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Bioactive Fractions from *Streptococcus Macedonicus* MBF 10-2 Produced In an Optimized Plant-Based Peptone Medium

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ABSTRACT

Postbiotic fractions of lactic acid bacteria have been reported for their potential applications as skin health's microbial therapeutics and may also appeal to consumers who wish to avoid animal-based products. We aim to establish the optimum plant-peptone fermentation of *Streptococcus macedonicus* MBF10-2, which possesses Bacteriocin Like-Inhibitory Substance activity in our previous study, to produce bacterial bioactive fractions. We evaluate their potential antibacterial and antioxidant actions and assess the preliminary safety for human skin application. Fermentation was carried out using plant peptone modified *de Man, Rogosa,* and Sharpe (MRS) medium, i.e., soy peptone and Vegitone, non-animal-carbon sources that substitute proteose peptone in MRS medium. Fractions of MBF10-2 lysate and cell-free supernatant were collected and processed as follows, i.e., cell disruption, fraction separation, and fractions freeze-drying. Fractions were confirmed for antibacterial properties by the agar well diffusion method and assess for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), while safety assessment was carried out by skin patch assay. Maximum growth of MBF10-2 achieved by fermentation in soy peptone- and in Vegitone-modified media was 9.00 and 7.99 g total cell mass, respectively. The antibacterial property of fractions was most effective against *Micrococcus luteus* T18. The lysate fraction exhibited a mild antioxidant potency (IC_{50} 840 μg/mL), and all bioactive fractions were proven safe and non-allergenic for human skins. *S. macedonicus* MBF10-2 postbiotics bioactive fractions were indicated as being safe for topical application. This is the first report on the production of a safe *S. macedonicus* bioactive postbiotic possessing mild antibacterial and mild-to-weak antioxidant.

Keywords: antibacterial; antioxidant; lysate; soy peptone; *Streptococcus macedonicus* MBF 10-2

INTRODUCTION

As the attention towards microbiome therapeutics increases, it has been intensively studied on how this treatment could influence human health, immunity, and different disease states, as well as on the possibility of the development of novel therapeutic agents, the modification of antibiotic policies, and implementation, and thereof improved health outcomes (ElRakaiby *et al*., 2014; Mimee, Citorik, and Lu, 2016; Reardon, 2014). The utilization of lyophilized bacterial extracts for therapeutic purposes has recently gained new interest despite its introduction back in the 1970s (Cazzola, Capuano, Rogliani, and Matera, 2012). The emergence of problems associated with the viable cell–based therapeutics, e.g., probiotics treatment, in terms of safety and stability prompted new development of living cell-free metabolites, often termed as postbiotics. Postbiotics are soluble factors containing metabolic by-products secreted by viable bacteria or obtained after bacterial lysis. Several soluble factors contained in postbiotics are short-chain fatty acid, enzymes, peptides, muropeptide from peptidoglycan, endopolysaccharides, exopolysaccharides, cell surface proteins, vitamin, plasmalogen, and organic acids (Konstantinov, Kuipers, and Peppelenbosch, 2013; Tsilingiri and Rescigno, 2012). The employment of postbiotics for health purposes

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possess advantages over viable bacteria or probiotics. Postbiotics are considerably safer than probiotics due to the absence of immunogenicity caused by administering live bacteria that can cause bloating, bacteremia, and transfer of antibiotic resistance genes (Doron and Snydman, 2015; Shenderov, 2013). Discoveries of antimicrobial and immunomodulatory activities from cell-free supernatant of lactic acid bacteria (LAB) cultures proved the therapeutic benefits of postbiotics (Iordache *et al*., 2008).

Lactic acid bacteria have been widely used in microbiology, biotechnology, food industries, and the health sector for their role in producing essential compounds such as lactic acid, ethanol, carbon dioxide, bacteriocin peptides, exopolysaccharides (EPS), oligosaccharides, enzymes, vitamins, and aromatic compounds (Fessard and Remize, 2017). Bacteriocin peptide is the main compound produced by many bacteria known to possess antimicrobial activity for various applications, including food preservation and health treatments (Cotter, *et al.* 2005; De Vuyst and Leroy, 2007). For decades,, many strains of LAB have been developed as bioactive agents for skin therapeutics due to the ability of several components of the bacteria, i.e., cell wall fragments, metabolites, and lysate containing lactic acid, to modulate skin's immune response and therefore increase protection by stratum corneum (Lew and Liong, 2012). Moreover, lactic acid produced by LAB can act as antimicrobial agents that inhibit the growth of several skin pathogens and increase ceramide production by keratinocytes (Lew and Liong, 2013).

