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A Novel Postbiotic Patch Formulation Using *Pediococcus acidilactici* BK01 for Anti-Acne Applications

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ABSTRACT

Acne is an inflammatory skin disorder often associated with infection by *Cutibacterium acnes*. Postbiotics in the form of cell-free supernatant (CFS) from *Pediococcus acidilactici* BK01 contain lactic acid, bacteriocins, and hydrogen peroxide, which exhibit potential antibacterial activity. This study aimed to formulate *P. acidilactici* BK01 CFS into a topical patch and evaluate its activity against *C. acnes*. Antibacterial activity and the lowest effective concentration by agar diffusion were determined using the well-diffusion method. The lowest concentration showing an inhibition zone (6.25%) was used to formulate patches via solvent-casting with HPMC K4M or PVA (2–4%) as polymers and glycerin, propylene glycol, or PEG 400 (0.2–0.6%) as plasticizers. The selected formulation was evaluated for antibacterial activity and physical characteristics. A combination of 4% PVA and 0.6% glycerin produced the best base and was used to prepare patches containing 6.25–75% CFS. The 65% CFS patch showed the highest inhibition against *C. acnes* ATCC 11827 (12.03 ± 0.373 mm), categorized as strong. The patch demonstrated acceptable organoleptic properties, thickness, folding endurance, pH, moisture content, and tensile strength. These findings indicate that the 65% CFS patch has potential as a topical anti-acne preparation.

Keywords: acne; cell-free supernatant; patch; *Pediococcus acidilactici*; postbiotics

Introduction

Acne is a multifactorial chronic inflammatory skin condition that is commonly encountered (1). Acne most frequently occurs in post-pubertal adolescents and affects 49.9% of the young people surveyed (2,3). Acne is characterized by hyperactive sebaceous gland secretion, hyperkeratosis of the hair follicle infundibulum, and the effect of bacterial colonization (4,5). Acne not only causes physical discomfort but can also lead to psychological issues that reduce self-confidence (2). Topical or systemic antibiotics still dominate current acne treatments. However, long-term use of antibiotics can lead to bacterial resistance (6,7). Therefore, alternative acne treatments derived from natural ingredients, such as postbiotics, need to be developed due to their safety profile compared to probiotics. One probiotic with potential as an anti-acne agent is Pediococcus acidilactici BK1, which can produce postbiotics in the form of cell-free supernatant containing bioactive compounds such as lactic acid, hydrogen peroxide, and bacteriocins (8).

A postbiotic lysate-based anti-acne cream formula has been developed by Bae et al. (2023). The results of the study showed that lysate from heat-treated Pediococcus acidilactici LM1013, which had been inactivated by thermal treatment, was proven to reduce the release of lipase from the phylotype IA strain of C. acnes. Lipase is one of the

virulence factors that produces oxidative stress in keratinocytes and perifollicular inflammation in acne. The content of unsaturated weak acids (65.13%) in heat-treated Pediococcus acidilactici LM1013 has also been shown to inhibit biofilm formation by Cutibacterium acnes and Staphylococcus aureus. Biofilms play a role in the antibiotic resistance of C. acnes by blocking the diffusion of antimicrobial agents (9).

The patch dosage form is chosen for acne treatment due to its ability to deliver medication directly to the skin, thereby improving patient compliance for long-term therapy (10,11). The risk of overdose or underdose can also be reduced because the patch releases active ingredients at the target site, and treatment can be stopped at any time. Patches also offer advantages over other topical formulations, such as creams or gels, due to their waterproof nature and ability to absorb fluids, making them effective in protecting against secondary infections. Previous studies have shown that leave-on formulations containing Heat-treated Pediococcus acidilactici LM1013 have produced positive results in improving acne conditions (9). So far, there have been no publications related to the formulation of anti-acne patches from CFS P. acidilactici BK01 postbiotics. Therefore, in this study, an effective and physically stable CFS P. acidilactici BK01 patch formula for acne treatment was developed. The best patch formula was selected based on evaluations of organoleptic properties, patch thickness, fold endurance, pH, moisture content, and tensile strength.

METHODS

Materials

The probiotic Pediococcus acidilactici BK01 was obtained from the Faculty of Animal Science, Andalas University; Clindamycin HCl 300 mg capsules were purchased from Bernofarm, (Indonesia), MRS broth and NA were purchased from Neogen (USA), BHI Agar (Merck, Germany), distilled water (Kafarma, Indonesia), HPMC K4M (Lawsim Zecha, Indonesia), polyvinyl alcohol (Chang Chun, China), propylene glycol (Dow Chemical Pacific, Singapore), PEG 400 (Sigma Aldrich, USA), glycerin (PT. Palapa Muda Perkasa, Indonesia), and Cutibacterium acnes ATCC 11827.

Production of Cell-Free Supernatant of *Pediococcus acidilactici* BK01

A total of 1 mL of *P. acidilactici* BK01 from a pure stock culture was inoculated into 9 mL of MRS broth in a test tube, then incubated at 37 °C for 24 hours (12). The rejuvenated *P. acidilactici* BK01 was centrifuged at 14,000 rpm for 5 minutes at 4^{0}C, and the supernatant was filtered using a 0.22 µm membrane filter (13).

