Indonesian Journal of Medical Laboratory Science and Technology

Open Access p-ISSN 2684-6748 | e-ISSN 2656-9825

Analysis of Exopolysaccharides in *Lactobacillus casei* group Probiotics from Human Breast Milk

Nur Kusmiyati[1], Yuni Puspitasari[2], Ulfah Utami[3], Anggeria Oktavisa Denta[4]

[1]Agricultural Product Technology Department, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, East Java, Indonesia

[2]Biology Study Program, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, East Java, Indonesia

[3]Biology Study Program, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, East Java, Indonesia

[4]Nursing Study Program, Politeknik Negeri Madura, Sampang, East Java, Indonesia

Correspondence:
Nur Kusmiati,
Jl. Veteran, Lowokwaru, Malang, East Java, Indonesia
Zip Code: 65145

Email: kusmiy4tinur@gmail.com

Received: April 16, 2022
Revised: January 6, 2023
Accepted: March 27, 2023
Published: April 29, 2023

DOI: 10.33086/ijmlst.v5i1.2872

Abstract

Exopolysaccharides get a lot of attention because they can improve the host immune system. Exopolysaccharide is a polysaccharide that is produced and secreted from microbes outside the cell, usually found on the outside of the bacterial structure. The *Lactobacillus casei* group from human breast milk is thought to have the ability to produce exopolysaccharides. The purpose of the study was to examine the exopolysaccharide of the *L. casei* group that was isolated from breast milk. The methods used include the gravimetric, the phenol-sulfuric acid and the Fourier Transform Infra-Red (FTIR). The results showed that the *L. casei* group could produce exopolysaccharides, and had high exopolysaccharide total sugar content. *Lactobacillus paracasei* had the highest exopolysaccharide and total sugar content of 3660 mg/L and 80.6%, respectively. The FTIR results of the *L. casei* group exopolysaccharides showed the presence of hydroxyl functional groups O-H (3425.76-3295.98 cm^{-1}), methyl C-H (2930.86-2856.70 cm^{-1}), carbonyl C=O (1660.11-1647.27 cm^{-1}), C-H (1456.16-1373.44 cm^{-1}) and C-O-C ether (1071.08-1056.82 cm^{-1}) which are specific characters of exopolysaccharides. Since the FTIR profile demonstrates that the *L. casei* group can produce exopolysaccharides, it has greater potential as a a probiotic.

Keywords

Exopolysaccharide, Functional Ggroup, *Lactobacillus casei*.

Citation: Kusmiyati N, Puspitasari Y, Utami U, Denta AO. Analysis of Exopolysaccharides in Lactobacillus casei group Probiotics from Human Breast Milk. Indones J Med Lab Sci Technol. 2023;5(1):29–41. DOI: 10.33086/ijmlst.v5i1.2872

INTRODUCTION

Changes in lifestyle and poor diet in the community can disrupt the balance of the digestive tract microbiota, resulting in a decrease in the body's immune system (1). One of the prevention efforts against this is by restoring the balance of the immune system, like improving the health of the

digestive tract. According to Ahamad *et al*. (2), the digestive tract is a reflection of the immune system because almost 80% of the components of the immune system are found in the digestive tract. Improving the health of the digestive tract can be done by consuming probiotics.

Lactobacillus is one of the largest genera of lactic acid bacteria (LAB) and is often used as a probiotic. Research related to Lactobacillus probiotics is limited to the requirements that must be met as a probiotic, namely acid and bile salt resistance, can adhere to intestinal cells and survive in the intestinal tract, able to produce antimicrobial compounds, antagonists against pathogens, safe in food and clinically, proven to affect health, and comes from humans (1). The advantage of the Lactobacillus genus is that it has been declared a safe microorganism when added to food because it does not produce toxins, otherwise known as Generally Recognized As Safe (GRAS) microorganisms (3). According to Teame *et al*. (4), several microorganisms are probiotic which can produce exopolysaccharides.

Exopolysaccharides (EPS) are sugar polymers or polysaccharides secreted by microorganisms out of cells. EPS has a health effect because it can improve the immune system, so it is essential to analyze probiotics in producing EPS. According to Ahamad *et al*. (2), the *Lactobacillus casei* group derived from human breast milk is eligible as a probiotic. *L. casei* group consisting of *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* can be used as candidates to produce EPS.

EPS analysis can be done through EPS production and total sugar analysis (5). In addition to these two analyzes, to ensure the compound is an EPS is also needed. One method that can be used is the characterization of the functional group of a compound using Fourier Transform Infra-Red (FTIR) spectrophotometry (6). EPS functional group analysis using FTIR can provide complete information about the functional groups contained in the structure of an EPS compound.

The exploration of probiotic microorganisms that produce EPS is increasing because the ability of these microorganisms to synthesize EPS is considered very important for health. This study aimed to analyze the exopolysaccharide profile of the *L. casei* group derived from human breast milk based on parameters of crude extract content, total sugar, and identification of exopolysaccharide functional groups using FTIR.

