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Development of *Bacillus stratosphericus* Lysate Concentrate to Control Sebum Secretion through In vitro Studies and Clinical Trial

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Abstract
The sebum on human skin is generated for various causes. The composition of the formed sebum increases the proliferation of *Cutibacterium acnes* (*C. acnes*) residing on the skin. As *C. acnes* proliferates, it produces skin irritants that stimulate the sebaceous glands, increasing sebum production. Skin troubles such as acne may occur. The lysate concentrates of *Bacillus stratosphericus* (*B. stratosphericus*), first discovered in the stratosphere, confirmed a 66.35% inhibition of Nitric Oxide (NO) production at 0.50 mg/ml concentration in vitro. Additionally, the growth inhibition efficacy of *B. stratosphericus* lysate concentrate (BSLC) against *C. acnes* was confirmed, showing a 95.1% inhibition of growth proliferation at a consistency of 0.50 mg/ml. Based on the in vitro results, the efficacy of BSLC in degrading and reducing sebum was confirmed by reacting it with artificial sebum to various concentrations. The results showed a concentration-dependent decrease in artificial sebum ccording to the efficacy results confirmed in vitro, a clinical trial was conducted to evaluate the daily sebum reduction efficacy of a serum formulation containing 50 mg/ml of BSLC. After a 4-week application, the test group containing BSLC determined a significant 28.68% reduction in sebum levels, demonstrating the practical implications of the research. In conclusion, BSLC is considered to have sufficient industrial value as a valuable ingredient for the cosmetics industry aimed at sebum improvement.

Keywords Acne, Anti-inflammatory, *Bacillus stratosphericus*, Fermentation, Sebum reduction

Introduction

Sebum in the skin is comprised of glycerides, free fatty acids, wax esters, and squalene [1, 2]. The formation of sebum is divided into intrinsic and extrinsic factors [3, 4]. Inherent factors involve hormones, which primarily begin to be secreted during puberty or due to hormonal imbalances in the body, stimulating the sebaceous glands and producing sebum [3]. Extrinsic factors include increased skin temperature due to ultraviolet (UV) exposure, which prompts sweating to regulate body temperature, stimulating the sebaceous glands and producing sebum [5]. Particularly during the summer, when ultraviolet (UV) radiation is intense, the stimulation of

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sebaceous glands increases, leading to heightened sebum secretion and, consequently, oily skin. When intrinsic and extrinsic factors stimulate sebum formation, the composition of the sebum, such as fatty acids, promotes the proliferation of *Cutibacterium acnes* (*C. acnes*), a resident skin microorganism [6]. *C. acnes* is a microorganism known as an acne-causing bacterium [2, 6–8]. As *C. acnes* proliferates on the skin, it creates metabolites such as short-chain fatty acids, which have been reported to trigger skin irritation and induce the expression of various inflammatory factors [6, 9, 10]. Therefore, side effects such as acne can occur on the skin when inflammation becomes severe [6, 11]. With its active research efforts, the cosmetics industry plays a crucial and valued role in improving or regulating sebum secretion to enhance skin texture, benefiting the end users and making them feel valued and important.

Numerous microorganisms inhabit human skin, maintaining a state of harmony and balance [12, 13]. However, many research results show that imbalances in the microbial community can lead to various bodily abnormalities [14]. Accordingly, research is actively underway from a microbiome perspective to maintain or restore the balance of microbial communities [15]. Thus, when excessive sebum production due to various factors leads to an imbalance in resident skin microorganisms, the proliferation of *C. acnes* increases, raising the probability of inflammation and acne development on the skin [8]. When sebum produced on the skin is removed promptly, the ratio of *C. acnes* on the skin is regulated, and inflammatory factor expression is inhibited, it can be anticipated that relatively healthy skin can be maintained [6, 16]. However, if any stage in the acne development process encounters issues, the likelihood of acne occurrence increases. Therefore, there is a need to develop materials that can control the microorganisms related to acne in the skin, associated inflammation, and the actual sebum, thereby resolving the causal relationship between the occurrence of acne.

The stratosphere is approximately 10 to 50 km above the Earth's surface and is also the highest altitude at which commercial airplanes typically operate [17]. While closest to the Earth, the stratosphere is classified as outside the troposphere and is characterized by the absence of clouds, low water content, and minimal nutrients. In addition, the stratosphere features a low-temperature and low-pressure environment composed of trace amounts of water vapor, hydrogen, nitrogen compounds, bromides, sulfuric acid-water aerosols, and methanol [17]. These conditions create an extreme environment where most microorganisms struggle to grow. Among these, nitrogen compounds, including ammonia nitrogen, nitrate nitrogen, inorganic nitrogen, and organic nitrogen, are known to be components of atmospheric pollutants. *Bacillus stratosphericus* (*B. stratosphericus*), a microorganism first discovered in the stratosphere, is characterized as facultative anaerobe [18]. *B. stratosphericus* has been reported to be able to grow at a low temperature of 8℃ and exhibit robust resistance to UV-B radiation. It can also grow under high salinity and alkaline pH conditions [18]. Additionally, it has been reported that *B. stratosphericus* utilizes nitrogen compounds for respiration and growth. *B. stratosphericus*, an extremophile microorganism, is known to produce various metabolic products, with a notable example being a lipopeptide that possesses both hydrophilic and lipophilic parts [19, 20]. The lipopeptide first discovered in *Bacillus subtilis* exhibits amphiphilic properties and can be used as a natural surfactant [21]. Lipopeptides are generally composed of a cycloaliphatic heptapeptide consisting of seven amino acids. One such lipopeptide, surfactin, has been reported to have anti-wrinkle effects on the skin [22, 23].