Preparation of Test Bacterial Suspension

A loopful of *Cutibacterium acnes* ATCC 11827 bacteria was suspended in 10 mL of 0.9% physiological NaCl, then homogenized using a vortex, and the turbidity adjusted to match the 0.5 McFarland standard. The 0.5 McFarland solution was prepared by mixing 0.05 mL of 1% with 9.95 mL of 1%, with an estimated bacterial count of 1.5 x (14).

Preparation of Control Solution

The negative control was prepared by dissolving 5.5 grams of Man Rogosa Sharpe (MRS) liquid medium in 100 mL of distilled water in an Erlenmeyer flask, mixing until homogeneous, and then sterilizing it by autoclaving at 121°C with a pressure of 15 psi for 15 minutes.

The positive control was prepared from 300 mg of Clindamycin HCl. One capsule of Clindamycin HCl was dissolved in distilled water in a 10 mL volumetric flask. It was then diluted in a 10 mL volumetric flask until a solution of Clindamycin HCl at two µg/50 µL (40 ppm) was obtained.

Antibacterial Activity Test of Cell-Free Supernatant *Pediococcus acidilactici* BK01

The antibacterial activity test of CFS *P. acidilactici* BK01 was conducted using the well diffusion method. A suspension of *C. acnes* bacteria (50 µL) was homogenized into 20 mL of Nutrient Agar in a Petri dish until solidified. After the agar solidified, wells of 8 mm diameter were made using a cork borer. Then, 50 µL of CFS *P. acidilactici* BK01 was placed into each well and incubated for 24 hours at 37°C (15). As a positive control, 50 µL of Clindamycin HCl at a concentration of 40 ppm was used, while the negative control was 50 µL of MRS broth medium. The inhibition zone diameter was measured using a caliper (12).

Determination of Lowest Effective Concentration by Agar Diffusion Method

CFS *P. acidilactici* BK01 was prepared at various concentrations, namely 100; 75; 65; 50; 25; 12.5; 6.25; 3.125; and 1.563%. A suspension of *C. acnes* bacteria (50 µL) was homogenized into 20 mL of Nutrient Agar in a Petri dish until solidified. Once the agar had solidified, 8-mm-diameter wells were created using a cork borer. Then, 50 µL of each concentration of CFS *P. acidilactici* BK01 was placed into each well and incubated for 24 hours at 37°C. As a positive control, 50 µL of Clindamycin HCl antibiotic at a 40 ppm concentration was used, and as a negative control, 50 µL of MRS broth medium was used. The lowest effective concentration was defined as the lowest concentration of CFS *P. acidilactici* BK01 that still produced an inhibition zone against *C. acnes* ATCC 11827 in the agar diffusion assay.

Polymer Selection

Polymer selection was evaluated based on organoleptic properties, thickness, pH, and folding endurance of the formed patch. The experimental composition used for polymer selection is shown in **Table 1** below.

Table 1. Experimental composition for polymer selection

Ingredients (%)	P1	P2	P3	P4	P5	P6
HPMC K4M	2	3	4	-	-	-
PVA	-	-	-	2	3	4
Water as	100	100	100	100	100	100

Selection of Plasticizer

The selection of plasticizer is evaluated based on organoleptic properties, tensile strength, thickness, and folding endurance of the formed patch. The trial compositions for selecting the plasticizer are shown in **Table 2**, using the chosen polymer, PVA 4%.

Table 2. Trial compositions for plasticizer selection

Ingredients (%)	L1	L2	L3	L4	L5	L6	L7	L8	L9
PVA	4	4	4	4	4	4	4	4	4
PEG 400	0.2	0.4	0.6	-	-	-	-	-	-
Propyleneglycol	-	-	-	0.2	0.4	0.6	-	-	-
glycerin	-	-	-	-	-	-	0.2	0.4	0.6
water ad	100	100	100	100	100	100	100	100	100

Patch Preparation

Patches containing CFS *P. acidilactici* BK01 were prepared using the solvent casting method. PVA 4%, glycerin 0.6%, and each concentration of CFS *P. acidilactici* BK01 for formulas F1, F2, F3, F4, F5, and F6 were weighed, then distilled water was added up to 10 grams, and the mixture was stirred until homogeneous. The mixture was then poured into molds. The patches were dried in an oven at 40°C for 48 hours. Once dry, the patches were removed from the molds and cut using an 8 mm cork borer. Patches with a diameter of 8 mm were tested for antibacterial activity and evaluated for organoleptic properties, thickness, folding endurance, pH, moisture content, and tensile strength.

Preparation of Controls

The negative control was prepared by formulating 4% PVA and 0.6% glycerin using distilled water up to 10 grams and mixing until homogeneous. The mixture was then poured into molds. Once dry, the patches were removed from the molds and cut using a cork borer with an 8 mm diameter.

The positive control was prepared by formulating 4% PVA, 0.6% glycerin, 4 mL of Clindamycin HCl solution with a concentration of 100 ppm, and adding distilled water to a total volume of 10 grams, resulting in a final Clindamycin HCl concentration in the patch of 2 µg/50 µL (40 ppm). The mixture was stirred until it was homogeneous and then poured into molds. Once dry, the patches were removed from the molds and cut using a cork borer with an 8 mm diameter.