MATERIALS AND METHODS

Materials used in this study are *L. casei* group (*L. casei*, *L. paracasei*, *L. rhamnosus*) isolated from human breast milk (7), *deMan Rogosa and Sharpe Broth* (MRSB)

(Himedia), *deMan Rogosa and Sharpe Agar* (MRSA) (Merck), sucrose, ethanol, trichloroacetic acid 10% (TCA), 70% alcohol (OneMed), spirits, distilled water, phenol 5%, concentrated H_2SO_4.

Bacterial Cultivation

This study used the *L. casei* group isolated from human breast milk in the study of Kusmiyati *et al*. (7). The cultivation of the *L. casei* group was carried out through culture. One ose of pure culture was taken and grown on 5 mL of MRSA-tilted media at 37 ºC for 24 hours. The rejuvenated species were used for inoculum stock preparation and exopolysaccharide (EPS) production analysis (8).

Exopolysaccharide production analysis

One ose of the isolate was inoculated into 20 mL of MRSB, which 5% sucrose had been added and incubated at 37 ºC for 24 hours. Inoculum was added to 20 mL of 10% TCA and homogenized with a 90 rpm shaker incubator for 30 minutes. Then it was centrifuged for 30 minutes at 4 ºC at 4500 rpm. The supernatant was taken and added to 96% cold ethanol twice. The sample was allowed to stand at 4 ºC for 24 hours, then centrifuged at 4 ºC at 4500 rpm for 30 minutes. The pellets were dried in an oven at 60 ºC and the EPS dry weight was weighed. EPS levels were calculated based on the method of Nurhasanah *et al*. (5) and are presented in Equation 1.

$$\text{EPS content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{EPS dry weight (mg)}}{\text{media volume (L)}}$$

Analysis of total sugar content

The total sugar content of EPS was determined by the phenol sulfuric acid method using glucose as the standard curve. The standard curve was made by means of 1 mL of a standard glucose solution containing 10, 20, 30, 40, 50 and 60 ppm. Glucose was put into a test tube and 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid were added. The solution was heated in a water bath at 40 ºC for 15 minutes and the absorbance was measured at 490 nm (9).

Exopolysaccharide: as much as 2 mg of crude EPS was dissolved in 50 mL of distilled water. A total of 1 mL of EPS solution was quickly added to 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid and left for 10 minutes. Next, the solution was heated in a water bath at 40 ºC for 15 minutes. The absorbance was measured at 490 nm (9).

Identification of exopolysaccharide compound profile with Fourier Transform Infra-Red (FTIR)

The Fourier Transform Infra-Red provides complete information about the functional groups contained in the structure of a compound. Thus, in this analysis, it can be ascertained that the isolate group can produce exopolysaccharides (10). 2 mg of dry exopolysaccharide was put into an agate mortar and mixed with 200 mg of potassium bromide (KBr). After that, the material is

ground until homogeneous. Next, the mixture is pressed and formed into pellets. The pellet is placed in the cell holder of the FTIR instrument. Dry exopolysaccharides and KBr were suppressed with a hydraulic suppressor with an FTIR spectrum at a wave number of 500-4500 cm^{-1} (11).

Data analysis

Data analysis was carried out descriptively, quantitatively and qualitatively. Quantitative data analysis used the product and total sugar of exopolysaccharides, while qualitative data analysis used the results of the FTIR profile of exopolysaccharide compounds. Product data and total sugar exopolysaccharide were tested for normality using Shapiro-Wilk and homogeneity tested using Levene Test. If the data is normal and homogeneous, then it is analyzed using the Analysis of Variance (ANOVA). If the variance in the data gives a significant difference, then the Duncan Multiple Range Test (DMRT) is carried out with a level of = 5%.

RESULTS

Exopolysaccharide production analysis

Exopolysaccharide production analysis showed that the *L. casei* group could produce exopolysaccharides (Figure 1). The calculation results show that *L. paracasei* can produce the highest exopolysaccharide, which is 3660 mg/L. Based on statistical analysis, the results were not different with *L. casei* and different ($P<0.05$) with *L. rhamnosus*.