Regarding *B. stratosphericus*, research has been conducted to evaluate the impact on plant growth based on antifungal activity and research related to fuel cells [24, 25]. Research on its use in pharmaceuticals, cosmetics, and food industries has not been undertaken. Consequently, in this study, we evaluated the anti-inflammatory efficacy and the growth inhibition of *C. acnes*, an acne-causing bacterium, using BSLC in vitro. In addition, to assess the direct degradation or removal efficacy of BSLC on sebum, we evaluated using artificial sebum. As a result, BSLC exhibited excellent growth inhibition of *C. acnes*, confirmed anti-inflammatory effectiveness, and a reduction in artificial sebum. Finally, a clinical trial was conducted to evaluate the effectiveness of BSLC in improving sebum levels on actual human skin. After four weeks of application, the serum formulation containing BSLC revealed significant improvement in sebum levels.

Materials and methods

Raw materials and reagents

The microorganism used in this study was the *Bacillus stratosphericus* (KCCM 13387P) strain isolated from LABIO, and the Tryptic Soy Broth (TSB) medium purchased at DB Difco was employed for fermentation. *Cutibacterium acnes* (*C. acnes*, KCTC 3320) was obtained from the Korean Collection for Type Cultures(KCTC). An AnaeroPack® (Mitsubishi Gas Chemical Co., Inc, Japan) and Reinforced Clostridial Medium (RCM, BD Difco, USA) were used to culture *C. acnes*. DMEM, FBS, and RPMI-1640 media were acquired from Welgene, Korea. Lipopolysaccharide (LPS), Dexamethasone (DEX), N-acetyl-L-cysteine (NAC), and Sodium dodecyl sulfate (SDS) dealing with Sigma-Aldrich were applied, and all other analytical reagents used in this study were of analytical grade.

Preparation of *Bacillus stratosphericus* lysate concentrate

The *B. stratosphericus* was cultured at 37±0.5℃ in an incubator (JSSI-200 C, JSR, Korea) for 72 h until reaching the stationary phase. After the culture was completed, glass beads (DAIHAN Scientific, 0.07–0.11 mm) were added at 10% of the total volume, followed by vortexing for 10 min to obtain a solution containing both cell lysate and ferment. Subsequently, the solution was centrifuged at 3,600 X *g* to separate the cell for 30 min. After that, the supernatant was filtered through a 0.2 μm membrane filter (Sartorius AG, Germany) to obtain a pure liquid form of the ferment and lysate. Finally, the *B. stratosphericus* lysate concentrate (BSLC) was obtained from the supernatant using a freeze dryer (ilShinBioBase, Korea).

Nitric oxide assay

BSLC was diluted and treated at different concentrations in RAW264.7 cells using an RPMI-1640 medium containing 1% FBS. *C. acnes* culture medium was used as a stimulant in this study. *C. acnes* was inoculated at a level of 0.1% into brain heart infusion broth (BHI) and incubated at 37℃ for three days. The culture was then centrifuged at 4,500 X g for 15 min. After removing the supernatant, 30 ml of PBS was added, and the mixture was homogenized by sonication for 30 min, followed by five cycles of freeze-thawing using liquid nitrogen. After sonication for 0.5 h, the mixture was collected by centrifugation. The supernatant was obtained and used as the stimulus. The positive control used was N-acetyl-L-cysteine (NAC). NAC was applied and cultured at 37℃ with 5% CO_2 for 24 h. After reacting with a medium containing NO applying Griess reagent, the absorbance was measured at 540 nm wavelength.

Table 1 Formulation used in the daily sebum improvement clinical trials

Phase	Ingredient	Placebo group Content (%)	Test group Content (%)
A	Water	Up to 100	Up to 100
	Ammonium Acryloyldimethyltaurate/VP Copolymer	0.1	0.1
	Ammonium Acryloyldimethyltaurate/Beheneth-25 Methacrylate Crosspolymer	0.1	0.1
A-1	Butylene Glycol	10	10
	Glycerin	6.0	6.0
	Panthenol	0.2	0.2
	Water, Butylene glycol, 1,2-hexanediol, Sodium Hyaluronate	0.5	0.5
	Disodium EDTA	0.02	0.02
B	Polyglyceryl-6 Distearate (and) Jojoba Esters (and) Polyglyceryl-3 Beeswax (and) Cetyl Alcohol	1.5	1.5
	Dimethicone	5.0	5.0
C	*Bacillus stratosphericus* lysate concentrate (BSLC)	-	5.0
E	Water	1.0	1.0
	Citric Acid	0.001	0.001

Growth inhibition assay for *C. acnes*

A micro-dilution assay using a 96-well plate was conducted to evaluate the growth inhibition of *C. acnes* at different concentrations of BSLC. First, *C. acnes* was precultured in RCM broth to achieve a 1×10^8 CFU/mL concentration. The culture was then diluted with RCM broth to obtain a final concentrate of 5×10^5 CFU/mL, and 100 μL of this suspension was inoculated into each well of the 96-well plate. After diluting BSLC with 0.75% NaCl, 100 μL of each concentration was added to the corresponding wells of the 96-well plate. After treating the samples, they were placed in a Gas pack and cultured at 37±0.5℃ for 96 h. After culturing, each well's Optical Density (OD) was measured at 600 nm utilizing a Spectrophotometer (Libra S22, Biochrom Ltd., UK). Subsequently, the solution in the well was spread on an RCM Agar plate to observe colonies of *C. acnes*.