Antibacterial Activity Test of the Patch

Testing was conducted on Petri dishes using Nutrient Agar media already seeded with *C. acnes* bacteria, including a positive control (a Clindamycin HCl patch) and a negative control (a patch without CFS *P. acidilactici* BK01). The patches were placed on the

Table 3. CFS *P. acidilactici* BK01 Patch Formulation

Ingredients (%)	Formula					
	F1	F2	F3	F4	F5	F6
CFS Pediococcus acidilactici BK01	6.25	12.5	25	50	65	75
PVA	4	4	4	4	4	4
glycerin	0.6	0.6	0.6	0.6	0.6	0.6
water ad	100	100	100	100	100	100

solidified media and then incubated at 37°C for 24 hours. Measurement of the inhibition zone was carried out by observing the clear color transition on the Petri dish. The inhibition zone test was performed in three repetitions (16).

Physical Evaluation of CFS Patch *Pediococcus acidilactici* BK01

1. Organoleptic

Organoleptic examination was observed visually, including the smell, color, shape, and texture of the resulting patch.

2. Patch Thickness

The patch preparation is expected to have uniform thickness. Patches are taken from each formula, and their thickness is measured at five different points using a digital micrometer; then the average is calculated. The requirement for patch thickness is that it must not exceed 1 mm (17).

3. Folding Endurance Test

For the folding endurance test, samples are taken from each formula and subjected to repeated folding until the patch breaks or tears. The sample meets the requirement if the number of folds exceeds 300 (17).

4. pH Test

Each patch formula is placed in a porcelain dish, and 5 mL of distilled water is added; it is then allowed to swell. The pH of the patch is then measured using a pH meter. A pH value that does not irritate the skin is between 4.5 and 6.5 (17).

5. Moisture Content

The moisture content of the patch is measured using a moisture analyzer at a temperature of 105°C. The percentage of moisture content will be displayed on the device screen (18).

6. Tensile strength

The tensile strength of the patch is determined using a Universal Testing Machine (10kN, RTI-1310). The patch is clamped between the upper and lower material clamps, and then a load or force is applied and pulled until it breaks. During testing, the tensile force and elongation are recorded to calculate the tensile strength (19).

RESULT AND DISCUSSION

The antibacterial activity test of CFS *P. acidilactici* BK01 was carried out against the bacterium *C. acnes* ATCC 11827. The method used was the well diffusion method because it is relatively simple, does not require high costs, and allows for observation of the sensitivity of antibacterial compounds. In the well diffusion method, antibacterial compounds will diffuse into the agar medium. If the compound can inhibit or kill the test microorganism, a clear zone will form around the well, known as the inhibition zone (8). The results of the antibacterial activity test of CFS *P. acidilactici* BK01 against *C. acnes* ATCC 11827 are presented in **Table 4** below. The inhibition zones produced by CFS *P. acidilactici* BK01 against *C. acnes* ATCC 11827 are shown in **Figure 1**.

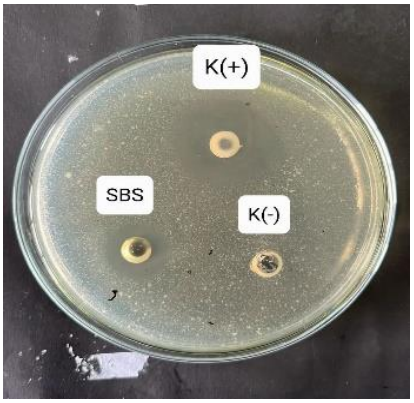

Figure 1. Inhibition zones produced by CFS *P. acidilactici* BK01 against *C. acnes* ATCC 11827 using the well diffusion method

Table 4. Results of the antibacterial activity test of CFS *P. acidilactici* BK01 against *C. acnes* ATCC 11827

Storage time (days)	Inhibition zone (mm)		
	CFS P. acidilactici BK01	Positive control	Negative control
1	13.72 ± 0.667	21.53 ± 0.485	0 ± 0
30	13.37 ± 0.826	22.20 ± 0.469	0 ± 0

Note: Positive control: Clindamycin HCl at a concentration of 40 ppm, Negative control: MRS broth medium

Antibacterial activity is classified based on the diameter of the inhibition zone, where inhibition zones of ≥ 20 mm are classified as very strong, 10–20 mm as strong, 5–10 mm as moderate, and ≤ 5 mm as weak (20). Based on this classification, the CFS of *P. acidilactici* BK01 exhibited strong antibacterial activity against C. acnes with an inhibition zone diameter of 13.72 ± 0.667 mm. The antibacterial activity of CFS *P. acidilactici* BK01 is attributed to several bioactive compounds, including lactic acid, bacteriocins, and hydrogen peroxide (21). Lactic acid diffuses through the bacterial cell membrane and dissociates within the cell, leading to a reduction in cytoplasmic pH and inhibition of bacterial growth (22). In addition, bacteriocins disrupt membrane permeability, causing leakage of intracellular components and impairing ATP efflux mechanisms in *C. acnes* (23). Hydrogen peroxide further contributes to antibacterial activity by denaturing proteins and interfering with nucleic acid synthesis and function, thereby inhibiting bacterial growth (24).

The negative control in this test is MRS broth medium. Test results showed that no inhibition zone formed around the wells. It demonstrates that MRS broth medium does not contain antibacterial compounds and thus does not affect the growth of the test bacteria. Testing with a negative control aims to determine whether the growth medium of *P. acidilactici* BK01 has any effect on *C. acnes*.