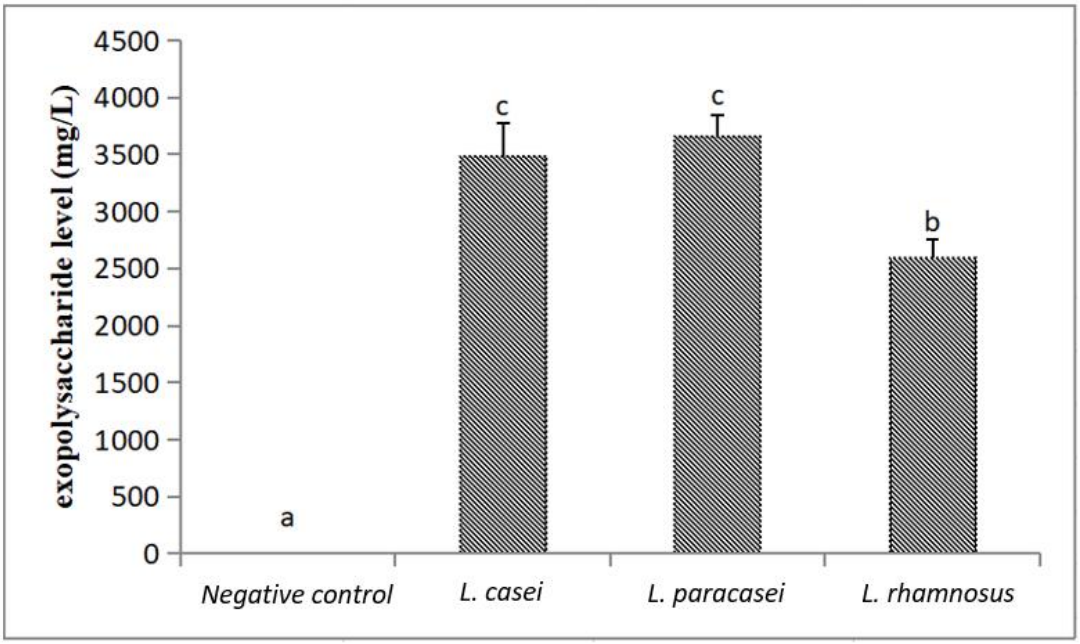

Figure 1. Exopolysaccharide content of the *L. casei* group

Exopolysaccharide total sugar analysis

The total sugar content of the exopolysaccharide group of *Lactobacillus casei* showed different results (Figure 2). The calculation results showed that the total sugar content of the highest exopolysaccharide in *L. paracasei* was 80.6%. This value was not significantly different in *L. casei* by 72.6%, and significantly different from *L. rhamnosus*. The research of Xu *et al*. (12) showed that the total sugar content of *L. casei* exopolysaccharide isolated from sauerkraut, northeastern China, by the phenol-sulfuric acid method was 88%.

Identification of exopolysaccharide functional groups using a Fourier Transform Infra-Red (FTIR) spectrophotometer

The analysis of exopolysaccharide spectra produced by the *L. casei* group showed different wave numbers (Figure 3). All species have functional groups indicating the presence of an exopolysaccharide in general, namely O-H (hydroxyl), C=O (carbonyl), C-H (methyl), and C-O-C (ether).

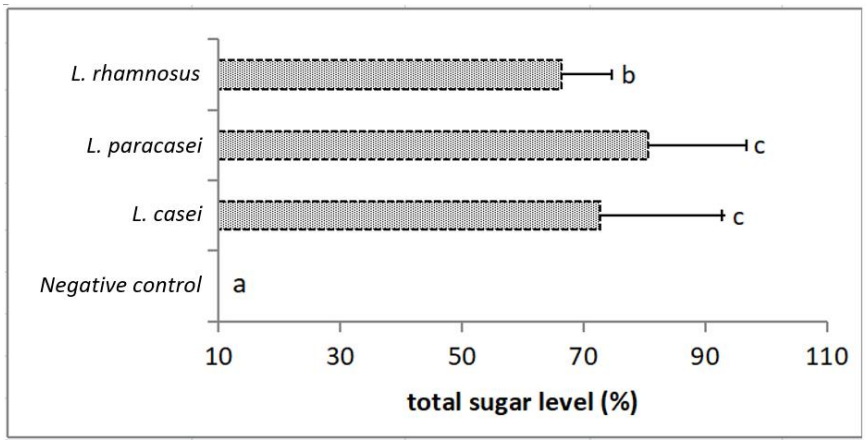

Figure 2. Total sugar content of the *L. casei* group of exopolysaccharides

The results of the exopolysaccharide spectra produced in the *L. casei* group of bacteria, namely the spectra at a wave number of 3295.98 cm^{-1}, indicated the presence of a hydroxyl group (O-H) with the type of *stretching* molecular vibration. The absorption band in the wave number region of 2926.59 and 2856.70 cm^{-1} represents the methyl group (C-H) with the type of *stretching* molecular vibration. The absorption band in the 1660.11 cm^{-1} region represents the *stretching* C=O vibration of the carboxyl group. Spectra at wave numbers 1453.31 and 1373.44 cm^{-1} indicate the presence of a group (C-H) with a *bending* molecular vibration type. The sharp absorption band at the wave number of 1071.08 cm^{-1} indicates the *stretching*

vibration of C-O-C of the ether functional group. The results of the FTIR spectra showed the presence of exopolysaccharides in the *L. casei* group.

An analysis of the results of the FTIR spectra of exopolysaccharides from the *L. casei* group regarding the similarity of the wave numbers obtained for each functional group is presented in Table 1. The *L. casei* group has eleven to twelve functional groups. *L. casei* had more functional groups than *L. paracasei* and *L. rhamnosus*.