Since the last decade, the intensive utilization of LAB strains in postbiotic therapy development for skin health has been reported, i.e., *Bifidobacterium longum* lysate as immunity enhancer for sensitive skin; *Vitreoscilla filiformis* lysate as prevention and improvement of hyper seborrhea in the scalp due to hypersecretion of sebum; and a mixture of *Lactobacillus acidophilus* NCFM, *Lactobacillus salivarius* Ls-33, as well as *Bifidobacterium lactis* 420 cell-free metabolites for modulation of skin diseases related to tight junction activity (Genard, 2015; Guéniche *et al*., 2010; Putaala, *et al.* 2016).

As a homofermentative strain of lactic acid bacterium (LAB) that produces lactic acid as a single end product, *Streptococcus macedonicus* (König and Fröhlich, 2017) is known to produce a food-grade lantibiotics called Macedocin and Macedovicin, bacteriocin peptide with an approximate molecular weight of 2,500 Da and 3,400 Da, exhibiting a broad spectrum of inhibition against several LAB, Gram-positive spoilage bacteria, and pathogenic bacteria (Georgalaki *et al*., 2002). A strain *S. macedonicus* MBF10-2, which was isolated from tofu by-product and has been known to produce exopolysaccharides such as glucans and fructans (Malik, *et al.*, 2009), was able to produce bacteriocin that inhibited the growth of *Micrococcus luteus, Streptococcus pyogenes, Lactococcus lactis, Leuconostoc mesentroides,* and *Weisella confusa* as reported (Grazia *et al*., 2017).

Previously, we reported a small-scale optimization of *Streptococcus macedonicus* MBF 10-2 lysate production in plant-based soy peptone modified-MRS and MRS Vegitone media employing Response Surface Methodology (RSM) to reduce the uses of animal-derived products in human use (Andyanti, Dani, Mangunwardoyo, Sahlan, and Malik, 2019). Such animal-derived issues have been rising since the emergence of a transmissible prion disease known as bovine spongiform encephalopathy (BSE) (Casalone and Hope, 2018). Other preferences, i.e., vegetarian and halal, are also accommodated as well.

In this study, MBF10-2 inactivated fractions of cell free-supernatant (CFS) and lysate were further characterized for their potential in modulating skin health to develop bacterial bioactive therapeutics. This study aims to authenticate the optimum bacterial fermentation and the preparation of bioactive fractions production of Strep. macedonicus MBF 10-2 in a plant-based de Man, Rogosa, and Sharpe (MRS) medium and evaluate its skincare potential as an antioxidant as well as its sensitivity towards the skin. Fermentation was carried out in plant-based MRS according to our previous study with an adjustment, and the cell disruption employed was optimized as described (Andyanti *et al*., 2019).

MATERIALS AND METHODS

Bacterial strains and growth condition

The strain used for bioactive fractions production is *Strep. macedonicus* MBF 10-2 (Malik *et al*., 2009), a facultative anaerobic strain of lactic acid bacterium that can grow generally in de Man, Rogosa, Sharpe (MRS) medium (De Man, Rogosa, and Sharpe, 1960) at 37°C. For antibacterial properties confirmation, indicator strains for MBF 10-2 bacteriocin activity reported in our previous study (Grazia *et al*., 2017) were used in this study, i.e., *Micrococcus luteus* T18, *Leuconostoc mesentroides* TISTR 120, and *Streptococcus*

pyogenes FF22. *M. luteus* T18 was routinely cultured at 37°C for 24-48h under aerobic conditions, while *Leuc. mesentroides* TISTR 120 and *Strep. pyogenes* FF22 were cultured at 37°C for 24-48h under anaerobic conditions. Strains that are normally live in human skin as commensal bacteria were also used in this study, i.e., *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Corynebacterium diphtheria*; all were routinely cultivated in 37 °C incubator for 24 h under anaerobic conditions.