Based on the results of antibacterial activity tests presented in **Table 4**, it can be observed that the positive control, Clindamycin HCl at a concentration of 40 ppm, exhibited an inhibition zone diameter of 21.53 ± 0.485 mm, which falls into the firm category. Meanwhile, CFS *P. acidilactici* BK01 had an inhibition zone diameter of 13.72 ± 0.667 mm, placing it in the strong category. It indicates that CFS *P. acidilactici* BK01 can inhibit the growth of *C. acnes* bacteria and thus has the potential to be developed as an anti-acne preparation.

Antibacterial activity testing was also conducted on CFS *P. acidilactici* BK01. The results in **Table 4** indicate that CFS *P. acidilactici* BK01 retained its antibacterial activity. CFS *P. acidilactici* BK01 exhibited stable antibacterial activity against *C. acnes* ATCC 11827. It is evidenced by the inhibition zone diameter of CFS *P. acidilactici* BK01 after one month of storage, which was 13.37 ± 0.826 mm. Compared to the first day, as shown in **Table 4**, CFS *P. acidilactici* BK01 had an inhibition zone diameter of 13.72 ± 0.667 mm. These findings indicate that the antibacterial compounds in CFS P. acidilactici BK01 remained stable during storage, consistent with previous studies reporting that antimicrobial compounds produced by lactic acid bacteria can maintain their activity after long-term storage (25).

The determination of the lowest effective concentration using the agar diffusion method aimed to identify the lowest concentration of CFS *P. acidilactici* BK01 that can still inhibit the growth of *C. acnes* ATCC 11827 (26). The concentration series tested were 100, 75, 65, 50, 25, 12.5, 6.25, 3.125, and 1.563%. The purpose of this variation in concentration is to serve as a basis for selecting the concentration of CFS *P. acidilactici* BK01 to be used in the patch formula. The results of the lowest effective concentration determination by the agar diffusion method are presented in **Table 5** below. The inhibition zones produced by different concentrations of CFS *P. acidilactici* BK01 are shown in **Figure 2**.

The diffusion-based antibacterial activity test was conducted using the well diffusion method because it allows direct interaction between CFS *P. acidilactici* BK01 and the agar medium, facilitating visual observation of inhibition zones. The positive control used was Clindamycin HCl at a concentration of 40 ppm. As shown in **Table 5**, the positive control produced an inhibition zone diameter ranging from 21.55 to 22.13 mm, classified as very strong. Meanwhile, the negative

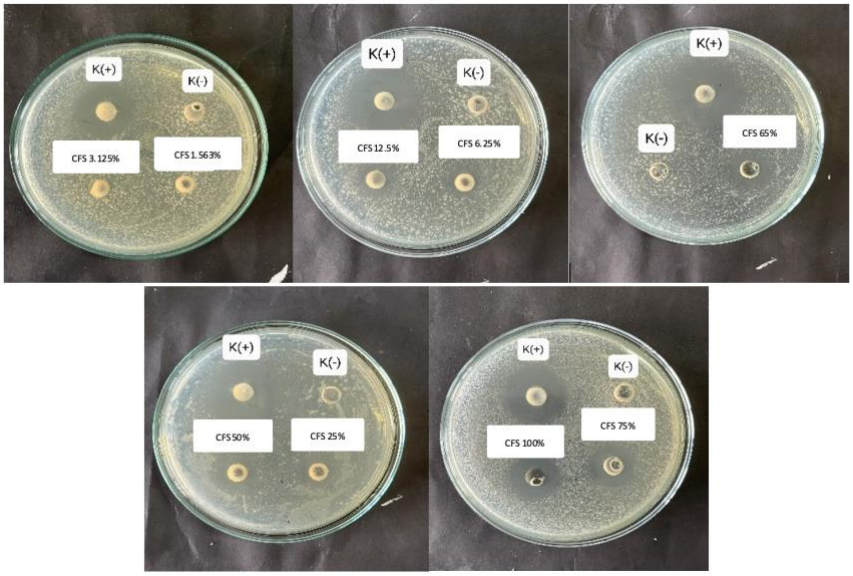

Figure 2. Results of the Determination of the Lowest Effective Concentration of CFS *P. acidilactici* BK01 Against *C. acnes* ATCC 11827 by Agar Diffusion Method.

control, which was MRS broth media, did not show any inhibition zone against *C. acnes* ATCC 11827.

Based on agar diffusion results presented in **Table 5**, the lowest concentration of CFS *P. acidilactici* BK01 that could still inhibit the growth of *C. acnes* ATCC 11827 was 6.25%, with an inhibition zone diameter of 10.72 ± 0.192 mm. The higher the concentration of CFS *P. acidilactici* BK01, the higher the content of antibacterial compounds. The larger diameter of the inhibition zone provides evidence for this. c Based on these results, patches containing CFS *P. acidilactici* BK01 were formulated at concentrations of 6.25%, 12.5%, 25%, 50%, 65%, and 75%.

Table 5. Results of the Determination of the Lowest Effective Concentration of CFS *P. acidilactici* BK01 Against C. acnes ATCC 11827 by Agar Diffusion Method.

