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# Screening of Microbial Strains Used to Ferment *Dendrobium officinale* to Produce Polysaccharides, and Investigation of These Polysaccharides' Skin Care Effects

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**Abstract:** The microbial fermentation of plants is a promising approach for enhancing the yield of polysaccharides with increased activity. In this study, ten microbial strains, *Lactiplantibacillus plantarum* CCFM8661, *Limosilactobacillus reuteri* CCFM8631, *Lactobacillus helveticus* M10, *Lacticaseibacillus rhamnosus* CCFM237, *Lactilactobacillus sakei* GD17-9, *Lacticaseibacillus casei* CCFM1073, *Bacillus subtilis* CCFM1162, *Bacteroides cellulosilyticus* FTJSI-E-2, *Bacteroides stercoris* FNMHLBEIK-4, and *Saccharomyces cerevisiae* HN7-A5, were used to ferment *Dendrobium officinale*. The skin care activity of the resulting polysaccharides (F-DOP) was evaluated in cultured HaCaT and RAW 264.7 cells, and a mouse model. The results indicated that *D. officinale* medium promoted strain proliferation, and fermentation significantly enhanced polysaccharide yield (up to 1.42 g/L) compared to that without fermentation (0.76 g/L). Moreover, F-DOPs, especially after CCFM8631 fermentation, exhibited an excellent ability to attenuate sodium dodecyl sulfate-induced HaCaT cell injury (from 69.04 to 94.86%) and decrease nitric oxide secretion (from 42.86 to 22.56 μM) in lipopolysaccharide-stimulated RAW 264.7 cells. In vivo, CCFM8631-FDOP reduced the transdermal water loss rate, skin epidermal thickness, and interleukin 6, and enhanced the expression of filaggrin, improving 2,4-dinitrofluorobenzene-induced skin damage. Therefore, considering viable cell counts, polysaccharide yields, and skin care efficacy in vitro and in vivo, CCFM8631 is the most suitable strain to enhance the skin care activity of DOPs and possesses promising potential for applications in the cosmetics industry.

**Keywords:** *Dendrobium officinale*; fermentation; *Limosilactobacillus reuteri* CCFM8631; polysaccharides



**Citation:** Tang, X.; Wang, B.; Mao, B.; Zhao, J.; Liu, G.; Yang, K.; Cui, S. Screening of Microbial Strains Used to Ferment *Dendrobium officinale* to Produce Polysaccharides, and Investigation of These Polysaccharides' Skin Care Effects. *Processes* **2023**, *11*, 2563. https://doi.org/10.3390/pr11092563

Academic Editors: Ali Demirci, Irfan Turhan and Ehsan Mahdinia

Received: 24 July 2023
Revised: 17 August 2023
Accepted: 24 August 2023
Published: 27 August 2023



## 1. Introduction

*Dendrobium officinale*, belonging to the family Orchidaceae, is widely distributed in several countries worldwide, including China, Japan, and Australia [1]. In China, *D. officinale* has been recognized as one of the most valuable traditional Chinese medicines (TCMs) for thousands of years. TCM practitioners believe that *D. officinale* offers a wide range of health benefits, such as fever reduction, stomach nourishment, and lifespan extension [2]. Modern pharmaceutical studies have revealed multiple bioactivities associated with *D. officinale*, such as immune-regulatory, antitumor, cardioprotective, and anti-aging bioactivities [3]. Owing to its exceptional nutritional value, *D. officinale* is considered a life-saving herb [4].

As a complex botanical matrix, *D. officinale* is rich in polysaccharides, flavonoids, alkaloids, pigments, and other small-molecule components [3,5,6]. Among these constituents, *D. officinale* polysaccharides (DOPs), such as glucomannan with 1,4-β-D-Manp and 1,4-β-D-Glcp, the main active component with antioxidant, moisturizing, and hair growth-promoting effects, have a great potential in functional foods and cosmetics [7]. However, studies investigating the structure–bioactivity relationship of polysaccharides

have suggested that the original polysaccharide structures present in plants may not exhibit optimal bioactivities [8]. Therefore, it is crucial to develop a suitable method to improve the yield and biological activity of DOPs.