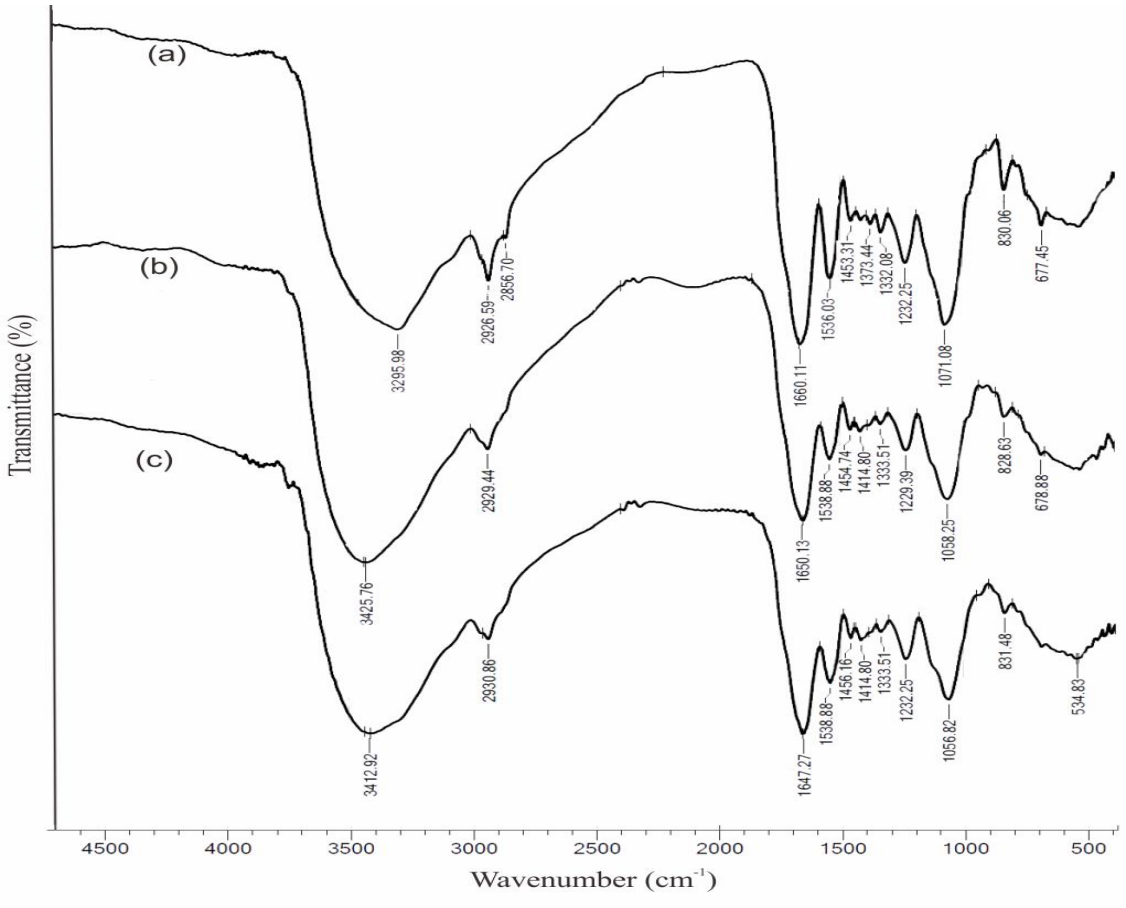

Figure 3. FTIR spectra of *L. casei* group exopolysaccharide; (a) *L. casei*, (b) *L. paracasei*, (c) *L. rhamnosus*.

Table 1. FTIR spectra of exopolysaccharides from the *L. casei* group

Functional groups	Vibration	Wave number (cm^{-1})			Wave number (cm^{-1}) (13)
		L. casei	*L. paracasei*	*L. rhamnosus*	
(O-H) Hydroxyl	*Stretching*	3295.9	3425.7	3412.9	3650-3200
(C-H) Methyl	*Stretching*	2926.5 and 2856.7	2929.4	2930.8	3000-2850
(C=O) Carbonyl	*Stretching*	1660.1	1650.1	1647.2	1850-1630
(C-H)	*Bending*	1453.3 and 1373.4	1454.7 and 1414.8	1456.1 and 1414.8	1465-1370
(C-O-C) Ether	*Stretching*	1071,08	1058.2	1056.8	1090-1000

Exopolysaccharide FTIR results in *L. casei* have similarities to previous studies. Differences in previous studies often occur in shifting wavenumbers but still show the similarity of functional groups. The *L. casei* group has all the functional groups required for a polysaccharide compound. According to Zhang *et al*. (14) functional groups that indicate the presence of an exopolysaccharide are O-H (hydroxyl), C=O (carbonyl), C-H (methyl), and C-O-C (ether) groups. This is following the research of Chambi *et al*. (15), who stated that FTIR analysis of exopolysaccharides had several carbohydrate-related peaks, including hydroxyl, methyl, carbonyl, and ether groups.

DISCUSSION

Exopolysaccharide production analysis

Genetics and individual phenotypes influence differences in the production of exopolysaccharides in LAB (16). Genetic traits are inherited traits of each bacterial species that are influenced by gene composition, while phenotypic traits tend to be influenced by environmental factors. In addition, the origin and species differences of LAB strains will be able to contribute to the richness and diversity of glucosyltransferase (gtf) genes, which will affect the diversity of enzymes in exopolysaccharide synthesis so that variations in exopolysaccharide production occur.

