan STIKes Bakti Tunas Husada Tasikmalaya.

17. Sulastri, A., Manguntungi, B., & Vanggy, L. R. 2020. Analisis Viabilitas Lactobacillus Lactis Pada Inovasi Media Dasar Pertumbuhan Alternatif dan Media Dasar Penepungan Bakteri Asam Laktat. Jurnal Tambora. 4(2).

18. Rosmania & Yanti, F. 2020. Perhitungan Jumlah Bakteri di Laboratorium Mikrobiologi Menggunakan Pengembangan Metode Spektrofotometri. Jurnal Penelitian Sains. 22(2):76-86.

19. Aviany, H., & Pujianto, S. 2020. Analisis Efektivitas Probiotik di Dalam Produk Kecantikan sebagai Antibakteri terhadap Bakteri Staphylococcus epidermis. Berkala Bioteknologi. 3(2).

20. Wulan, R., Meryandini, A., & Sunarti, T. C. 2017. Potensi Limbah Cair Industri Tapioka sebagai Media Pertumbuhan Starter Bakteri Asam Laktat Pediococcus pentosaceus E.1222. Jurnal Sumberdaya HAYATI. 3(1):27-33.

21. Nurjannah, L., Suryani, Achmadi, S. S., & Azhari, A. 2017. Produksi Asam Laktat Oleh Lactobacillus delbrueckii subsp. Bulgaricus Dengan Sumber Karbon Tetes Tebu. Jurnal Teknologi dan Industri Pertanian Indonesia. 9(1).

22. Lizayana, Mudatsir, & Iswadi. 2016. Densitas Bakteri pada Limbah Cair Pasar Tradisional. Jurnal Ilmiah Mahasiswa Pendidikan Biologi. 1(1):95-106.

23. Seniati, Marbiah, & Irham, A. 2019. Pengukuran Kepadatan Bakteri Vibrio harveyi Secara Cepat Dengan Menggunakan Spektrofotometer. Agrokompleks. 19(2).

24. Rohman, A., Dwiloka, B., & Rizqiati, H. 2019. Pengaruh Lama Fermentasi Terhadap Total Asam, Total Bakteri Asam Laktat, Total Khamir, dan Mutu Hedonik Kefir Air Kelapa Hijau (Cocos nucifera). Jurnal Teknologi Pangan. 3(1):127-133.

25. Aniriani, G. W., Apriliani, N. F., & Sulistiono, E. 2018. Hidrolisis Polisakarida Xilan Jerami Menggunakan Larutan Asam Kuat Untuk Bahan Dasar Produksi Bioetanol. 2018. Jurnal Ilmiah Sains. 18(2).

26. Salma, Prajapati, J. B., Hati, S., Sreeja, V., & Trivedi, J. 2017. Deproteinated Cheese Whey Medium for Biomass Production of Probiotic Lactobacillus helveticus MTCC 5463. International Journal of Current Microbiology and Applied Sciences. 6(3):174-187.

27. Noor, Z., Cahyanto, M. N., Indrati, R., & Sardjono, S. 2017. Skrining Lactobacillus plantarum Penghasil Asam Laktat untuk Fermentasi Mocaf. AGRITECH. 37(4).

28. Syachroni, Maruddin, F., Yuliati, F. N., & Mukhlisah, A. N. 2020. Karakteristik Mikrobiologi dan Kimiawi Susu Fermentasi Menggunakan Kultur Campuran Lactobacillus plantarum dan Lctobacillus acidophillus. Jurnal Sains dan Teknologi Peternakan. 1(2).

29. Bawole, K. V., Umboh, S. D., & Tallei, T. E. 2018. Uji Ketahanan Bakteri Asam Laktat Hasil Fermentasi Kubis Merah (Brassica oleracea L.) pada pH 3. Jurnal MIPA Unsrat Online. 7(2):20-23.

30. Zelvi, M., Suryani, A., & Setyaningsih, D. 2017. Hidrolisis Eucheuma cottonii Dengan Enzim K-Karagenase Dalam Menghasilkan Gula Reduksi Untuk Produksi Bioetanol. Jurnal Teknologi Industri Pertanian. 27(1):33-42.

