Development of Skin Microbiome Technology in Skin Care Products as a Solution to Maintain and Improve Health Skin

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Abstract. This proposed research is part of a series of studies in an effort to develop and apply skin microbiome technology to skincare products so that skin health can be maintained. The role of the skin microbiome is very important so that the balance on the skin surface needs to be maintained to provide protection or as a skin barrier. However, the problem that arises is that the presence of the skin microbiome can decrease over time, this can be triggered by the presence of our bodies such as genes, diet, lifestyle, age, environment, exposure to UV rays and the use of skincare which actually reduces the balance of variations in the skin microbiome. Therefore, there is a need for microbiome therapy to restore skin function and health. The aims of this study were (1) to find an emulgel formula containing prebiotics, parabiotics and postbiotics from Lactobacillus which is safe and capable of providing protection to the skin in accordance with SNI requirements and contains active ingredients that contribute as antibacterial pathogens that cause skin infections (2) to find Emulgel lotion formula enriched with prebiotics, parabiotics and postbiotics that have antibacterial activity of pathogens that cause skin infections, (3) determine the level of safety, preference, moisture, smoothness and antibacterial activity in vitro. Methods: This research method includes lactobacillus fermentation as a starter culture, manufacture of parabiotics and postbiotics, emulgel formulations, antibacterial tests, and physical evaluation of emulgels. Results: The results of this study are expected to obtain emulgel cosmetic preparations enriched with prebiotic, parabiotic and postbiotic based skin microbiome technology that can be applied topically to avoid skin damage due to unbalanced skin microbial conditions. Conclusions: These results suggest that prebiotic, parabiotic and postbiotic of Lactobacillus casei have use to made the emulgel cosmetic.

Keywords: Emulgel · Lactobacillus Lactis · Prebiotic · Parabiotic · Postbiotic

1 Introduction

The skin is one of the largest organs that almost completely covers the surface of the body. The skin functions as a barrier / protection of the body from various pathogens and injuries. Given the importance of skin function for the body, we must pay attention to skin

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health by providing care and nourishing the skin properly. One of the important functions of the skin is as a habitat for the growth of millions of microorganisms such as bacteria, fungi and viruses that form a colony in the stratum and pores. This coexistence is known as the microbiome [1] [2] [3]. The skin microbiome will maintain a balance with the surrounding community, so that it can defend against pathogens from outside, and prevent infection in the body [3] [4]. With age, the composition of the microbiome changes due to exposure to UV rays and various chemicals. These changes in the composition of the microbiome also accelerate skin aging, because the microbiome and skin cells in the body interact with each other. The composition of the microbiome depends on the environment in which the bacteria grow, the sex of a person, and age [6] [7] [3] The composition of the skin microbiome greatly determines the health condition of our skin, therefore the presence of the skin microbiome is very important to maintain its sustainability.

In general, the research results used for the development of skin care products are natural ingredients derived from plants whose active compounds are utilized to provide certain bioactivity. The use of microorganisms as a source of active ingredients for the development of skin care products has not been widely reported, therefore it is necessary to explore this topic. One of the bacteria that can be used for skin therapy purposes or can be used as an active ingredient in skin care is lactobacillus. Lactobacillus is one of the lactic acid bacteria known as endogenous inhabitants of healthy skin, when inflammatory skin conditions are often associated with a disturbed skin microbiome. Lactobacillus-based probiotics are currently being explored as new treatments for inflamed skin through topical skin application against pathogens and inflammation. According to the research results [8] showed that lactobacillus can inhibit the growth of pathogenic bacteria and has immunomodulatory activity to reduce inflammation. The results of Lactobacillus plantarum fermentation can also inhibit the process of melanogenesis in cells melanoma B16F10.

Various efforts have been made for the purpose of treating and maintaining health, but the selection of inappropriate cosmetic products will actually worsen skin health conditions. This is because many cosmetic products contain preservatives and antimicrobials, causing the balance of the skin microbiome to change both in terms of quantity and diversity. Therefore, the development of skin microbiome technology needs to be explored regarding its bioactivity in maintaining skin health and the components of chemical compounds that are responsible.

2 Materials and Methods

2.1 Microorganism Material And Parabiotic-Postbiotic Preparation

The main ingredient that will be used in this research is *Lactobacillus lactis*. Other ingredients include Nutrient agar (NA) bacterial growth media, Muler Hinton Agar (MHA), and ingredients for the formulation of emulgel formulas including ethanol, span 80, paraffin (liquid), propylene glycol, tween 80, methylparaben, propylparaben, carbopol 940, triethanolamine. And perfume.

2.2 Methods

All procedures were carried out in a sterile condition (Class II biohazard cabinet) and aseptic techniques are applied. Good microbia culture practice guidance was exercised during the whole process.