Fermentation of *S. macedonicus* MBF 10-2 in a 2-L flask bioreactor

A frozen working cell bank was used to inoculate 18 mL soy peptone modified-MRS broth in two sterile glass test-tubes. After overnight incubation, the 20mL of bacterial inoculum was inoculated into 180mL of soy peptone modified-MRS broth and incubated at 37°C for 24h without shaking. Then 200mL of the inoculum was used to inoculate 1800mL soy peptone modified-MRS broth aseptically in a 2L flask fermenter using BioFlo®/CelliGen® 115 benchtop fermenter [Eppendorf, Germany]. The fermenter was run at 37°C for 11h, according to previous optimization (Andyanti *et al*., 2019). Bacterial cells were harvested by centrifugation at 8,000rpm, 4°C for 20mins, and then the cell pellet was kept frozen at -20°C. The cell-free supernatant (CFS) was divided; one for direct freeze-drying using Buchi Lyovapor™ L-300 [Buchi, Switzerland], and the other one for fractionation using Centrifugal Filter Units, Ultra-15 Amicon®, [Thermoscientific, USA] at 3, 10, and 30kDa molecular weight cut-off (MWCO) prior to freeze-drying.

Bacterial cell disruption

The bacterial cells disruption was carried out by both mechanically and mechanic-enzymatic combination, by using an ultrasonic homogenizer [Sartorius Stedim, Germany] and by adding lysozyme in a 50mL falcon tube using a ¼" probe (6 mm). Frozen cell pellets were suspended in Tris-HCl pH 8.0 buffer solution 3X of cell pellet weights and were homogenized using a vortex mixer. A 0.01% (v/v) protease inhibitor phenylmethylsulphonyl fluoride (PMSF) per sample was added, homogenized, and incubated on ice for 5min prior to cell disruption. The mechanical lysis was done by using an ultrasonicator for 40 cycles with the duration of 75s with a pause of 15s per cycle, and a duty cycle of 0.5s, at an amplitude of 75%. Whereas, mechanic-enzymatic lysis was carried out by first adding 2mg/mL lysozyme subsequently after the addition of PMSF, which then homogenized and incubated on ice for 30min prior to treatment with ultrasonicator. The lysate was obtained by separating cell debris (pellets) using a refrigerated centrifuge at 8,000 rpm and 4°C for 40min; the supernatant fraction (lysate) was kept in a 10mL vial in -20°C prior to freeze-drying (Andyanti *et al*., 2019).

Antibacterial property

Strep. macedonicus MBF 10-2 lysate and CFS antibacterial property against indicator strains were confirmed by performing agar well diffusion assay as described (Boateng and Diunase, 2015) with some modifications. *Leuconostoc mesenteroides* TISTR 120 and *Micrococcus luteus* T18 were used as indicator strains, grown in MRS agar and in Tryptic Soy Agar, respectively. The cell amount applied (10^7 CFU/mL) was prepared according to (McFarland, 1907) by using McFarland III as the reference and added to subsequent agar medium in ratio 1:4 (v/v). Agar wells were prepared by using a sterile stainless-steel cylinder (diameter 6mm). Each well base formed was then sealed with 20µL of molten agar medium. Fractions with various concentrations were deposited into the wells, i.e., 200,000ppm, 20,000 ppm, 2,000ppm, and 200ppm, at 50µL each. Nisin [activity: 10^6 IU/g from Sigma-Aldrich Pte Ltd.] as a positive control and sterile water as a blank reference were used. The plates were incubated overnight in an upside position, and the zone of inhibition formed was observed and determined by using calipers (Kronvall, *et al.,* 2003).

DPPH radical scavenging assay

Antioxidant activity of *Strep. macedonicus* MBF 10-2 fractions were measured using DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging test. A total of 100µL sample volume was added with 100µL of 0.5mM DPPH solution on 96 well-plate and was incubated at room temperature in a dark place for 30min. The absorbance was then measured by using a spectrophotometer at 517nm. Sterile soy-peptone modified-MRS medium was used as a blank reference, and ascorbic acid was used as a positive standard sample. All assays were carried out in triplicates (Xing *et al*., 2015).

Skin sensitivity test

Subject recruitment was carried out by a purposive sampling method. Healthy subjects aged 18-25 years with Indonesian ethnicities of Chinese, Papuans, and Javanese who provided informed consent were recruited for the skin patch test. All participants must not have tattoos or wound marks on their arms. Participants must be free from skin diseases (i.e., atopic dermatitis, psoriasis, and severe skin inflammations). The study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. KET-430/UN2.F1/ETIK/PPM.00.02/2020).