Artificial sebum reduction assay

The artificial sebum (PICKERING Laboratories, USA) was liquefied by heating to 70℃ [26]. Afterward, it was mixed with water, Sodium Dodecyl Sulfate (SDS), and BSLC in a weight ratio 1:1 for each component. The mixed solution was vortexed for 2 h at 50℃. After that, the solution was allowed to settle for 5 min. Then, the mixed solution was separated by centrifugation at 12,032 x *g* for 15 min at 25℃, and only the upper phase was collected. The collected oil layer was re-separated using centrifugation, then cooled at 4℃ for 15 min, and the weight was measured.

Preparation of serum formulation for clinical trial

The serum used in the clinical trial contained 5% BSLC, while the serum of the placebo group was formulated with water instead of 5% BSLC (Table 1). After heating the Phase A-1 to 72±2.5℃, add Phase A-1 to Phase A and mix using an Agi-mixer at 1,000 X *g* and 72±2.5℃ for 3 min. Then, Phase B dissolved at 75℃ and added to Phase A. Phase C and Phase D were mixed at 1,000 X *g*, 25℃ for 2 min, and then added to Phase A. Finally, Phase E is applied to adjust the pH of the final formulation.

Clinical trial

A clinical trial was conducted through the One Advanced Technology Center (OATC) institution to evaluate the clinical efficacy of BSLC (IRB No. 2018071702-2206-HR-158-01). A total of 40 Korean adult women (average age 44.45±9.60 years) were enrolled in the study, with 20 trial

subjects in the test group and placebo group, to evaluate the efficacy of daily sebum improvement. The effectiveness evaluation was assessed by analyzing the sebum amount analysis values before and after four weeks of use of the test product and control group as variables. To evaluate the daily sebum improvement, a photograph was measured in the facial area using Janus-III, and the left nose area was measured using Sebumeter® SM815 before and after four weeks of use of the investigational product. The amount of sebum output per unit area (μg/cm^2) absorbed in a special opaque plastic tape mounted on a sebumeter cassette was used for analysis value.

Statistical analysis

ANOVA One-way analysis was performed to evaluate differences in various groups after three replicate tests for in vitro research. The SPSS Statistic 25 (SPSS, USA) software was used as a statistical analysis program for a clinical trial at the OATC institute. The Shapiro-Wilk test assessed the Normality test, and a Paired t-test was applied for parametric data. In contrast, the Wilcoxon signed-rank test was used for non-parametric data comparisons between time points. The parametric test method, the Independent two-sample t-test, and the non-parametric test method, the Mann-Whitney U test, were used to compare groups.

Results

Concentration of *Bacillus stratosphericus* lysate through fermentation

Bacillus stratosphericus (*B. stratosphericus*) was cultured in TSB medium and harvested on the third day, one day after reaching the stationary phase. After culturing, the Optical Density (600 nm) was measured to be 8.82, and the pH was 8.8 (Fig. 1). After culturing, the BSLC was obtained at 7.03±0.5 g/L by freeze-drying with cell lysate and ferment. Using the obtained BSLC, we conducted evaluations of its anti-inflammatory effects, inhibition of *C. acnes* growth, and artificial sebum reduction effects, as well as clinical assessments on human subjects.

Evaluation of anti-inflammation efficacy for BSLC

A micro-dilution assay using a 96-well plate was conducted to evaluate the growth inhibition of *C. acnes* at different concentrations of BSLC. First, *C. acnes* was precultured in RCM broth to achieve a 1×10^8 CFU/mL concentration. The culture was then diluted with RCM broth to obtain a final concentrate of 5×10^5 CFU/mL, and 100 μL of this suspension was inoculated into each well of the 96-well plate. After diluting BSLC with 0.75% NaCl, 100 μL of each concentration was added to the corresponding wells of the 96-well plate. After treating the samples, they were placed in a Gas pack and cultured at 37±0.5℃ for 96 h. After culturing, each well's Optical Density (OD) was measured at 600 nm utilizing a Spectrophotometer (Libra S22, Biochrom Ltd., UK). Subsequently, the