Concentration of CFS P. acidilactici BK01 (%)	Inhibition zone (mm)		
	CFS P. acidilactici BK01	Positive control	Negative control
100	13.63 ± 0.506	22.13 ± 0.227	0 ± 0
75	13.51 ± 0.147	22.13 ± 0.227	0 ± 0
65	12.17 ± 0.183	21.74 ± 0.417	0 ± 0
50	12.00 ± 0.201	21.55 ± 0.291	0 ± 0
25	11.69 ± 0.245	21.55 ± 0.291	0 ± 0
12.5	10.86 ± 0.223	21.96 ± 0.416	0 ± 0
6.25	10.72 ± 0.192	21.96 ± 0.416	0 ± 0
3.125	0 ± 0	21.73 ± 0.325	0 ± 0
1.563	0 ± 0	21.73 ± 0.325	0 ± 0

Note: Positive control: Clindamycin HCl at a concentration of 40 ppm; Negative control: MRS broth media

In the formulation of the cell-free supernatant patch of *Pediococcus acidilactici* BK01, polymers and plasticizers were selected. Polymers were chosen based on organoleptic evaluation, thickness, pH, and folding endurance of the resulting patches. Meanwhile, plasticizers were selected based on organoleptic evaluation, folding endurance, thickness, and tensile strength of the resulting patches. The preferred polymers and plasticiz ers were then used in the formulation of patches containing CFS *P. acidilactici* BK01.

The polymers used in the patch formulation were HPMC K4M and PVA at concentrations of 2%, 3%, and 4%. Each polymer concentration was prepared by dispersing the polymer in distilled water, then pouring it into molds. After drying, polymers were selected based on organoleptic observation, thickness, pH, and folding endurance. The results of the polymer observations are shown in **Table 6**.

Based on **Table 6**, the evaluation results of formulas P1 to P6 in terms of homogeneity, thickness, and folding endurance all meet the requirements. Formulas using the HPMC K4M polymer form opaque patches. Meanwhile, formulas using the PVA polymer result in transparent patches, making them more aesthetically pleasing when applied to the skin. PVA was selected because it provides greater clarity and better physical properties compared to Na-CMC, HPMC, and gelatin (28). **Table 6** shows that formulas P4 and P5 produced wrinkled and uneven patches.

On the other hand, formula P6 produced patches that were not wrinkled, resulting in a smoother, more aesthetically pleasing, and

Table 6. Results of polymer selection

Formulations	Evaluation parameter					
	Organoleptic			Thickness (mm)	pH	Folding endurance
	Texture	Color	Homogeneity			
P1	Smooth surface without wrinkles	Opaque	Homogeneous	0.036 ± 0.002	6.89 ± 0.064	>500
P2	Smooth surface without wrinkles	Opaque	Homogeneous	0.051 ± 0.002	7.25 ± 0.056	>500
P3	Smooth surface without wrinkles	Opaque	Homogeneous	0.075 ± 0.001	6.25 ± 0.055	>500
P4	Smooth surface with wrinkles	Transparent	Homogeneous	0.032 ± 0.001	6.12 ± 0.006	>500
P5	surface with wrinkles	Transparent	Homogeneous	0.054 ± 0.003	5.87 ± 0.006	>500
P6	Smooth surface without wrinkles	Transparent	Homogeneous	0.077 ± 0.002	5.61 ± 0.010	>500
Desired target	Smooth surface without wrinkles	Transparent	Homogeneous	< 1 (17).	4-6 (40).	>300 (18).

Note: P1: HPMC K4M 2%, P2: HPMC K4M 3%, P3: HPMC K4M 4%, P4: PVA 2%, P5: PVA 3%, P6: PVA 4%

more comfortable patch when applied to the skin surface, as shown in **Figure 3**. Based on the pH testing results, Sformula P6 has a pH of 5.61, which is close to the pH of the active ingredient in the patch formulation, namely CFS *P. acidilactici* BK01. Therefore, formula P6 was selected as the chosen polymer based on the evaluation of organoleptic properties, thickness, pH, and folding endurance.

The selected polymer, as indicated in **Table 6**, was then used for plasticizer selection. The formulation was made using the plasticizers glycerin, propylene glycol, and PEG 400 with varying concentrations of 0.2%, 0.4%, and 0.6%. Each plasticizer concentration was prepared by dispersing PVA polymer and plasticizer in distilled water and then pouring the mixture into molds. After drying, the selection of the plasticizer was carried out based on organoleptic evaluation, folding endurance, thickness, and tensile strength. The observation results for the plasticizers are shown in **Table 7**.

Table 7. Results of plasticizer selection

Formulations	Evaluation parameter					
	Organoleptic			Folding endurance	Thickness (mm)	Tensile strength (MPa)
	Texture	Color	Homogeneity			
L1	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.077 ± 0.004	3.388 ± 1.408
L2	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.081 ± 0.003	5.321 ± 1.649
L3	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.079 ± 0.002	4.069 ± 0.484
L4	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.075 ± 0.007	5.867 ± 0.922
L5	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.076 ± 0.005	5.101 ± 1.544
L6	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.081 ± 0.004	4.714 ± 1.015
L7	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.078 ± 0.004	5.726 ± 0.257
L8	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.081 ± 0.008	4.501 ± 0.731
L9	Smooth surface without wrinkles	Transparent	Homogeneous	> 500	0.093 ± 0.013	6.657 ± 1.474
Desired target	Smooth surface without wrinkles	Transparent	Homogeneous	> 300 (18).	< 1 (17).	Nilai tertinggi (41).