Fractions of lysate and CFS of *Strep. macedonicus* MBF 10-2 at 5% suspension were applied on the arm skin and covered by a waterproof skin patch. Sterile water was used as a blank reference. After 48 h, the patch was removed, and skin reactivity was observed visually in the next 24h.

Data analysis

Antibacterial property, antioxidant activity and skin sensitivity were analyzed by performing simple data analysis of triplicate measurements. Calculation of inhibition percentage of antioxidant activity was carried out by:
Inhibition (%) = (1-Sample absorbance/Control absorbance) x 100 %. Whilst, skin reactivity assay was employed to assess skin sensitivity, by using International Contact Dermatitis Research Group (ICDRG) guidelines for grading (Lazzarini, *et al.,* 2013).

RESULT AND DISCUSSION

Performance of plant-based nitrogen sources in *Streptococcus macedonicus* MBF10-2 Fermentation

To determine the best plant-based medium for optimum cell growth of *Strep. macedonicus* MBF10-2, the fermentation was conducted in two different media containing different plant-peptone sources, i.e., soy peptone modified-MRS, and MRS proteose peptone vegetable (Vegitone), resulted pH decreases with increasing time during the fermentation, reaching the lowest endpoint of around 4.5 after 11h and 17h, respectively. Dissolved oxygen (DO) decreased until six hours and then remained stagnant after. The two consecutive-cycle fermentations in a 2L fermenter yielded an average cell pellet wet mass of 8.20g, 9.00g, and 7.99g in standard MRS, soy peptone modified-MRS, and MRS Vegitone, respectively, showing that soy peptone modified-MRS was the best, and therefore was chosen as the optimum medium for *Strep. macedonicus* MBF 10-2 fermentation.

The importance of monitoring the supernatant pH and dissolved oxygen (DO) levels were due to lactic acid production observation during the homofermentative pathway; the deterioration of supernatant pH confirmed the metabolism of dextrose into lactic acid by glycolysis (Vuyst and Tsakalidou, 2008; König and Fröhlich, 2017). Results showed that the level of DO halted after 6h, which marked the commencement of the stationary phase of *Strep. macedonicus* MBF 10-2 growth and the production of non-growth associated products, which is in agreement (Dunford, 2012).

Following the medium optimization of peptone source for MRS, fermentation of *Strep. macedonicus* MBF10-2 was continued in soy peptone modified-MRS medium for 11h for two consecutive cycles. An adequate amount of starter culture prepared by staged pre-inoculation steps from the same frozen stock batch as a fixed working cell bank ensures a low risk of contamination and uniformity in cell procurement (Yang and Sha, 2019).

Production of *Streptococcus macedonicus* MBF 10-2 fractions

Potential activities and yield possessed by the fractions of CFS and cell lysate were assayed, while, in order to gain a reproducible yield of bacterial bioactive fraction, the process of lysis chosen was considered based on those parameters. Two different lysis method was carried out, i.e., mechanical and mechanical-enzymatic lysis.

The number of cycles and pH of lysis buffer that was optimized for effective lysis of *Strep. macedonicus* MBF 10-2, and judgment was made by visual density and microscopic observation of lysed cell is 40 cycles of ultrasonication and pH 8.0 of lysis buffer, which is considered as the best lysis condition. Comparison of cell lysates obtained by ultrasonication and by a combination of ultrasonication and enzymatic lysis were observed under the microscope; the latter displayed a denser color than that of yielded by mechanically only, indicates the more complete breaking of cells. Freeze-drying of lysate fraction resulted as white hygroscopic powder, while of cell-free supernatant fractions, a yellowish hygroscopic crude powder formed (Figure 1).

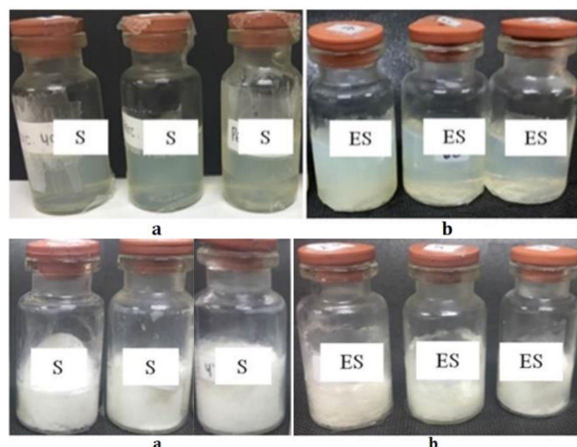

Figure 1. The lysate production process of *Streptococcus macedonicus* MBF10-2 fermentation using both mechanical and enzymatic methods for disruption and freeze-drying. A) after disruption and separation by centrifugation, the lysate appeared as cloudy liquid-fraction; and B) after freeze-drying, the lysate powder turned into white to yellowish white. S, by ultrasonication; ES, by enzymatic and ultrasonication treatments.