Anindita (16) reproted that four strains of *Weisella confusa* derived from breast milk produced different exopolysaccharides, namely *W. confusa* AS3 (1883 mg/L), *W. confusa* AS14 (1570 mg/L), *W. confusa* AS18 (1369 mg/L) and *W. confusa* AS21 (1480 mg/L). These results indicate that the ability to produce exopolysaccharides is influenced by differences in strains. The same species have varying capabilities in the production of exopolysaccharides. Fatih (17) added that the differences in the production of exopolysaccharides were caused by the different enzyme activities of each species.

The exopolysaccharide content of *L. casei* isolated from palm sap was lower (106.33 mg/L) (10) compared to the fermented colostrum kefir (1340 mg/L). In another study, *L. paracasei* exopolysaccharide levels were lower after 48 hours of fermentation (376.4 mg/L) (18) compared to fermented yoghurt (932.0 mg/L) (19). This study showed that *L. paracasei* produced an exopolysaccharide of 3660 mg/L, which is a fairly high value compared to existing studies. The production of exopolysaccharides in the *L. casei* group resulted in a high value because the MRSB medium was added with 5% sucrose. According to Malick *et al*. (20), bacterial culture conditions such as medium composition, pH, temperature, and incubation period have been shown to

significantly affect exopolysaccharide production.

The exopolysaccharide content of *L. rhamnosus* with the addition of hydrogen peroxide (H_2O_2) supplementation after 24 hours of incubation was 567 mg/L (21). The same study showed an increase in exopolysaccharides when given the addition of calcium chloride ($CaCl_2$) and H_2O_2 after 12 hours, namely 2498 mg/L. Research by Rajoka *et al*. (22) showed that *L. rhamnosus* could produce 1808 mg/L exopolysaccharides, while the study of Bertsch *et al*. (23) *L. rhamnosus* could produce 1095 mg/L exopolysaccharides without the addition of media supplementation.

Another factor that affects the production of exopolysaccharides is incubation time. In this study, the incubation time used was 24 hours. Incubation time must pay attention to the adequacy of nutrients in the media to ensure that bacteria can still grow by producing exopolysaccharides and do not reach a static or death phase when harvested. According to Angelin & Kavitha (24), exopolysaccharides are synthesized in the logarithmic or final logarithmic, and the stationary phase. However, maximum production occurs only in the late logarithmic phase rather than the stationary phase. According to Fan *et al*. (25), the production of exopolysaccharides is often associated with the growth phase of bacteria. Some exopolysaccharides are only synthesized in the late logarithmic or stationary growth phase, while others are synthesized during the microbial growth process. The quantity of exopolysaccharide produced varies between strains, media composition, and culture conditions such as pH, temperature and carbon ratio.

According to Al-Manhel (26), the concentration of carbon sources is one of the most important factors in achieving high levels of exopolysaccharide production. In this study, the 5% sucrose addition increased the production of exopolysaccharides more than previous studies, which were without the addition of sucrose. According to Bibi *et al*. (27) *Weisella cibaria* C43-11 grown in liquid media and enriched with 10% sucrose showed high exopolysaccharide production. The addition of excessive sugar in the culture medium also increased the production of LAB exopolysaccharides. The increase in sucrose concentration in MRS media was suitable for exopolysaccharides production in *Lactobacillus confusus* TISTR 1498. The exopolysaccharide produced by the *L. casei* group in this study showed that the *L. casei* group had the potential to be developed other than as a probiotic.

Exopolysaccharide total sugar analysis

The total sugar content of exopolysaccharides is influenced by the amount of sugar contained in the production medium and the activity of the invertase

enzyme, which plays a role in converting the substrate into its constituent sugar monomers (28). According to Imran *et al.* (29), the total sugar content in the exopolysaccharide *L. plantarum* NTMI05 was 95.45% and *L. plantarum* NTMI20 was 92.35%. This indicates the presence of total sugar in the exopolysaccharide produced by each different strain. In the same study, the total sugar content of *L. helveticus* MB2-1 was obtained at 95.45%. In another study, the total sugar content of *L. pentosus* 14FE was 81.38%, *L. plantarum* 47FE was 83,28%, *L. pentosus* 68F was obtained 85.19%, indicating that all exopolysaccharides consist of carbohydrates (30).

The total sugar content of *Bacillus subtilis* exopolysaccharide in the research of Razack *et al*. (31) with media without supplementation showed a yield of 53%, while media supplemented with 2% sucrose showed a yield of 76%. This indicates that the medium has an affect on the total sugar content of the exopolysaccharide.