Note: L1: PVA 4% + Propylene glycol 0.2%, L2: PVA 4% + Propylene glycol 0.4%, L3: PVA 4% + Propylene glycol 0.6%, L4: PVA 4% + PEG 400 0.2%, L5: PVA 4% + PEG 400 0.4%, L6: PVA 4% + PEG 400 0.6%, L7: PVA 4% + Glycerin 0.2%, L8: PVA 4% + Glycerin 0.4%, L9: PVA 4% + Glycerin 0.6%

Based on **Table 7**, the evaluation results of formulas L1 to L9 in terms of organoleptic properties, fold endurance, and thickness meet the requirements. The formulas using the plasticizer propylene glycol are challenging to remove from the mold. In contrast, formulas using the plasticizers glycerin and PEG 400 are easier to remove from the mold. It is related to the characteristics of the plasticizers used. Glycerin and PEG 400 have hygroscopic properties that increase the moisture content of the patch (29). The tensile strength test results in **Table 7** show that patches using the plasticizer glycerin have the highest tensile strength values.

Figure 3. Results of polymer selection for patch formulation.

Meanwhile, the lowest tensile strength was observed in formulas containing propylene glycol. Tensile strength is influenced by the type and concentration of the plasticizer, as well as the molecular size, number of hydroxyl groups, and compatibility between the plasticizer and polymer (30). Previous studies have also reported that PVA based films containing glycerin exhibit better mechanical properties than those formulated with propylene glycol or PEG 400 (31). Based on the physical evaluation results, formula L9 containing glycerin was selected because it formed a homogeneous, transparent patch with a smooth texture, did not wrinkle, and was easily removed from the mold, as shown in **Figure 4**. In addition, formula L9 exhibited the highest tensile strength value of 6.657 ± 1.474 MPa.

Figure 4. Results of plasticizer selection for patch formulation

Based on the results of polymer selection, formula P6 with a PVA concentration of 4% was chosen. Furthermore, based on the results of plasticizer selection, formula L9 with a glycerin concentration of 0.6% was determined. The selected polymer and plasticizer were then formulated into patches with CFS *P. acidilactici* BK01 concentrations of 75%, 65%, 50%, 25%, 12.5%, and 6.25%, based on the results of the lowest effective concentration determination in **Table 5**. The patch formulas are presented in **Table 8** below.

Table 8. Formulation of CFS *P. acidilactici* BK01 patch

Ingredients (%)	Formulations						Note
	F1	F2	F3	F4	F5	F6	
CFS Pediococcus acidilactici BK01	6.25	12.5	25	50	65	75	Active ingredients
PVA	4	4	4	4	4	4	Polymer
glycerin	0.6	0.6	0.6	0.6	0.6	0.6	Platicizer
water ad	100	100	100	100	100	100	Solvent

The antibacterial activity test was conducted to evaluate the ability of the patch preparations to inhibit the growth of C. acne ATCC 1182 bacteria. In this study, antibacterial activity testing was performed on patches F1, F2, F3, F4, F5, and F6. The results of the antibacterial activity test for the CFS *P. acidilactici* BK01 patch are presented in **Table 9** below.

The results of the antibacterial activity test for patches F1, F2, and F3 showed that the CFS *P. acidilactici* BK01 patches did not exhibit any inhibition zones, as indicated by the growth of *C. acnes* ATCC 11827 bacteria on the patches. Meanwhile, F4, F5, and F6 had inhibition zones ranging from 10.61 to 12.05 mm, which fall into the strong category. Inhibition zones of the patch formulations are shown in **Figure 5**. As shown in **Figure 5**, inhibition zones were observed only in F4–F6, whereas F1–F3 did not exhibit visible inhibition zones.

The negative control, which was a patch without CFS P. acidilactici BK01, did not exhibit any inhibition zone against C. acnes ATCC 11827. On the other hand, the positive control patch containing Clindamycin HCl at a concentration of 40 ppm showed a very strong inhibition zone, with inhibition diameters ranging from 21.20 to 23.17 mm, indicating the antibacterial activity of clindamycin. The positive control patch was prepared using PVA 4% and glycerin 0.6%, with the incorporation of 4 mL Clindamycin HCl solution (100 ppm) and distilled water added up to 10 g, resulting in a final Clindamycin HCl concentration equivalent to 2 µg/50 µL (40 ppm) in the patch. However, the uniformity of clindamycin distribution within the patch matrix was not evaluated in this study. The absence of an inhibition zone in the patch without CFS *P. acidilactici* BK01 as the negative control indicates that the antibacterial activity

Table 9. Results of the antibacterial activity test of the CFS *P. acidilactici* BK01 patch

Treatment	Inhibition zone (mm)					
	F1	F2	F3	F4	F5	F6
Patch CFS P. acidilactici BK01	0 ± 0	0 ± 0	0 ± 0	10.61 ± 0.365	12.03 ± 0.373	12.05 ± 0.485
Positive control	23.17 ± 0.448	22.76 ± 0.130	21.20 ± 0.772	22.69 ± 0.325	22.50 ± 0.145	21.85 ± 0.298
Negative control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Note: Positive control: Clindamycin HCl 40 ppm patch; Negative control: patch without CFS P. acidilactici BK01