Table I. Minimum Inhibitory Concentration (MIC) of cell lysate against indicator strains and cell free supernatant

Bacteria strains	**MIC (μg/mL)**		
	Lysate(mec)	**Lysate (mec+enz)**	**CFS**
Leuc. mesenteroides TISTR 120	1,000	50,000	250
M. luteus T18	250	500	31.25
Strep. pyogenes FF-22	>200,000	>200,000	>200,000
Sta. aureus	>200,000	>200,000	100,000
Sta. epidermidis	>200,000	>200,000	100,000
C. diphtheriae	200,000	200,000	100,000

The higher yield was observed in lysate attained by a combination of ultrasonication and enzymatic method (~10.95%), which is 0.88% higher than that of ultrasonication only, thus confirmed that the lysozyme indeed helped the disruption efficiency of the bacterial cell (Table I).

Antibacterial properties

Result of the agar well diffusion assay, which was able to accommodate larger sample volumes (50-150μL) against indicator strains, was performed for both MBF 10-2 lysate and CFS fractions. Indicator strains were inhibited by *Strep. macedonicus* MBF 10-2 as previously reported, i.e., *Leuc. mesenteroides* TISTR 120 and *M. luteus* T18 (Grazia *et al*., 2017). Optimization of cell amount used was carried out, which was determined by visual observation as a lawn of colonies observation, and then converted it in to score. The optimum cell amount for *Leuc. mesentroides* TISTR 120 was $1x10^6$CFU/mL of inoculum for 24h incubation time in microaerobic condition at 32°C, while for *M. luteus* T18 was $1x10^7$ CFU/mL for 48h in aerobic condition at 37°C. The most active inhibition was shown against *M. luteus* T18 as a clear inhibition zones (6–18.2mm), (Table II). Thus, *M. luteus* T18 was chosen as the major indicator strain throughout the study, while Nisin was used as positive bacteriocin reference. All MBF10-2 CFS and lysate fractions were shown to be able to inhibit the growth of *M. luteus* T18.

The inhibition activity in comparison to Nisin showed that the relative activity of lysate and cell-free supernatant fractions against *M. luteus* T18 were 3.83; 3.72; 1.87; and 2.46IU/mg for CFS fraction; sterile-filtered CFS fraction; lysate by ultrasonication fraction; and lysate by an ultrasonication-lysozyme fraction, respectively, with an inhibition zones diameter limited between 12.0 and 13.5mm (Table II). Relative potency to Nisin was in (IU/mg).

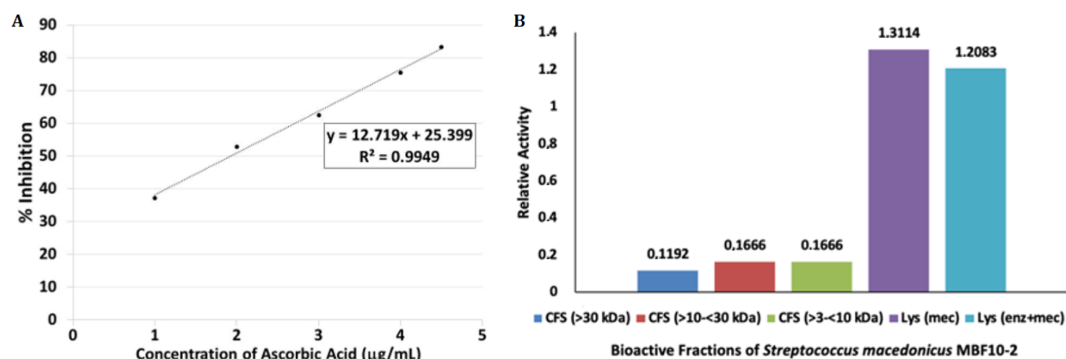

Figure 2. Antioxidant activity of *Streptococcus macedonicus* MBF10-2 fractions using DPPH. A) standard curve of reference standard of ascorbic acid; B) relative activity of *Streptococcus macedonicus* MBF10-2 fractions.