Exopolysaccharide production and exopolysaccharide sugar content obtained from the three bacteria in the *L. casei* group showed varying results due to different species or strains. According to Nurhasanah *et al*. (5) differences in yield and total sugar content of exopolysaccharides due to differences in strains between species. Differences in bacterial strains cause the number of metabolites produced to also be different. Fatih (17) added that the number of exopolysaccharides produced by different LAB was caused by inherited traits or genetic traits. The analysis of the total sugar exopolysaccharide of the *L. casei* group in this study showed a relationship between the total sugar content of the exopolysaccharide and the crude production of exopolysaccharides. This analysis supports the presence of polysaccharides produced by the *L. casei* group and improves the quality of the probiotics of the *L. casei* group.

Identification of exopolysaccharide functional groups using a Fourier Transform Infra-Red (FTIR) spectrophotometer

The FTIR results of this exopolysaccharide can provide information that the *L. casei* group is capable of producing exopolysaccharides. Exopolysaccharides can be used as a form of self-defence by the *L. casei* group. In addition, exopolysaccharides in lactic acid bacteria can also be used to improve the host immune system. According to Jurášková *et al*. (32), exopolysaccharides produced by lactic acid bacteria can increase macrophage activity and cytokine production and stimulate the formation of immunoglobulin-A (IgA).

According to Mundiri *et al*. (33), exopolysaccharides are known to have the potential to act as immunomodulators that play a role in the innate immune system in

digestion. Exopolysaccharides of lactic acid bacteria can enhance the innate immune system through the role of gut-associated lymphoid tissue (GALT). The use of exopolysaccharides as immunomodulators has been widely carried out and has been proven in-vitro and in-vivo to increase macrophage activity, cytokine production and ability to stimulate IgA formation. In the research of Inturri *et al*. (34), exopolysaccharides from lactic acid bacteria, *Bifidobacterium longum* can increase the production of IFN- γ , IL-1 β , and IL-6. According to Domingos-Lopes *et al*. (35), exopolysaccharides from the lactic acid bacteria *Leuconostoc citreum* L3C1E7 can suppress the synthesis of allergen-specific IgE and exopolysaccharides from lactic acid bacteria have been shown to have immunomodulatory activity with non-toxic effects. According to Rajoka *et al*. (36), the exopolysaccharides of Lactobacillus contain various functional groups (such as hydroxyl groups, phosphate groups, and carbonyl groups), which help to exert their immunomodulatory, antimicrobial, antioxidant, and anticancer activities. Exopolysaccharides from Lactobacillus can be used as nutritional and therapeutic agents to regulate the immune system, which can help fight various diseases such as cancer, diabetes, and hypertension. Therefore, the addition of Lactobacillus as exopolysaccharide agents immunomodulatory in functional foods has potential in the future.

CONCLUSIONS

The *L. casei* group was able to produce exopolysaccharides (EPS). *L. paracasei* was able to produce the highest EPS (3660 mg/L) and total sugar (80.6%). Confirmation by FTIR showed that the *L. casei* group could produce EPS characterized by hydroxyl, methyl, carbonyl and ether functional groups. This study shows that the *L. casei* group has more value than just being a probiotic.

AUTHOR CONTRIBUTIONS

Nur Kusmiyati: substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Yuni Puspitasari: rafting the article or revising it critically for important intellectual content. Ulfah Utami and Anggeria Oktavisa Denta: agreement to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGEMENTS

This research was funded by Lembaga Penelitian dan Pengabdian Masyarakat (LP2M) UIN Maulana Malik Ibrahim Malang through Bantuan Operasional Perguruan Tinggi Negeri (BOPTN) Litapdimas Kementerian Agama Indonesia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Othman NZ, Mohd Din ARJ, Mohammad Z, Rosli MA, Sarmidi MR. Statistical optimization of medium compositions for high cell mass and exopolysaccharide production by *Lactobacillus plantarum* ATCC 8014. Appl Food Biotechnol. 2018;5(2):87–96. DOI: 10.22037/afb.v5i2.19299
2. Ahamad N, Kar A, Mehta S, Dewani M, Ravichandran V, Bhardwaj P, et al. Immunomodulatory nanosystems for treating inflammatory diseases. Biomaterials. 2021;274(April):120875. DOI: 10.1016/j.biomaterials.2021.120875
3. Sasikumar K, Kozhummal Vaikkath D, Devendra L, Nampoothiri KM. An Exopolysaccharide (EPS) from a *Lactobacillus plantarum* BR2 with potential benefits for making functional foods. Bioresour Technol. 2017;241:1152–6. DOI: 10.1016/j.biortech.2017.05.075
4. Teame T, Wang A, Xie M, Zhang Z, Yang Y, Ding Q, et al. Paraprobiotics and postbiotics of probiotic lactobacilli, their positive effects on the host and action mechanisms: A review. Front Nutr. 2020;7(October). DOI: 10.3389/fnut.2020.570344
5. Nurhasanah, Fu'adah IT, Satria H, Yuwono SD. Analisis eksopolisakarida dari bakteri asam laktat hasil fermentasi kefir kolostrum. [Analysis of exopolysaccharides from lactic acid bacteria resulting from colostrum kefir fermentation.] Anal Environ Chem. 2020;5(1):65–73. DOI: 10.23960/aec.v5.i1.2020.p65-73
6. Khan SA, Khan SB, Khan LU, Farooq A, Akhtar K, Asiri AM. Fourier Transform Infrared Spectroscopy: fundamentals and application in functional groups and nanomaterials characterization. Handb Mater Charact. 2018;(September):1–613. DOI: 10.1007/978-3-319-92955-2
7. Kusmiyati, N., Wicaksono, S.T., Sukarno, A.S. Isolation and characterization of probiotic lactic acid bacteria from human breast milk. Nova Biotechnol Chim. 2022;21(2): e1053. DOI: 10.36547/nbc.1053
8. Sulastri A, Manguntungi B, Vanggy LR. Analisis Viabilitas *Lactobacillus lactis* pada Inovasi Media Dasar Pertumbuhan Alternatif dan Media Dasar Penepungan Bakteri Asam Laktat. [Viability analysis of *Lactobacillus lactis* on innovation of alternative growth base media and lactic acid bacterial flourishing basic media] J TAMBORA. 2020;4(2):16–22. DOI: 10.36761/jt.v4i2.635
9. Ma'unatin A, Harijono H, Zubaidah E, Rifa'i M. The Isolation of exopolysaccharide-producing lactic acid bacteria from lontar (*Borassus flabellifer* L.) sap. Iran J Microbiol. 2020;12(5):437–44. DOI: 10.18502/ijm.v12i5.4605
10. Winahyu DA, Primadiamanti A. Bioaktivitas Antioksidan Lotion Senyawa Eksopolisakarida dari Mikroalga Spirulina sp. [Bioactivity antioxidant lotion exopolysaccharide compound from microalgae *Spirulina* sp]Anal Anal Environ Chem. 2020;5(02):169–77.
11. Pinaria, Y. W., Antara, N. S., Putra, G. G., & Sujaya IN. Characterization of exopolysaccharide produced by *Lactobacillus casei* AL15 isolated from sap of *Arenga pinnata*. J Nat Sci Res. 2016;6(22):7–12. Available from: www.iiste.org
12. Xu X, Qiao Y, Peng Q, Shi B, Dia VP. Antioxidant and Immunomodulatory properties of partially purified exopolysaccharide from *Lactobacillus casei* isolated from Chinese Northeast Sauerkraut. Immunol Invest. 2021;00(00):1–18. Available DOI: 10.1080/08820139.2020.1869777
13. Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan JR. Introduction to spectroscopy. 3P rd P Ed. Paint Testing Manual. 2016. p. 545-545–2.
14. Zhang Z, Zhou Z, Li Y, Zhou L, Ding Q, Xu L. Isolated exopolysaccharides from *Lactobacillus rhamnosus* GG alleviated adipogenesis mediated by TLR2 in mice. Sci Rep. 2016;6(May):1–14. DOI: 10.1038/srep36083
15. Chambi D, Romero-Soto L, Villca R, Orozco-Gutiérrez F, Vega-Baudrit J, Quillaguamán J, et al. Exopolysaccharides production by cultivating a bacterial isolate from the hypersaline environment of Salar De Uyuni (Bolivia) in

pretreatment liquids of steam-exploded quinoa stalks and enzymatic hydrolysates of curupaú sawdust. Fermentation. 2021;7(1):1–16. DOI: 10.3390/fermentation7010033