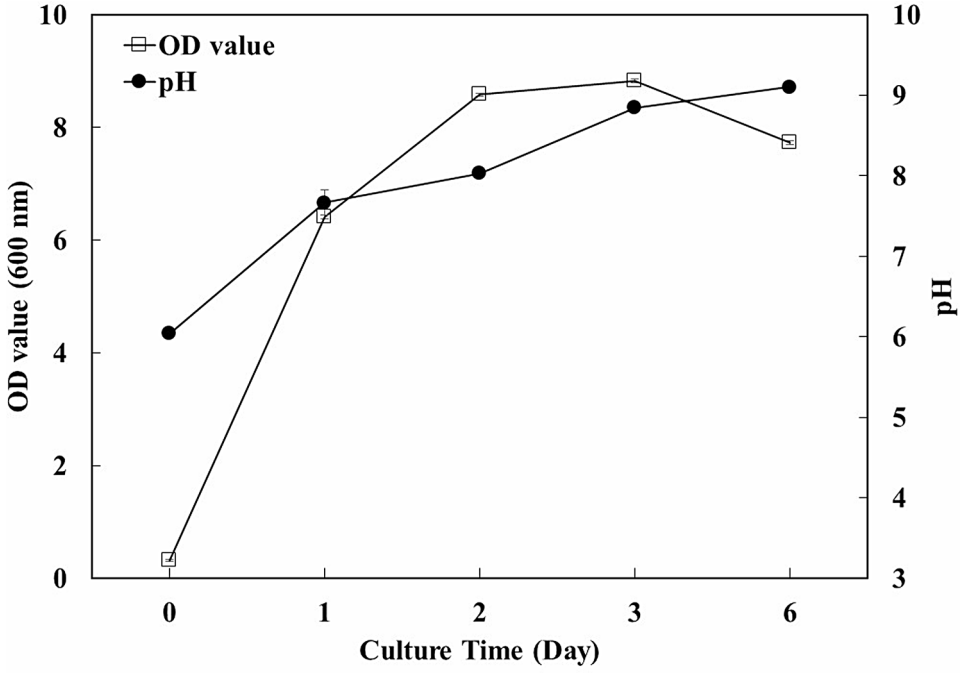

Fig. 1 Growth pattern of *Bacillus stratosphericus*. The culture was conducted over a total of 6 days, reaching the stationary phase starting from day 2. After day 3, it was observed that the culture entered the death phase. On day 3, the culture was terminated, and the lysate concentrate obtained was used for this study. As the culture progresses, a pattern of gradual increase in pH is observed

solution in the well was spread on an RCM Agar plate to observe colonies of *C. acnes*.

To determine the anti-inflammatory efficacy of BSLC, the inhibition of Nitric Oxide (NO) production by RAW264.7 macrophages was assessed. Lipopolysaccharide (LPS) is generally used as a stimulant to confirm its efficacy in inhibiting NO expression. However, the potential of utilizing *C. acnes* ferment (CAF), associated with sebum, as an alternative irritant was also investigated. The stimulus concentration of CAF was determined based on the amount of NO production induced by LPS in RAW264.7 cells when CAF was treated at various concentrations.

The results indicated that NO production increased concentration-dependent when CAF was applied to cells at concentrations ranging from 30 to 80 mg/ml, confirming its potential use as an irritant. It was confirmed that the concentration of CAF that produced NO levels exceeding those induced by LPS was 80 mg/ml. Therefore, 80 mg/ml CAF was established as the stimulus concentration for evaluating the anti-inflammatory efficacy of BSLC (Fig. 2). After setting the CAF and its concentration, Dexamethasone (DEX) and N-Acetyl-L-Cysteine (NAC), commonly used as positive controls, were conducted to determine their suitability for use as positive controls (Fig. 3).

NAC, a potent antioxidant that produces the essential glutathione, is widely used as an anti-inflammatory agent. After treating with CAF at 80 mg/ml, applying 20 mM NAC resulted in the generation of 7.4 μM NO, indicating approximately 84.96% (100- (7.4 / 49.2×100)) inhibition of NO production. This determines that NAC can be employed as a positive control (Fig. 3). In contrast, DEX did not act as a positive control in RAW264.7 cells stimulated by CAF. Eventually, NAC demonstrated high anti-inflammatory efficacy against the irritant, leading to its selection as the final positive control.

The NO production inhibition efficacy of BSLC was examined at different concentrations. As a result, BSLC at a consistency of 0.5 mg/ml demonstrated a NO production inhibition of 66.35% (100 - (14.2 / 42.2×100)) (Fig. 4). It was confirmed that BSLC inhibited NO production concentration-dependent, from 0.05 to 0.5 mg/ml. Based on the results of NO production inhibition, BSLC exhibits excellent anti-inflammatory efficacy by suppressing the irritation caused by the acne-causing bacterium *C. acnes*.

Confirmation of *C. acnes* growth inhibition effect

To evaluate the growth inhibition efficacy of BSLC on *C. acnes*, another cause of excessive sebum secretion, the study was conducted by treating *C. acnes* with various concentrations of BSLC ranging from 0.1 to 0.5 mg/ml. The experimental results showed that a 0.1 mg/ml BSLC concentration inhibited *C. acnes* growth by 65.2% compared to the control. A 0.5 mg/ml BSLC concentration resulted in only 4.9% of *C. acnes* growth. As the concentration of BSLC increased, its effect on inhibiting the growth of *C. acnes* also increased. At 0.5 mg/ml BSLC, a growth inhibition rate of approximately 95% (100% −4.9%) was observed (Fig. 5).

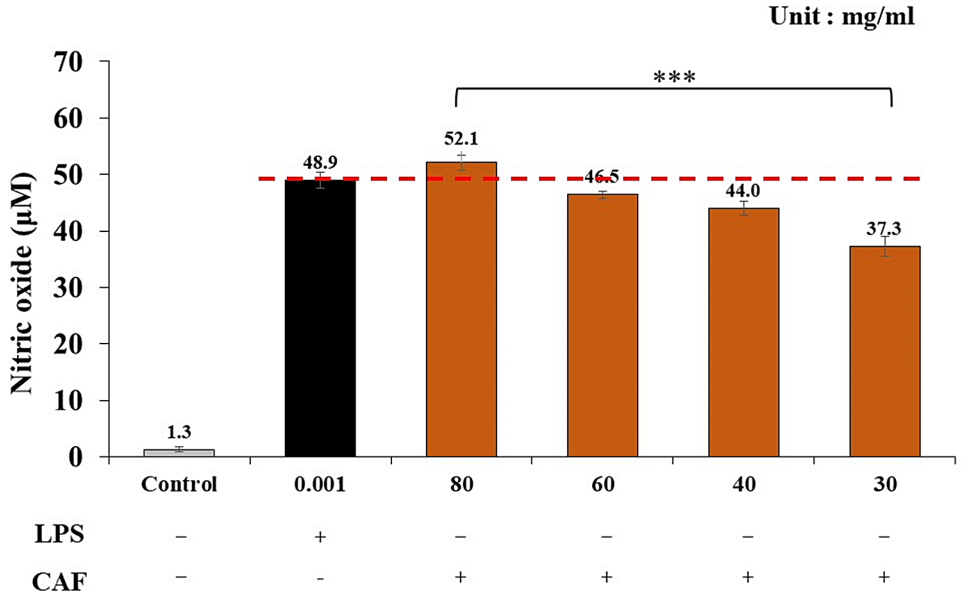

Fig. 2 Results of irritant concentration for *C. acnes* ferment. Control (gray): cells were not treated with any irritant; LPS (black): cells were applied with only LPS; *C. acnes* ferment (CAF) at concentrations of 30, 40, 60, and 80 mg/mL were added to the cell for the irritant (brown). The data is reported in triplicate as means SD ($n = 3$). One-way ANOVA analysis was performed between groups: *** $p < 0.001$