Table II. Inhibition of DPPH for antioxidant assay of MBF10-2 fractions

Samples	Relative activity*	Inhibition (%)
CFS fraction >30 kDa	0.1192	49.673
CFS fraction 10-30 kDa	0.1666	50.844
CFS fraction 3-10 kDa	0.1666	46.605
Lysate (mec)	1.3114	51.483
Lysate (mec+enz)	1.2083	49.431

*Relative activity to reference of Ascorbic acid

It is shown that the antibacterial potencyof *Strep. macedonicus* MBF 10-2 against *M. luteus* T18 were indeed significantly lower than that of Nisin. However, the bacterial lysate and CFS fractions from *Strep. macedonicus* MBF 10-2 all demonstrated equal potencies, confirming the release of intracellular bacteriocin by lysis. In comparison, lysate fraction of combination of the enzymatic and mechanic method showed higher antibacterial strength than that of mechanic-lysate only. Thus, significant higher efficiency in cell lysis by the combination of ultrasonication-lysozyme is confirmed.

Antioxidant properties

For bioactive agents applied in skincare pharmaceutics, antioxidant properties of *Strep. macedonicus* MBF 10-2 lysate and CFS fractions were determined; the IC_{50} values was measured based on DPPH assay in relative Unit Activity (UA) using ascorbic acid as reference (Figure 2 A). *Strep. macedonicus* MBF 10-2 lysate showed low IC_{50} values, i.e., 1.3114UA and 1.2083UA, for lysate fractions obtain by sonication and by the combination of sonication and lysozyme, respectively, which revealed a mild antioxidant. Meanwhile, CFS fractions showed no potential antioxidant activity as indicated by IC_{50} values 0.1192UA; 0.1666UA; and 0.1666UA for CFS fractions >30kDa, 10-30kDa and 3-10kDa, respectively. All unit activity relative of fractions (Figure 2 B), whereas inhibition potency of samples (Table II).

Overall, postbiotic *Strep. macedonicus* bioactive fractions, which require high concentration to reach the effective antioxidant potencies, are none reported. The exact mechanisms of postbiotic bioactivities have not been fully elucidated, however, a greater antioxidant capacity was observed in lysate rather than CFS fractions. This finding reveals that *Strep. macedonicus* MBF 10-2 postbiotic possessed mild to low antioxidant probiotic than *Bifidobacterium animalis* (Shen, *et al.,* 2011), while it is more appropriate as the ingredients of daily skincare bioactive agents. *B. animalis* 01 cell-free supernatant was observed for its highest inhibitory activity of 73.11%, while the cell lysate's inhibitory potential was only 27.72%. Shen *et al* (2011) however, did not apply freeze drying prior to the assay, thus the exact IC_{50} value of B remained unknown.

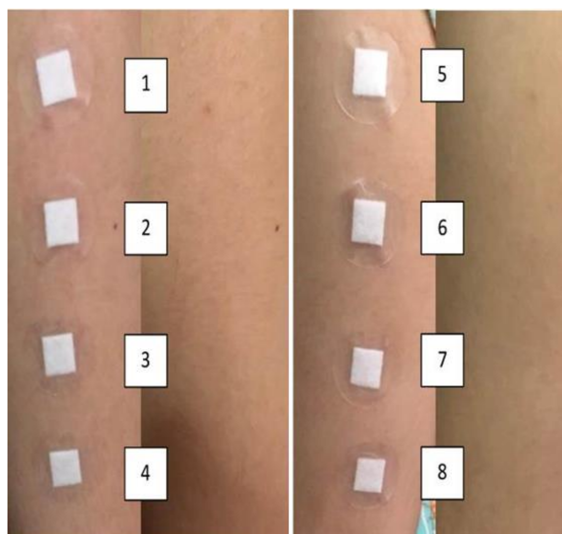

Figure 3. Skin sensitivity assay of *Streptococcus macedonicus* MBF10-2 fractions on the skin of Indonesian young adult volunteers.