16. Anindita NS. Identifikasi glukosiltransferase (gtf) penyandi eksopolisakarida pada strain *weisella confusa* probiotik asal air susu ibu (ASI). [Identification of exopolysaccharide encoding glucosyltransferase (gtf) in the weisella confusa probiotic strain from breast milk]. J Pangan dan Agroindustri. 2020;8(2):75–85. DOI: 10.21776/ub.jpa.2020.008.02.3
17. Fatih MT. Produksi eksopolisakarida oleh bakteri asam laktat asal susu kacang tanah terfermentasi. [Production of exopolysaccharides by lactic acid bacteria from fermented peanut milk] Malang: UIN Maulana Malik Ibrahim Malang. 2020. Available from: http://etheses.uin-malang.ac.id/id/eprint/24215
18. Bhat B, Bajaj BK. Hypocholesterolemic potential and bioactivity spectrum of an exopolysaccharide from a probiotic isolate *Lactobacillus paracasei* M7. Bioact Carbohydrates Diet Fibre. 2019;19(December 2018):100191. DOI: 10.1016/j.bcdf.2019.100191
19. Li XW, Lv S, Shi TT, Liu K, Li QM, Pan LH, et al. Exopolysaccharides from yoghurt fermented by *Lactobacillus paracasei*: Production, purification and its binding to sodium caseinate. food hydrocoll. 2020;102(December 2019):105635. DOI: 10.1016/j.foodhyd.2019.105635
20. Malick A, Khodaei N, Benkerroum N, Karboune S. Production of exopolysaccharides by selected Bacillus strains: optimization of media composition to maximize the yield and structural characterization. Int J Biol Macromol. 2017;102:539–49. DOI: 10.1016/j.ijbiomac.2017.03.151
21. Ng IS, Xue C. Enhanced exopolysaccharide production and biological activity of *Lactobacillus rhamnosus* ZY with Calcium and Hydrogen Peroxide. Process Biochem. 2017;52:295–304. DOI: 10.1016/j.procbio.2016.10.006
22. Rajoka MSR, Jin M, Haobin Z, Li Q, Shao D, Jiang C, et al. Functional Characterization and biotechnological potential of exopolysaccharide produced by *Lactobacillus rhamnosus* strains isolated from human breast milk. LWT - Food Sci Technol. 2018;89:638–47. DOI: 10.1016/j.lwt.2017.11.034
23. Bertsch A, Roy D, LaPointe G. Enhanced exopolysaccharide production by *Lactobacillus rhamnosus* in co-culture with *Saccharomyces cerevisiae*. Appl Sci. 2019;9(19). DOI: 10.3390/app9194026
24. Angelin J, Kavitha M. Exopolysaccharides from probiotic bacteria and their health potential. Int J Biol Macromol. 2020;162:853–65. DOI: 10.1016/j.ijbiomac.2020.06.190
25. Fan Y, Wang J, Gao C, Zhang Y, Du W. A Novel exopolysaccharide-producing and long-chain n-alkane degrading bacterium *Bacillus licheniformis* strain DM-1 with potential application for in-situ enhanced oil recovery. Sci Rep. 2020;10(1):1–10. DOI: 10.1038/s41598-020-65432-z
26. Al-Manhel AJA. Production of exopolysaccharide from local fungal isolate. Curr Res Nutr Food Sci. 2017;5(3):338–46. DOI: 10.12944/CRNFSJ.5.3.19
27. Bibi A, Xiong Y, Rajoka MSR, Mehwish HM, Radicetti E, Umair M, et al. Recent advances in the production of Exopolysaccharide (EPS) from *Lactobacillus* spp. and its application in The Food Industry: A Review. Sustain. 2021;13(22):1–19. DOI: 10.3390/su132212429
28. Azizah FR. Pengaruh penambahan sukrosa pada air kelapa dan lama fermentasi terhadap produksi eksopolisakarida oleh *Leuconostoc mesenteroides*. [Effect of adding sucrose to coconut water and fermentation time on exopolysaccharide production by *Leuconostoc mesenteroides*]. Malang: UIN Maulana Malik Ibrahim Malang. 2019. Available from: http://etheses.uin-malang.ac.id/id/eprint/15207
29. Imran MYM, Reehana N, Jayaraj KA, Ahamed AAP, Dhanasekaran D, Thajuddin N, et al. Statistical optimization of exopolysaccharide production by *Lactobacillus plantarum* NTMI05 and NTMI20. Int J Biol Macromol. 2016;93:731–45. DOI: 10.1016/j.ijbiomac.2016.09.007
30. Saif FAA, Sakr EAE. Characterization and Bioactivities of Exopolysaccharide produced from probiotic *Lactobacillus plantarum* 47FE and *Lactobacillus pentosus* 68FE. Bioact Carbohydrates Diet Fibre. 2020;24(April):100231. DOI: 10.1016/j.bcdf.2020.100231
31. Razack SA, Velayutham V, Thangavelu V. Medium optimization for the production of exopolysaccharide by *Bacillus subtilis* using synthetic sources and agro wastes. Turkish J Biol. 2013;37(3):280–8. DOI: 10.3906/biy-1206-50
32. Jurášková D, Ribeiro SC, Silva CCG. Exopolysaccharides Produced by lactic acid bacteria: from biosynthesis to health-promoting properties. Foods. 2022;11(2). DOI: 10.3390/foods11020156
33. Mundiri NA, Megantara I, Anggaeni TTK. Kajian pustaka: pemanfaatan eksopolisakarida bakteri asam laktat probiotik asal produk pangan fermentasi sebagai imunomodulator. [Literature

review: utilization of probiotic lactic acid bacteria exopolysaccharide from fermented food products as an immunomodulator] Indones Med Veterinus. 2020;9(5):849–59. DOI: 10.19087/imv.2020.9.5.849

34. Inturri R, Mangano K, Santagati M, Intrieri M, Di Marco R, Blandino G. Immunomodulatory effects of *Bifidobacterium longum* W11 produced exopolysaccharide on cytokine production. Curr Pharm Biotechnol. 2017;18(11):883–9. DOI: 10.2174/1389201019666171226151551

35. Domingos-Lopes MFP, Nagy A, Stanton C, Ross PR, Gelencsér E, Silva CCG. Immunomodulatory Activity of exopolysaccharide producing *Leuconostoc citreum* strain isolated from pico cheese. J Funct Foods. 2017;33:235–43. DOI: 10.1016/j.jff.2017.03.054

36. Rajoka MSR, Wu Y, Mehwish HM, Bansal M, Zhao L. Lactobacillus Exopolysaccharides: New perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health. Trends Food Sci Technol. 2020;103(March):36–48. DOI: 10.1016/j.tifs.2020.06.003