Microbial fermentation has emerged as a promising biomodification technology for natural products and has garnered increasing attention. During fermentation, microorganisms produce a large number of extracellular enzymes, such as proteases, cellulases, glycosidases, and pectinases. These enzymes rupture plant cells and accelerate the dissolution of active ingredients, resulting in improved polysaccharide yield. Additionally, during fermentation, microorganisms can transform the original polysaccharides into novel fermented polysaccharides with enhanced bioactivity [9]. A previous study where *Panax ginseng* was fermented with *Saccharomyces cerevisiae* GIW-1 reported that fermentation increased the yield of polysaccharides while also enhancing their in vitro antioxidant capacity (by scavenging hydroxyl and superoxide anion free radicals) and in vivo anti-inflammatory effects (by reducing tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 content) [10]. *Bacillus* sp. DU-106 fermentation altered the Mw and monosaccharide composition of DOPs, which enhanced the immunoregulatory ability of DOPs [11].

The skin, the largest organ in the body, plays a critical role in protecting the internal environment and maintaining homeostasis [12]. However, prolonged exposure to UV radiation, air pollution, and harmful organisms can damage the skin structure, leading to abnormalities in the skin barrier. Unfortunately, a compromised function of the skin barrier is often accompanied by dysregulated dermal immune responses, which in turn exacerbate damage to the skin barrier, creating a vicious cycle [13]. DOPs, which help maintain the integrity of the skin barrier and normalize immune responses, have the potential to promote skin health [14]. However, reports on the yields and bioactivities of polysaccharides derived from strains fermenting *D. officinale* are limited and fragmented. Therefore, the objective of this study was to obtain a strain that enhanced the skin care properties of DOPs. This study aimed to facilitate the application of microbial fermentation in herbal medicine and provide a theoretical foundation for the development of novel skin care products.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

The stems of *D. officinale*, provided by Nutri-Woods Bio-tech Co., Ltd. (Beijing, China), were crushed (60 mesh). Yeast extract FM528 was purchased from Angel Yeast Co., Ltd. (Yichang, China). Sodium dodecyl sulfate (SDS), methyl thiazolyl tetrazolium (MTT), and dimethyl sulfoxide (DMSO) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Lipopolysaccharide (LPS) was obtained from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). The enzyme-linked immunosorbent assay (ELISA) kits for nitric oxide (NO), filaggrin (FLG), and interleukin (IL)-6 were purchased from Jiangsu Meibiao Biotechnology Co., Ltd. (Yancheng, China). The other chemicals used in this study were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

### 2.2. Screening of Strains for Fermenting DOPs

#### 2.2.1. Activation and Culture of Strains

The Lactobacillus (*L. plantarum* CCFM8661, *L. helveticus* M10, *L. rhamnosus* CCFM237, *L. reuteri* CCFM8631, L. sakei GD17-9, *L. casei* CCFM1073), Bacillus (*B. subtilis* CCFM1162), Bacteroides (*B. cellulosilyticus* FTJSI-E-2, *B. stercoris* FNMHLBEIK-4), and yeast (*S. cerevisiae* HN7-A5) strains were obtained from Culture Collection of Food Microorganisms of Jiangnan University and cultured in specific media. Lactobacillus strains were cultured in MRS medium containing 20 g/L glucose, 5 g/L yeast extract, 10 g/L tryptone, 10 g/L beef extract, 2 g/L sodium acetate, 0.58 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L $MnSO_4 \cdot H_2O$, 2 g/L ammonium citrate dibasic, 2.6 g/L $K_2HPO_4 \cdot 3H_2O$, and 1 mL/L Tween 80. Bacillus strains were cultured in LB medium containing 10 g/L tryptone, 10 g/L yeast extract, and 10 g/L NaCl. Bacteroides strains were cultured in BHI medium containing 38.5 g/L brain heart

infusion, 1 g/L L-cysteine, 1 g/L hemin, and 1 g/L vitamin K. Saccharomyces cerevisiae was cultured in YPD medium containing 10 g/L yeast extract, 10 g/L tryptone, and 20 g/L glucose. Each strain was streaked onto its corresponding solid medium and incubated to obtain single colonies. After incubation, a single colony was picked and inoculated twice into liquid medium to obtain highly viable seed cultures for further experiments.