Skin sensitivity test

Due to its prospect to be used as skin bioactive ingredients, *Strep. macedonicus* MBF 10-2 postbiotics were tested for their skin reactivity. From twelve Indonesian subjects, none of them showed any signs of erythema, infiltrations, papules, or vesicules (Figure 3). In such wise, *Strep. macedonicus* MBF 10-2 postbiotics were regarded safe for topical application without signs of allergies or irritation. Without any sensitivity reaction observed, a further repeated open application test (ROAT) would not be needed (Lazzarini *et al*., 2013).

Optimization of media composition has been carried out by RSM previously (Andyanti *et al*., 2019), and in this study, the amount of peptone and dextrose added have been adjusted to achieve optimum production of the lactic acid and the antibacterial substances by *Strep. macedonicus* MBF 10-2. Carbon and nitrogen contents in the medium are essential nutritional parameters in bacterial growth and synthesis of intra- and extracellular metabolites (Dinarvand *et al*., 2013). Protein synthesis is prone to decrease C/N ratios due to shifts in transcript level of certain genes required for the uptake and metabolism of carbon sources and the formation of by-products (Kumar and Shimizu, 2010; Truong, Koch, Yoon, Everard, and Shanks, 2013). Soy peptone modified-MRS uses enzymatically digested soybean meal as primary nitrogen source while MRS Vegitone uses pea flour treated with fungal enzymes as the primary nitrogen source. According to the manufacturer, total nitrogen content in animal-based peptone used in standard MRS medium was ≥10%, while total nitrogen content in plant-based peptone was ≥8% and ≥10% for soy peptone and vegitone, respectively. Organic and inorganic nitrogen sources are known to improve cell growth and synthesis of enzymes. Carbon limitation with adequate amount of nitrogen have showed maximum enzyme production due to channeling of more nitrogen for cell growth and intra- and extracellular enzymes production (Dinarvand *et al*., 2012; Dinarvand *et al*., 2012).

Several studies highlighted the advantages of soy peptone as a nitrogen source for the production of various metabolites. Soy whey-based medium facilitated higher phytase activity of *Saccharomyces cerevisiae* and improved galactosidase and antibacterial activities in *Lactobacillus plantarum* (Roopashri and Varadaraj, 2014). Growth of *L. plantarum* in animal-free medium containing liquid acid protein residue of soybean, soy peptone, corn steep liquor, and raw yeast extract demonstrated improvement in biomass and lactic acid production (Coghetto, Vasconcelos, Brinques, and Ayub, 2016). Substitution of Brain-Heart Infusion (BHI) and sheep blood by soy peptone in the culture medium of *Streptococcus zooepidemicus* showed no metabolic deviations and preserved hyaluronic acid production (Benedini and Santana, 2013). Li *et al*. compared different nitrogen sources in the fermentation medium of *Streptococcus thermophilus* for exopolysaccharide (EPS) production and obtained the highest EPS yield when using soy peptone (Li *et al*., 2016). Nitrogen supplementation using soy peptone exhibited protease production by *Bacillus mojavensis* despite being lower than yeast extract (Hammami, Bayoudh, Abdelhedi, and Nasri, 2018). Another study has also stated soy peptone as the more desirable peptone source for the cultivation of probiotics such as *Lactobacillus* and *Bifidobacterium* in the vegetarian application (Heenan, Adams, Hosken, and Fleet, 2002). Besides the ability to maintain cell activity in metabolite production, economic considerations also encouraged the utilization of soy peptone as bacto-peptone replacement, as soy peptone offers a remarkably cheaper nitrogen source alternative to the standard bacto-peptone (Saha, 2006).

Several factors should be considered in determining cell disruption methods for large-scale production in compare to lab-scale, i.e., the force

required, efficiency, product stability, and safety standards. Among all cell disruption methods, ultrasonication and enzymatic disruption are the most commonly applied in research- and industrial-scale (Phong *et al*., 2018). The Ultrasonication method employs cavitation shear force to break down bacterial cells and release intracellular substances. This method provides advantages such as the ability to be scaled-up, used in continuous operation, and ability to be combined with other methods, despite the need for cooling to prevent overheating and preserve product stability (Bystryak, Santockyte, and Peshkovsky, 2015; Pchelintsev, Adams, and Nelson, 2016). Enzymatic disruption utilizes lysozyme, a glycoside hydrolase that catalyzes the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan, causing damage in cell wall integrity (Wohlkönig, Huet, Looze, and Wintjens, 2010). Utilization of enzymatic disruption alone can be expensive and less efficient, despite the low energy required. Hence, combination of ultrasonication and lysozyme may serve maximum efficiency in bacterial cell lysis.