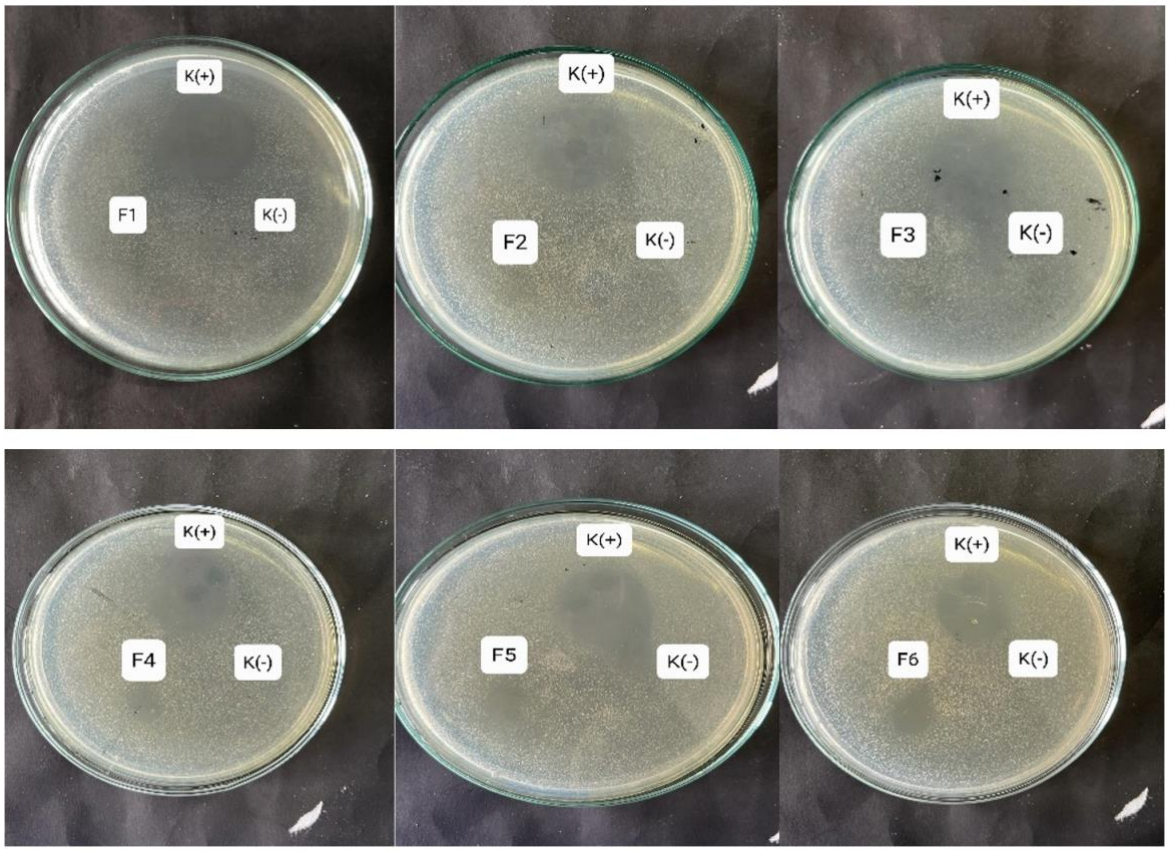

Figure 5. Inhibition zones produced by CFS *P. acidilactici* BK01 patch formulations (F1–F6) against *C. acnes* ATCC 11827

originated from CFS *P. acidilactici* BK01 and not from the patch base. This antibacterial activity may be attributed to the presence of lactic acid, bacteriocins, and hydrogen peroxide in CFS P. acidilactici BK01

Based on **Tables 2 and 9**, differences were observed between the agar diffusion results and the antibacterial activity test results of the CFS *P. acidilactici* BK01 patch at concentrations of 6.25% to 25%. It is due to the changes in pH that occur in the preparations. CFS *Pediococcus acidilactici* BK01 has a low pH, typically ranging from 3 to 4. The antibacterial activity produced by CFS has been shown to originate from its high organic acid content, especially lactic acid, as this activity decreases when the CFS is neutralized (21). After CFS is formulated into a patch with the addition of polymers and plasticizers, the pH increases to near neutral. This increase in pH causes some of the lactic acid to ionize into the lactate form, which has lower antibacterial

activity because it is less able to diffuse through the bacterial cell membrane. In addition, the release of active compounds from the patch matrix occurs slowly and gradually; therefore, at CFS concentrations of 6.25% to 25%, the amount of active compound released is not yet sufficient to exert an inhibitory effect against *C. acnes*. It differs from Clindamycin HCl, which still exhibits antibacterial activity at a concentration of 40 ppm, in both liquid and patch forms, because Clindamycin HCl is relatively stable and maintains its antibacterial activity across a wide pH range (32). The absence of inhibition zones in patches containing 6.25–25% CFS may be associated with physicochemical changes after incorporation into the patch matrix. The antimicrobial activity of CFS *P. acidilactici* BK01 is mainly attributed to organic acids, particularly lactic acid. Previous studies reported that the antimicrobial mechanism of lactic acid depends on the undissociated form, which can penetrate bacterial membranes, reduce intracellular pH, and disrupt membrane functions. Increased pH may shift lactic acid into its dissociated form (lactate), thereby reducing its antimicrobial activity and membrane permeability (33). In addition, incorporation into a polymeric patch matrix may alter the release behavior of active compounds. Transdermal and matrix type patches are known to provide controlled or sustained release, and the release profile is strongly influenced by polymer composition and matrix characteristics. Controlled release from the polymer matrix may reduce the immediate diffusion of active compounds into agar media, especially at lower concentrations, resulting in the absence of observable inhibition zones (34). However, the present study did not evaluate pH changes between liquid CFS and patch extract, release profiles, or quantify lactic acid and bacteriocin contents after patch formulation.