31. Azhari, F., Winarsa, R., Siswanto, Muzakhar, K., Utarti, E., Sutoyo, &

Arimurti, S. 2021. Growth of Lactobacillus casei FNCC0900 in Media Based Umbi Porang Plant (Amorphallus muelleri BI.). BERKALA SAINTEK. 9(2):86-94.

32. Macwan, S. R., Parmar, S. C., Prajapati, J. B., & Aparnathi, K. D. 2016. Evaluation of Cheddar Cheese Whey and Paneer Whey as a Growth Medium for Lactic Acid Bacteria. Journal of Pure and Applied Microbiology. 10(2):1261-1268.

33. Kusuma, G. P. A. W., Nocianitri, K. A., & Pratiwi, I. D. P. K. 2020. Pengaruh Lama Fermentasi Terhadap Karakteristik Fermented Rice Drink Sebagai Minuman Probiotik Dengan Isolat Lactobacillus sp. F213. Jurnal Itepa. 9(2): 182-193.

34. Mulyani, S., Sunarko, K. M. F., & Setiani, B. E. 2021. Pengaruh Lama Fermentasi terhadap Total Asam, Total Bakteri Asam Laktat, dan Warna Kefir Belimbing Manis (Averrhoa carambola). Jurnal Ilmiah Sains. 21(2):113-118.

35. Manzano, M. A. M., Porchas, G. R. R., Velazquez, D. A. G., Llanez, M. J. T., Porchas, M. M., Sifuentes, C. O. G., Cordova, A. F. G., & Cordoba B. V. 2020. Cheese Whey Fermentation by Its Native Microbiota: Proteolysis and Bioactive Peptides Release with ACE-Inhibitory Activity. Fermentation. 6(19).

36. Zulaika, E., & Wahyuningsih, N. 2018. Perbandingan Pertumbuhan Bakteri Selulolitik Pada Media Nutrient Broth dan carboxy Methyl Cellulose. Jurnal Sains dan Seni ITS. 7(2).

37. Wulandari, F., Nazaruddin, & Amaro, M. 2021. Pengaruh Jenis Bakteri Asam Laktat dan Lama Fermentasi Terhadap Mutu Fisik, Kimia, Organoleptik, dan Mikrobiologi Tepung Mocaf. Prosiding SAINTEK LPPM Universitas Mataram. (3).

38. Mulyani, H., Sundowo, A., Filailla, E., & Ernawati, T. 2019. Pengaruh Penambahan Starter dan Waktu Inkubasi: Dark Coklat (Theobromo cacao L.) Terhadap Aktivitas Antioksidan dan Kualitas Minuman Probiotik. Jurnal Teknologi Pangan dan Gizi. 18(1): 25-32.

39. Hill, D., Sugrue, I., Tobin, C., Hill, C., Stanton, C., Ross, R. P. 2018. The Lactobacillus casei Group: History and Health Related Applications. Frontiers in Microbiology.

40. Lusi, Periadnadi, & Nurmiati. 2017. Pengaruh Dosis Gula dan Penambahan Ekstrak Teh Hitam Terhadap Fermentasi dan Produksi Nata De Coco. Jurnal Metamorfosa. 4(1): 126-131.

41. Putri, S. N. Y., Syahrani, W. F., Utami, C. V. B., Safitri, D. R., Arum, Z. N., Prihastari, Z. S., & Sari, A. R. 2021. Pengaruh Mikroorganisme, Bahan Baku, dan Waktu Inkubasi pada Karakter Nata: Review. Jurnal Teknologi Hasil Pertanian. 14(1): 62-74.

Kurniawati, L., Kusdiyantini, E., & Wijanarka. 2019. Pengaruh Variasi Suhu dan Waktu Inkubasi Terhadap Aktivitas Enzim Selulase dari Bakteri Serratia marcescens. Jurnal Akademika Biologi. 8(1).