Nisin, a bacteriocin peptide of lantibiotics group produced by *Lactococcus lactis*, which was used throughout this study as an antibacterial bacteriocin reference, has been established as a food preservative by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) (Food *et al*., 2017). As the most characterized bacteriocin produced by LAB, Nisin is commonly used as a reference for positive control in bacteriocin studies.

The earliest characterized bacteriocin-producing *Strep. macedonicus* strain, which is also the representative genomic information for *Strep. macedonicus,* is strain ACA-DC 198. This strain was found to produce two lantibiotic peptides named Macedocin and Macedovicin. Macedocin is coded by a 15,171 bp region in *Strep. macedonicus* ACA-DC 198 chromosome, possessing 99% similarity to Streprococcin A-FF22 gene cluster from *Streptococcus pyogenes* with no potential pathogenicity traits against human (Georgalaki *et al*., 2002; Papadelli, *et al*., 2007; Tagg, *et al*., 1973). A complete genome sequencing of *Strep. macedonicus* ACA-DC 198 had been carried out ahead of the discovery of Macedovicin (Papadimitriou *et al*., 2012). The approximately 14 kb of gene cluster accountable for the biosynthesis of Macedovicin was identified by annotating against Thermophillin 1277 coding gene, a bacteriocin from lacticin 481 groups of lantibiotics possessing >99% similarity to that of Bovicin HJ50 (Dufour, *et al*., 2007; Kabuki *et al*., 2011; Liu *et al*., 2009).These bacteriocin peptides exhibited bactericidal activity, shown by loss of viability of susceptible indicator strain *Lactobacillus sakei* subsp. sakei LMG 13558^T with the absence of cell lysis, confirmed by the unchanged optical density of the culture (Georgalaki *et al*., 2002; Tagg *et al*., 1973).

Although bacteriocin-like inhibitory substance (BLIS) of *Strep. macedonicus* MBF 10-2 has yet to be characterized individually, multiple BLIS activity by *Strep. macedonicus* MBF 10-2 ranging from <3kDa to ≥30kDa has been demonstrated, thus proving similarity to the characterized *Strep. macedonicus* ACA-DC 198 strain. Subsequent genomic characterization of *Strep. macedonicus* MBF 10-2 is in the progressive process to reveal the gene clusters responsible for the biosynthesis of bacteriocin peptides or BLIS of *Strep. macedonicus* MBF 10-2 lysate displaying antibacterial activity.

The probiotic cell lysates, in addition to bacteriocin, contains hyaluronic acid, sphingomyelinase, lipoteichoic acid, exopolysaccharides, peptidoglycan, lactic acid, acetic acid and/or diacetyl, which provide a broad biologic activity that can be harnessed to provide skin benefits such as improving atopic eczema, atopic dermatitis, healing of burn and scars, skin-rejuvenating properties, improving skin innate immunity and protecting against photodamage (Kober and Bowe, 2015; Lew and Liong, 2013). Those could serve as bioactive agents in treating skin unhealthiness by occupying the same niche as the potential pathogenic microbiota, and affect acidification of surrounding as well as by inhibit the growth of pathogens as described (Aguilar-Toalá *et al*., 2018; Argenta, Satish, Gallo, Liu, and Kathju, 2016). The bioactive fractions prepared in this study appeared to give their protective effect by compounds that mimic the beneficial and therapeutic effects of probiotics with various mechanisms of action.

CONCLUSIONS

The best plant-based MRS medium to produce higher cell mass was obtained by fermentation of *Strep. macedonicus* MBF 10-2 in soy peptone modified-MRS, confirming the ability to replace animal-based bacto peptone for efficient cell growth. Cell disruption by a combination of mechanic and enzymatic method yielded a greater

amount of lysate fractions with antibacterial potency against indicator strains. Although topical application of MBF 10-2 bioactive fractions recommended as safe based on the skin sensitivity patch test, however, MBF 10-2 bioactive fractions showed only mild to very low antioxidant capacity. Hence, it is recommended to develop the postbiotics *Strep. macedonicus* MBF 10-2 bioactive fractions in a stable formulation that can preserve the bioactive compound's potential for the microbiome therapeutics in skincare pharmaceutics.

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