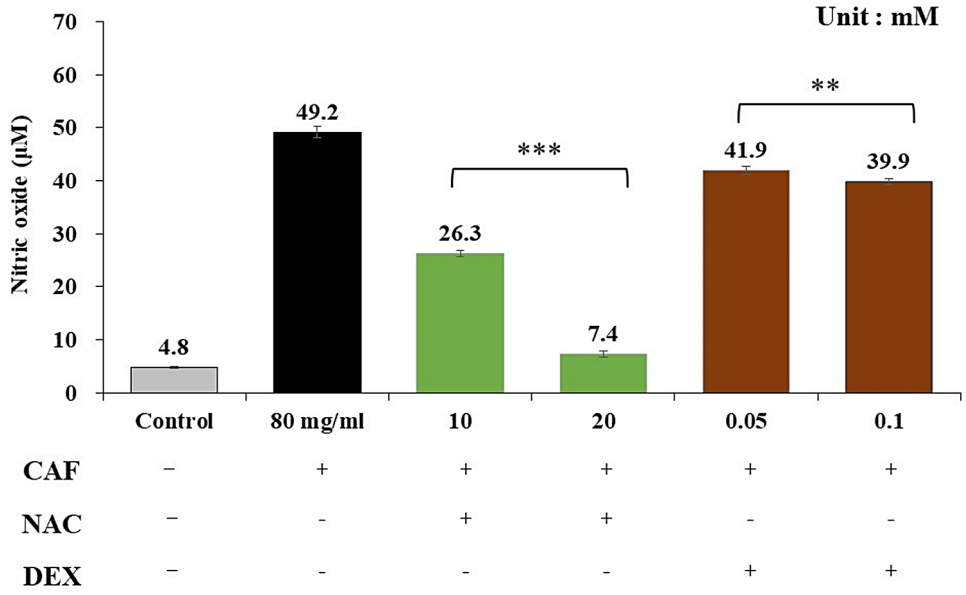

Fig. 3 Results of positive control substance. Control (gray): non-treatment of any irritant on cells; CAF (black): treatment with only CAF as an irritant on cells; NAC (green) at concentrations of 10, 20 mM, and DEX (brown) at concentrations of 0.05, 0.1mM were added to the cell for the positive control. The data is reported as means SD ($n = 3$). one-way ANOVA analysis between groups: ** $p < 0.01$, *** $p < 0.001$

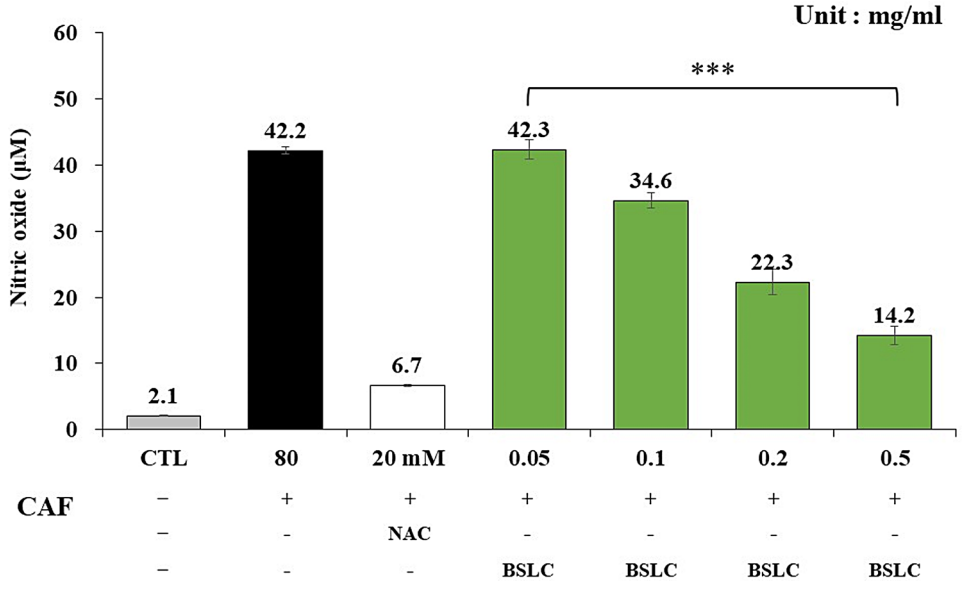

Fig. 4 Anti-inflammatory effects of BSLC. Control (gray): no treatment with any irritant; CAF (black): irritant; NAC (white): positive control. Each BSLC of 0.05, 0.1, 0.2, and 0.4 mg/ml were applied to the cells (green). The mean values and SD are recorded for all data tested ($n = 3$) with independent cells. One-way ANOVA analysis was employed between groups: *** $p < 0.001$

Assessment of artificial sebum reduction

Based on the in vitro results of BSLC's anti-inflammatory efficacy and *C. acnes* growth inhibition, BSLC was applied to artificial sebum to determine its potential for reducing sebum levels. The reduction was confirmed by treating artificial sebum with BSLC concentrations of 10, 30, and 50 mg/ml. Sodium dodecyl sulfate (SDS), known for effectively breaking down oily components, was used as the positive control. It was confirmed that the amount of sebum was reduced concentration-dependent on the BSLC, and the level of sebum was reduced by about 66.1% at a concentration of 50 mg/ml BSLC (Fig. 6).