Based on the antibacterial activity test, the F5 and F6 patch formulas showed inhibition zone diameters that were not significantly different. From the pH test results, F5 had a pH value of 4.76 ± 0.02, while F6 had a pH of 4.48 ± 0.01. The required pH range for preparations applied to the skin is 4.5–6.5 (17). Therefore, F5 with a 65% CFS *P. acidilactici* BK01 concentration was selected as the best formula for further physical evaluation.

Patch evaluation is conducted to determine the physical properties of the selected formula that has been formulated; this is crucial to ensure quality, comfort, and acceptability for users. Physical evaluation of the patch includes organoleptic testing, thickness, folding endurance, pH, and tensile strength. The results of the physical assessment for the F5 patch are presented in **Table 10** below.

An organoleptic examination is conducted to visually observe the shape, odor, surface condition, and color of the patch. The resulting patch preparation has a distinctive smell, a smooth and homogeneous texture, and is yellow in color, as shown in **Figure 6**. Next, the thickness test aims to assess the uniformity of the patch thickness produced. The results of the thickness test reflect the consistency of the patch solution that has been poured into the mold. Additionally, this test is used to determine whether the mold and the environment in which it is placed can produce patches with uniform thickness. Patch thickness is influenced by the size of the mold, the solution volume, and the total amount of solids in the solution. Thinner patches can increase the penetration rate of the active ingredient into the skin, as the amount of medium required for the active ingredient transfer becomes more limited (18). According to **Table 10**, the thickness test result for the patch is 0.095 ± 0.001 mm. The formulated patch thickness meets the requirement of being less than 1 mm (17).

Table 10. Results of the physical evaluation of F5 patch CFS *P. acidilactici* BK01

Test	Result of physical evaluation	Desired target
Organoleptic		
a. Form	Thin film	Thin film
b. Color	Yellow	Yellow
c. Odor	Characteristic odor	Characteristic odor
d. Texture	Smooth	Smooth
e. Homogeneiy	Homogeneous	Homogeneous
Thickness (mm)	0.095 ± 0.001	< 1 (17).
Folding endurance	> 500	> 300 (18).
pH	4.76 ± 0.02	4.5-6.5 (17).
% Moisture content	4.50 ± 0.281	< 10 (16).
Tensile strength (MPa)	6.057 ± 1.019	Highest value (41).

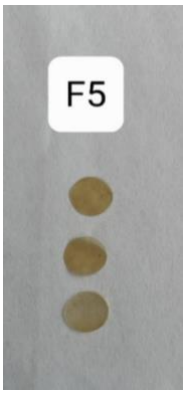

Figure 6. Appearance of the selected CFS *P. acidilactici* BK01 patch formulation (F5)

The folding endurance test is performed to measure the patch's fold capacity. The folding endurance of the preparation is > 500. This finding aligns with the specified requirement, which is more than 300-fold (18). Patch elasticity is affected by the addition of glycerin as a plasticizer, intended to enhance flexibility. Glycerin forms an elastic layer on the polymer, making it more resistant to tearing. In general, the more elastic the polymer, the higher its folding endurance (35). In previous studies, the use of plasticizers in conjunction with polymers has been shown to increase the folding endurance value of patches by enhancing their flexibility (36).

The pH test was conducted using a pH meter, and the pH of the patch preparation obtained was 4.76 ± 0.02. Measuring pH is important because if the pH of the preparation does not match the skin's pH, it can irritate (37). Based on the test results, the formulated patch has a pH that is suitable for the skin's pH range, which is 4.5–6.5.

The moisture content test on the patch aims to determine the patch's stability during storage. The result of the moisture content test on the patch, as shown in **Table 10**, is 4.50 ± 0.281%. It shows that the patch meets the moisture content requirement of <10% (16). A low moisture content in the preparation is recommended so that the resulting patch is stable, not easily brittle, and less susceptible to bacterial contamination (38).

The tensile strength test results show that patches containing CFS *P. acidilactici* BK01 have a tensile strength of 6.057 ± 1.019 MPa, while patches without CFS have a tensile strength of 6.657 ± 1.474 MPa. Based on these results, it is evident that the addition of CFS results in a decrease in the tensile strength of the patch. This decrease is due to the metabolite compounds in CFS, such as organic acids, which can affect the polymer matrix structure of the patch. Organic acids cause the formation of two phases in the film, resulting in instability of the particle structure, which disrupts the homogeneity of the matrix and decreases the tensile strength of the film (39).

CONCLUSION

The selected anti-acne patch formula contains 4% PVA, 0.6% glycerin, and 65% postbiotics cell-free supernatant of *Pediococcus acidilactici* BK01. The postbiotics cell-free supernatant of *Pediococcus acidilactici* BK01, when formulated into a patch, can inhibit the growth of acne-causing bacteria (*Cutibacterium acnes* ATCC 11827) with an inhibition diameter of 12.03 mm, which falls into the strong category.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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