#### 2.2.2. Fermentation of *D. officinale*

For the fermentation process, *D. officinale* (40 g/L) was used as the single carbon source (replacing glucose) to prepare *D. officinale*-based MRS, LB, BHI, and YPD media. The activated strains ($10^7$ CFU/mL) were inoculated into the corresponding *D. officinale*-based medium. The *Lactobacillus*, *Bacillus*, and *Bacteroides* strains were cultured at 37 °C and pH 6.0 for 16 h, and the yeast was cultured at 30 °C and pH 6.0 for 48 h. Sterile water was used instead of the microorganisms in the unfermented group. At the end of fermentation, the cell counts of the strains were determined using the plate dilution method [15], and the fermentation solutions were collected for polysaccharide extraction.

#### 2.2.3. Extraction of DOPs and F-DOP

Polysaccharides' extraction was performed using an ultrasonic-assisted method, according to a previous report [16]. The *D. officinale* fermentation solution was sonicated at a power level of 500 W for 10 min. The resulting supernatant was collected by centrifugation (8000× *g* for 15 min), deproteinated using Sevag reagent, precipitated by adding four volumes of ethanol at 4 °C for 24 h, and lyophilized to obtain DOPs (polysaccharides from non-fermented *D. officinale*) and F-DOPs (polysaccharides from fermented *D. officinale* solution). The total carbohydrate content was determined using the anthrone–sulfuric acid method [17].

### *2.3. Evaluation of F-DOPs Skin Care Effects In Vitro*

#### 2.3.1. Cell Culture

Human-immortalized keratinocytes (HaCaT cells) and mouse mononuclear macrophages (RAW 264.7 cells) were purchased from China Center for Type Culture Collection (Wuhan, China). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum (FBS) (Gibco, Billings, MT, USA) and incubated at 37 °C in a 5% $CO_2$ atmosphere. Prior to use, the polysaccharide samples were dissolved in the culture medium and sterilized by passage through a 0.22 μm membrane filter.

#### 2.3.2. Cytoprotection of SDS-Injured HaCaT Cell

SDS, the most-used anionic alkyl sulfate surfactant, was used to induce skin barrier damage [18]. Log-phase HaCaT cells ($5 \times 10^3$ cells/well) were collected, seeded in a 96-well plate, and divided into three groups: (1) control group (medium), (2) model group (SDS), and (3) treated group (DOP/F-DOP + SDS). After incubating for 12 h, the cells were pretreated with various polysaccharide samples (1000 μg/mL) for 24 h. Subsequently, the cells were exposed to SDS (50 μg/mL) for 24 h. Cell survival was determined using the CCK-8 assay.

#### 2.3.3. Anti-Inflammation in LPS-Induced RAW 264.7 Cell

The anti-inflammatory properties of the polysaccharide samples were characterized using an NO content assay in LPS-stimulated RAW 264.7 cells [19]. Logarithmic phase cells ($5 \times 10^3$ cells/well) were collected, seeded in a 96-well plate, and divided into three groups: (1) control group (medium), (2) model group (LPS), and (3) treated group (DOP/F-DOP + LPS). After incubation for 12 h, the RAW 264.7 cells were pretreated with various polysaccharide samples (1000 μg/mL) for 24 h. The cells were then stimulated with LPS (5 g/mL) for 24 h. The culture supernatants were collected, and the NO content was measured using a commercial NO assay kit, according to the manufacturer's instructions.

### 2.4. Evaluation of F-DOPs Skin Care Effects In Vivo

#### 2.4.1. Preparation of F-DOP-Based Ointment

The ingredients of the DOP/F-DOP ointments are listed in Table 1. Briefly, the oil phase (shark squalene and emulsifier), aqueous phase I (DOP/F-DOP, glycerin, and water), and aqueous phase II (nipagin ethyl ester and water at 90 °C) were prepared. Subsequently, aqueous phases I and II were slowly added to the oil phase and stirred continuously to obtain a DOP/F-DOP-based ointment [20].

**Table 1.** *Dendrobium officinale* polysaccharide (DOP)-based ointment composition and function of each ingredient in the formulation.

| Added Material | Amount Added (g) | Function |
|---|---|---|
| Unfermented/Fermented DOP | 1.50 | The main drug |
| Shark squalene | 3.90 | Oil phase |
| Emulsifier (Montanov S) | 2.10 | Surfactant |
| Glycerin | 2.40 | Water phase, humectant |
| Nipagin ethyl ester | 0.03 | Preservative |
| Distilled water | Volume to 30.00 | Solvent |