Clinical trial for BSLC serum

A clinical trial was conducted to evaluate the improvement of daily sebum levels on human skin based on the in vitro results demonstrating anti-inflammatory and *C. acnes* growth inhibition efficacy and the reduction

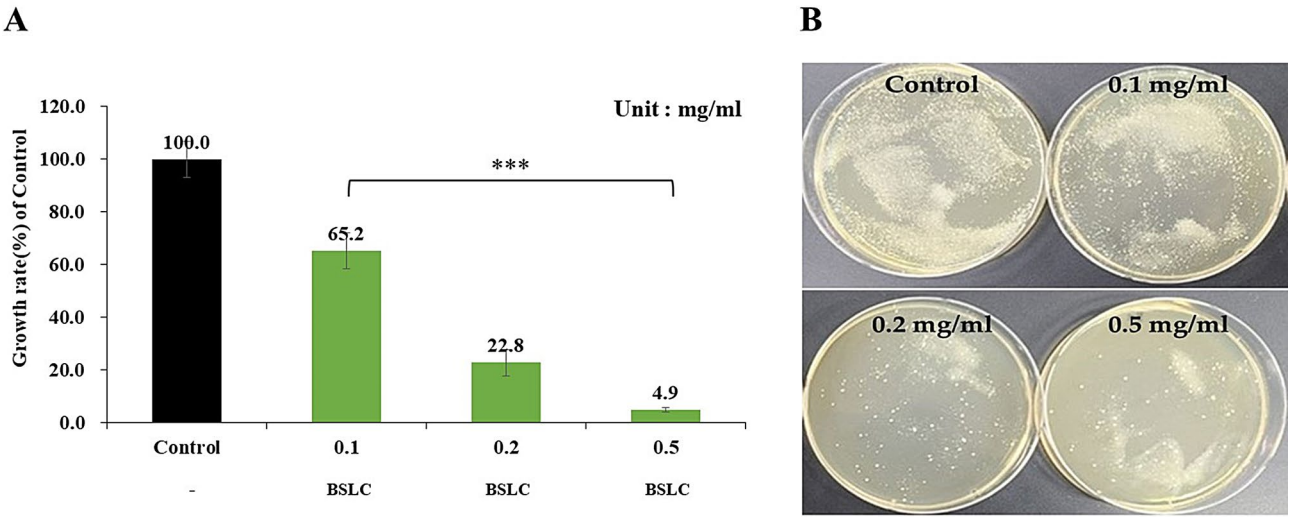

Fig. 5 Results of *C. acnes* growth inhibition efficacy. (**a**) Control (black): No treatment was applied during the cultivation of *C. acnes*. The results were obtained by treating *C. acnes* culture with BSLC at different levels from 0.1 to 0.5 mg/ml, followed by incubation for four days and measurement at OD 600 nm (green). This study was conducted in triplicate, and statistical analysis was applied in a one-way ANOVA protocol between groups. *** $p < 0.001$; (**b**) Control: No treating. Colonial patterns of *C. acnes* after spreading the *C. acnes* culture on a Petri dish and treating it with different concentrations of BSLC

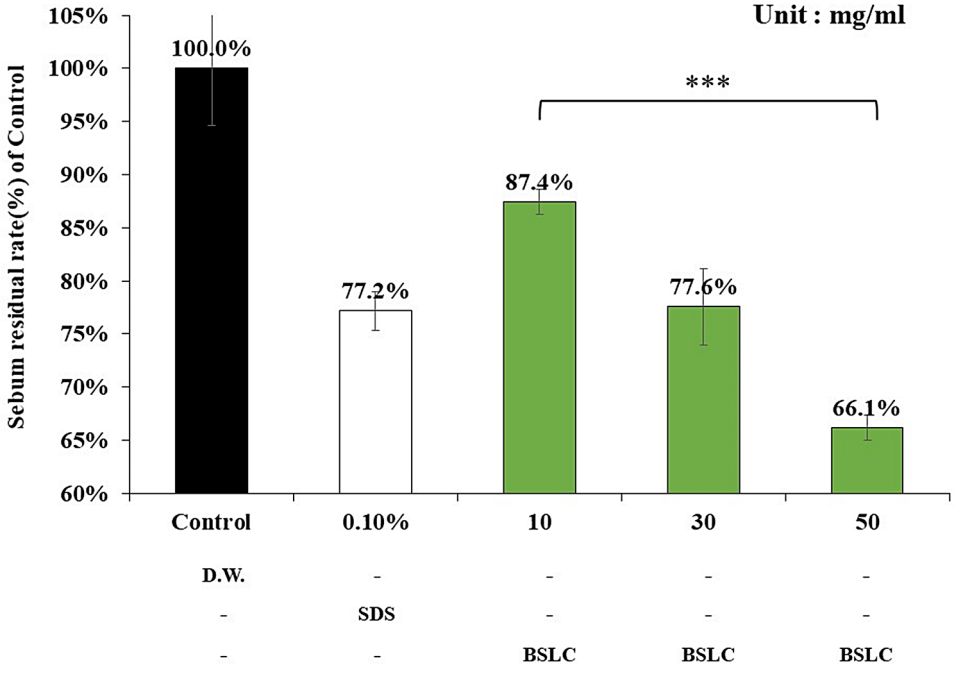

Fig. 6 Artificial sebum reduction effect of BSLC. Control (blank): It is treated with water only for artificial sebum; SDS (white): positive control. BSLC at 10, 30, and 50 mg/ml concentrations were treated with artificial sebum (green). All data were obtained from three repeated tests, and *p*-values for the groups were determined using one-way ANOVA statistical analysis. *** $p < 0.001$

in artificial sebum for BSLC. The clinical trial results revealed that compared to before product use, the sebum level in the test group (serum containing 5% BSLC) decreased by 28.68% (100 - (179.7/251.95×100)) after four weeks of use. The effective ingredient was not included in the placebo group (without BSLC), so the change rate after four weeks of use was not statistically significant. Ultimately, BSLC was an effective cosmetic ingredient for improving daily sebum levels (Fig. 7).