2.2.1 Parabiotic Preparation Procedure

Lactobacillus lactis culture was fermented on MRSB media which amounted to 108 CFU/g (ml). The culture was incubated at 37°C for 18 days as a starter culture which is the basis for producing prebiotics and parabiotics. Isolate in centrifugation at 4000xg for 10 minutes, then separated the metabolites to get organic acid/ SCFA, bacteriocin, H2O2. The biomass was taken using tyndalization and dried, and stored in a freezer at 4°C. These dead cells are known as Parabiotics.

2.2.2 Postbiotic Preparation Procedure

The culture of fermented isolates was separated by biomass to obtain metabolites in the form of supernatant free from bacterial cells by lysing. Then it is filtered and dried in a freezer at 4°C. These products are known as Postbiotics.

2.2.3 Emulgel Preparation Procedure.

Emulgel was made in four formulas, namely: 0% active ingredient as formula 0 (control), 2% prebiotic as formula 1, parabiotic 2% as formula 2, postbiotic 2% as formula 3.

Prebiotics, parabiotics and postbiotics in ethanol were mixed with the oil phase. (span 80 in liquid paraffin). The aqueous phase is prepared by dissolving tween 80 in water. Methylparaben and propylparaben as preservatives were dissolved in propylene glycol and then added to the aqueous phase. Each phase was heated to a temperature of 70°C, mixed in the aqueous phase, stirred, then allowed to stand until it reached room temperature until an emulsion was formed. Then 1% gelling agent of Carbopol 940 was dissolved in the aqueous phase and stirred, then triethanolamine was added. Emulsion formulas of prebiotics, parabiotics and postbiotics, paraffin (liquid), span 80, and tween 80 were mixed into the aqueous phase of Carbopol 940 (Table 1).

2.2.4 Physical Evaluation Of Emulgel

Organoleptic Test. Emulgel preparations were evaluated organoleptically including observing color changes, smelling aromas and textures of the preparations. The specifications for emulgel preparations that must be met are having a homogeneous preparation color, fragrant aroma and liquid texture.

Test Homogeneity. This homogeneity test was carried out by means of an emulgel preparation that had been made on a clean and dry slide to form a thin layer and then covered using an object glass, then observed under a microscope, seen the color was uniform or not. Emulgel preparations were declared homogeneous if the results of the observation using a microscope the texture of the preparations looked flat and there were no lumps.

Table 1. Formulas of emulgel enriched of prebiotic, parabiotic, and postbiotic from Lactobacillus lactis

Componen	Formula 0	Formula 1	Formula 2	Formula 3
Prebiotic	0	1	0	0
Parabiotic	0	0	1	0
Postbiotic	0	0	0	1
Ethanol	1	1	1	1
Span 80	1,25	1,25	1,25	1,25
Paraffin (liquid)	3,75	3,75	3,75	3,75
Propylene glycol	2,5	2,5	2,5	2,5
Tween 80	0,25	0,25	0,25	0,25
Methylparaben	0,015	0,015	0,015	0,015
Propylparaben	0,004	0,004	0,004	0,004
Carbopol 940	0,25	0,25	0,25	0,25
Triethanolamine	0,25	0,25	0,25	0,25
Perfume (drops)	2	2	2	2
Aquadest (ad)	50	50	50	50

* Formula 0: Base of Emulgel (w/w), Formula 1: Emulgel of prebiotic (Inulin), 2% (w/w), Formula 2: Emulgel of Parabiotic *Lactobacillus lactis*, 2% (w/w), Formula 3: Postbiotic *Lactobacillus lactis*, 2% (w/w).

Measurement of pH. The pH test was carried out using a pH meter at room temperature. The newly made emulgel preparation was directly measured by measuring the pH by placing the emulgel preparation into a glass beaker then measuring the pH with a pH meter that had previously been calibrated with a standard buffer solution (pH 4 and pH 7). According to SNI 06-4085-1996, emulgel can be said to be good if it has a pH of 8-11.

Viscosity Test. Measurement of the viscosity of the emulgel preparation using a Brookfield type RV viscometer, the preparation was put into a 250 mL beaker glass, then the spindle was lowered into the preparation to the specified limit. Measurements were made at a speed of 50 rpm and then the scale was read when the moving red needle stabilized. Then the viscosity value is calculated [9]. The viscometer in a good emulgel preparation is 500-20,000 cP [10]

Spreadability Test. The transparent glass is placed on the graph paper. On the glass is placed 0.5 g of emulgel preparation then covered with another transparent glass and left for ± 5 seconds to get the diameter of the area formed. Then proceed with increasing the load on the transparent glass with a load of 50, 100, 200, and 500 g respectively and observing the diameter of the area formed. The specification of the preparation is that the emulgel preparation can be spread easily and evenly.

Emulsion Type Test. A total of 1 drop of lotion preparation was placed on an object glass plus 1 drop of methylene blue solution, mixed evenly, observed under a microscope,

Table 2. Numerical scale on the stock assessment test

Hedonic scale	Numerical scale
Really like	5
Like	4
Neutral	3
Dislike	2
Very dislike	1

a homogeneous blue color was formed in the outer phase indicating the formation of an oil-in-water (w/o) emulsion.