Discussion

Acne occurs when inflammatory factors are expressed due to various causes, leading to skin irritation [7]. Acne can be categorized into non-purulent and purulent acne [27]. In severe cases, acne can cause pain, prompting

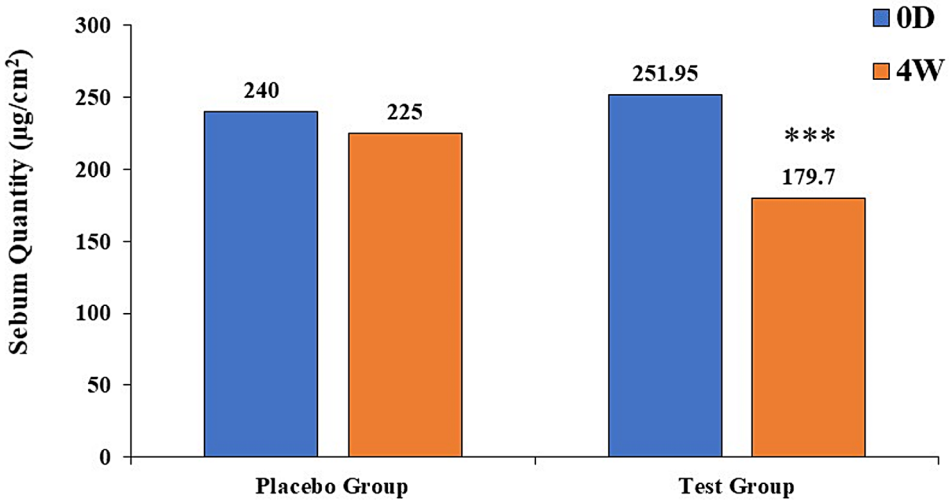

Fig. 7 Clinical trial results of daily sebum reduction with 5% BSLC serum. The results of sebum level analysis at week 0 (blue) and week 4 (brown) between the placebo and test group were obtained from a trial involving 20 Korean adults. The SPSS was applied as a statistical analysis program for a clinical trial

the use of medical treatments or functional cosmetics to manage it. On the other hand, preventing acne occurrence in advance would make it easier to maintain a healthy skin texture. The correlation between sebum, skin microbiome, and acne has been revealed through numerous studies [13]. *C. acnes*, which exists on the skin, is an opportunistic microorganism and does not permanently harm the skin. Recent research has revealed that *C. acnes* plays a beneficial role in maintaining the balance of the skin microbiome [28]. The skin hosts many microorganisms that coexist and maintain a balance. However, many studies have shown that an imbalance in the microbiome can lead to various side effects. Hence, when sebum production increases due to internal or external factors, *C. acnes* utilizes the sebum to grow more than other microorganisms, disrupting the balance of the skin microbiome. As a result, *C. acnes* becomes a harmful microorganism on the skin, leading to skin troubles such as acne. Therefore, the balance of the skin microbiome can be maintained by removing sebum from the skin or regulating the growth of *C. acnes*, continuously reducing the expression of inflammatory factors and minimizing the occurrence of acne.

In this study, the anti-inflammatory efficacy test against stimuli generated by *C. acnes* and the growth inhibition research of *C. acnes* were performed in vitro, applying the BSLC, a microorganism first discovered in the stratosphere. The anti-inflammatory effect was assessed by measuring the nitric oxide (NO) levels produced in RAW264.7 cells. Instead of applying the generally stimulant LPS for the NO assay, the stimulant employed was CAF. Subsequently, Dexamethasone was used to set the positive control; however, since it did not reduce the stimulation, it was deemed unsuitable as a positive control.

When 20 mM of N-acetyl-L-cysteine (NAC) was applied, it was confirmed to effectively inhibit NO production, leading to the selection of NAC as the positive control. Based on the results, the anti-inflammatory efficacy of BSLC was confirmed, and it was also observed that BSLC inhibited the growth of *C. acnes* in a dose-dependent manner. Furthermore, we investigated whether BSLC could directly reduce artificial sebum. It was confirmed that BSLC, in a dose-dependent manner, could directly decrease the amount of artificial sebum. Therefore, since BSLC has demonstrated efficacy against *C. acnes*, sebum, and inflammation, it is considered a potential total solution for issues related to sebum or acne (Fig. 8).

The stratosphere is an extreme environment where humans cannot live [17]. In particular, it has no water, oxygen, or nutrients and intense UV radiation. Therefore, it is an environment where most microorganisms find survival difficult. However, *B. stratosphericus* can grow at low temperatures and resist UV-B. Additionally, *B. stratosphericus* can utilize nitrogen compounds in the stratosphere for respiration and growth. Most microorganisms in extreme environments often possess defensive mechanisms to adapt and survive in such conditions. Microorganisms living in extreme environments, such as *Deinococcus radiodurans*, are known for their remarkable defensive mechanisms [29, 30]. A prominent example is their robust DNA repair system, which enables them to withstand severe conditions like radiation and desiccation. Thus, it can be expected that *B. stratosphericus* also produces metabolites to adapt to its extreme environment. Among the various metabolites of *Bacillus stratosphericus*, lipase and surfactin are known as representatives. Lipase, which can decompose lipids, and surfactin, a lipo-peptide with surfactant properties, can

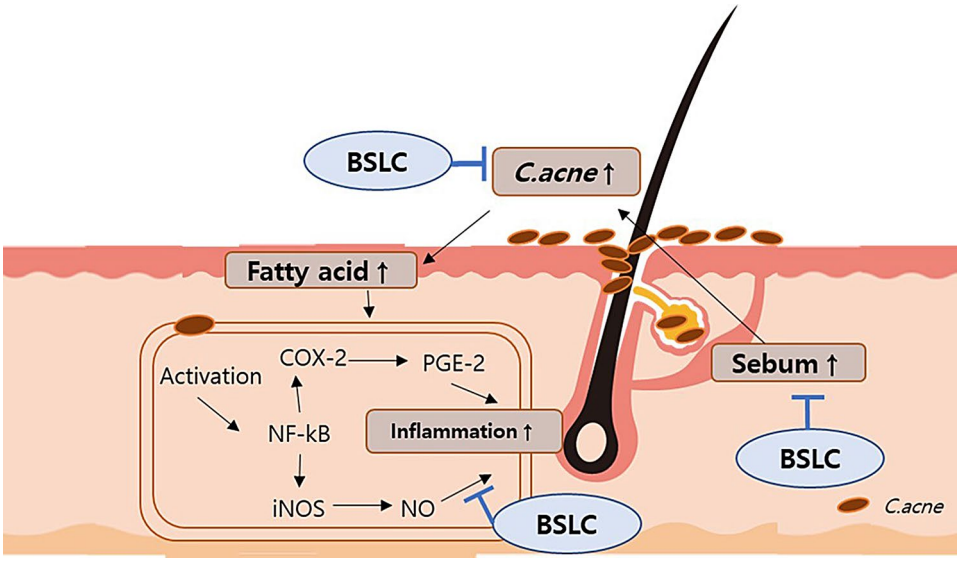

Fig. 8 Mechanism of sebum reduction for BSLC. By preparing BSLC and evaluating its anti-inflammatory effects, inhibition of *C. acnes* growth, and reduction of sebum using artificial sebum, we can predict its impact on the mechanisms of sebum production in human skin

be produced by different *Bacillus* species. For surfactin, an amphiphilic lipo-peptide is known to disrupt the cell membrane of *C. acnes*, causing osmotic imbalance and thereby inhibiting its growth [31]. Notably, literature has confirmed the possibility of *Bacillus stratosphericus* to produce these compounds [20]. Therefore, *Bacillus stratosphericus* was selected as a potential candidate microorganism capable of reducing sebum composed of lipid complexes [32, 33]. Also, *Lactobacillus* species, known for their ability to produce organic acids, generally exhibit antimicrobial activity against *C. acnes*. However, *Lactobacillus* strains typically display low pH levels during cultivation, and the acidic culture medium tends to inhibit the growth of various microorganisms [34]. Despite this, the low pH culture medium can cause significant irritation to the skin and increase ionic strength, leading to issues such as decreased viscosity in cosmetic formulations, which poses challenges for commercialization [35]. In contrast, the culture medium of *Bacillus stratosphericus* is reported to have a neutral pH level, which is expected to mitigate these issues.

We undertook studies to explore its potential in controlling skin sebum by utilizing *B. stratosphericus*, an extremophile microorganism. Based on the results confirmed in vitro, a clinical trial was conducted to assess the daily sebum improvement. After four weeks of application, it was confirmed that the sebum level in the skin was reduced. Therefore, it was determined that BSLC, based on its anti-inflammatory and *C. acnes* growth inhibitory effects, can inhibit sebum production and improve skin texture. Accordingly, BSLC is expected to have a splendid potential material for use as a sebum-improving ingredient in the cosmetics industry.

It is true that while *B. stratosphericus* has been discovered by experts searching for new extremophiles, but it has not been studied as extensively as *Lactobacillus sp.* or *Saccharomyces sp.* Therefore, this study aimed to investigate whether *B. stratosphericus* could directly or indirectly improve sebum control. The anti-inflammatory effects, inhibition of *C. acnes* growth, and reduction of artificial sebum by *B. stratosphericus* lysate concentrate (BSLC), along with the demonstrated clinical efficacy, suggest that BSLC has significant potential for application in various industries. Since 'Bacillus ferment' is a registered and applicable ingredient in the cosmetics industry, this research suggests that the developed BSLC could be effectively utilized as a sebum-controlling ingredient. However, to enhance the additional value of this developed material, it will be crucial to analyze the unknown active components of BSLC. If this analysis confirms the efficacy of these components, it could open opportunities not only in the cosmetics industry but also in other sectors, such as the medical industry.

Authors Contribution

Conceptualization, H.K., Y.G.C. and K.Y.H.; methodology, H.K.; formal analysis, H.K., S.G.Y., J.H.H; investigation, H.K.; validation, S.G.Y, J.H.H.; writing original draft preparation, H.K.; writing review and editing, H.K., Y.S.K., Y.G.C., and K.Y.H.; visualization, H.K., J.H.H., and K.Y.H.; supervision, Y.G.K. and K.Y.H.; project administration, H.K.; funding acquisition, K.Y.H. All authors have read and agreed to the published version of the manuscript.

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