Hedonic Test. According to Charpenter et al. (2000), the hedonic test is an acceptance test that aims to evaluate the panelists' acceptance of the product with the parameters of aroma, sensation on the skin and color of the preparation using 20 panelists aged 17 to 25 years who are treated to samples of lotion preparations. The rating scale can be seen in Table 2.

2.2.5 Evaluation of The Antibacterial Activity of Emulgel

Suspension of *S. aureus* with a concentration of 1.5 x 10^5 bacterial cells/mL which has been prepared by following 0.5 McFarland standard, 1 ml was put into a sterile petri dish and 15 mL of Muller Hinton medium was poured, the mixture was homogenized by shaking and the media was allowed to harden. Wells are made using a sterile cork drill. This well will be filled with emulgel preparation, positive control of levofloxacin 5 g/well and negative control of distilled water using a micropipette. Placement of wells in the media in order to have its own conditions such as, each well must have the same distance of 2 cm from the edge of the cup and the distance between the wells is 3 cm and a depth of 4 mm. After the whole process was completed, all the petri dishes were put in an incubator at 37°C for 18-24 hours. The zone of inhibition that appears on each agar is then measured using a caliper.

2.3 Statistical Analysis

Statistical analysis was performed with Microsoft Excel LTSC Professional Plus 2021 software for Windows. The results are expressed as mean±SD, to determine the difference between each concentration variation and the percentage of cell viability.

3 Results and Discussion

3.1 Characteristics Of Emulgel

In Table 3 it is known that the emulgel treatment was followed by a test proganoleptic. The basic formula and formula 3 have a semi-solid form, smooth texture, and have a fresh aroma, but formulas 2 and 3 which contain parabiotic and post biotic have an acidic

aroma which is the aroma character of *L. lactis*. The color character resulting from the manufacture of emulsion on the basic emulgel and F1 is white, while formulas 2 and 3 are cream colored. The color difference in formulas 2 and 3 is yellowish because it is produced from the MRSA medium used in culturing *L. lactis* (Figure 1).

Table 4 shows that the emulgel formulation of probiotics, prebiotics and postbiotics *L. lactis* complies with SNI, which has a pH of 7, which in this case is in accordance with SNI which is in the range of 4.5-8. In the dispersion test, the emulgel has a wide range, ranging from 8.43 to 9.10. The type of emulsion is in the form of oil in water, is homogeneous, and the incubation at room temperature for two weeks is still stable.

Table 5 is the inhibition test of the emulgel using S. aureus. In this table it can be seen that the formulation of emulgel of Probiotics, Prebiotics and Postbiotics *L. lactis* has the potential to inhibit *Staphylococcus aureus*. Inhibition ranged from 6.33-8.33. Formulation F3 which contains posbiotics is greater than formulations 2 and 3 which contain prebiotics and parabiotics.

Table 3. Organoleptic test

Organoleptic Parameter	**Result**			
	Base/F0	F1	F2	F3
Form	Semi solid	Semi solid	Semi solid	Semi solid
Texture	Fine	Fine	Fine	Fine
Scent	Fresh (perfume)	Fresh (perfume)	Fresh (perfume) & lactic acid bacteria smell (<)	Fresh (perfume) & lactic acid bacteria smell (>)
Color	White	White	Creamy white (<)	Creamy white (>)

Fig. 1. Emulgel enriched of prebiotic, parabiotic, and postbiotic

Table 4. Physical properties

Parameter		**Result**			**Requirement**
	Base	F1	F2	F3	
pH	7	7	7	7	4,5–8
Spreadability (Average of spreadability (cm^2) + SEM)	8.43 ± 0,06	8.50 ± 0,00	8.67 ± 0,06	9.10 ± 0,10	-
Emulsion Type	o/w	o/w	o/w	o/w	-
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Stability	Stabil	Stabil	Stabil	Stabil	Stabil

Table 5. Antibacterial activity

Sampel	**Average of Inhibition Zone (mm) ± SEM**	**Inhibition Zone Category**
Base/F0	0	0
F1	6,33 ± 0,58	Moderate
F2	7,67 ± 0,58	Moderate
F3	8,83 ± 0,29	Moderate

* Formula 0: Base of Emulgel (w/w), Formula 1: Emulgel of prebiotic (Inulin), 2% (w/w), Formula 2: Emulgel of Parabiotic *Lactobacillus lactis*, 2% (w/w), Formula 3: Postbiotic *Lactobacillus lactis*, 2% (w/w).

4 Conclusion

This study obtained emulgel cosmetic preparations enriched with prebiotic, parabiotic, and postbiotic-based skin microbiome technology that can be applied topically to avoid skin damage due to unbalanced skin microbial conditions.

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Conflict of Interest Statement. We declare that we have no conflict of interest.

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