#### 2.4.2. Animals and Experimental Design

Specific pathogen-free BALB/c male mice (6–8 weeks old, 18–20 g) were purchased from the Guangdong Medical Laboratory Animal Center (Foshan, China). The mice were housed under standard conditions with ad libitum access to standard food and water. The housing environment maintained a constant temperature of 25 ± 2 °C, humidity of 50 ± 10%, and a 12-h light/dark cycle. Following acclimation for one week, the mice were prepared for experimentation. The dorsal skin of each mouse, measuring 4 cm × 2 cm, was shaved. The mice were then randomly divided into five groups, each containing five mice: (A) normal control group (NC), (B) model control group (MC), (3) unfermented DOP cream treatment group (DOP), (C) CCFM8631-FDOP cream treatment group (CCFM8631), and (D) prednisolone cream treatment group as a positive control (PC). The experimental animal use license was approved by SYXK (Guangdong) 2018-0186.

#### 2.4.3. Induction of Skin Damage Model

The 2,4-dinitrofluorobenzene (DNFB)-induced skin damage in vivo model was established as previously described [21]. Briefly, 100 µL of 0.25% DNFB (*w*/*v*) dissolved in a 3:1 mixture of acetone/olive oil was painted onto the dorsal skin of each mouse on days 1 and 4. Furthermore, the same skin area was exposed to 100 µL of 0.2% DNFB on days 7 and 10 to induce skin injury (MC group). The NC group was treated with acetone/olive oil only. The DOP, CCFM8631, and PC groups were administered DOP/F-DOP-based ointment or prednisolone cream in DNFB-injured mice twice a day from day 7 to day 15, respectively.

#### 2.4.4. Macroscopic Observation and Transepidermal Water Loss (TEWL) Test

On day 16, all mice were transferred to a room with controlled temperature (23 ± 1 °C) and humidity (50 ± 10%). The dorsal skin of each mouse was photographed to evaluate the morphological changes in tissue appearance. Moreover, TEWL, an important indicator reflecting the integrity of the skin barrier and tissue gas exchange with the environment, was measured by the Tewameter® TM 300 (Courage & Khazaka, Cologne, Germany).

#### 2.4.5. Hematoxylin and Eosin (H&E) Staining

At the end of the experiment, all mice were euthanized by cervical dislocation. Dorsal skin samples were collected and divided into two sections. One portion was immediately immersed in 4% paraformaldehyde for histological observation, and the other was stored at −80 °C for further analysis. The fixed skin samples were embedded in paraffin, sliced into 6-µm-thick sections, and stained with hematoxylin and eosin for histopathological assess-

ment, as described in a previous study [22]. The H&E-stained sections were observed under an optical microscope, and the epidermal thickness was measured using ImageJ software.

#### 2.4.6. Biochemical Assays

Skin tissue samples were weighed (0.1 g), homogenized in normal saline (1 mL), and centrifuged at 12,000× *g* for 15 min at 4 °C to obtain the tissue supernatant. Protein content was determined using the BCA method using bovine serum albumin (BSA) as a standard [23]. The levels of FLG and IL-6 were measured using ELISA kits.

### *2.5. Statistical Analysis*

All results of this study are expressed as means ± standard deviation (SD). Statistical significance was determined using SPSS software (version 23.0) with one-way analysis of variance followed by Tukey's test. Origin 2023 software was employed for the preparation of graphs. Different letters mean $p < 0.05$, which was considered statistically significant for all analyses.

## 3. Results and Discussion

### *3.1. Determination of Viable Cell Counts and Polysaccharide Yields*

The ten strains were cultivated in *D. officinale*-based medium, and the viable cell counts after fermentation are shown in Table 2. Supplementation with *D. officinale* increased the proliferation of various strains. The viable cell counts of *B. cellulosilyticus* FTJSI-E-2, *B. subtilis* CCFM1162, *L. casei* CCFM1073, *B. stercoris* FNMHLBEIK-4, *L. plantarum* CCFM8661, *L. reuteri* CCFM8631, and *L. sakei* GD17-9 were 130 × $10^7$, 100 × $10^7$, 45 × $10^7$, 35 × $10^7$, 19 × $10^7$, 16 × $10^7$, and 10 × $10^7$ CFU/mL, respectively.

**Table 2.** Viable cell counts and polysaccharide yields from *D. officinale* fermented by different strains.

| Strains | Viable Cell Counts (×$10^7$)/(CFU/mL) | Polysaccharides Yield (g/L) |
|---|---|---|
| *L. plantarum* CCFM8661 | 19.0 ± 1.41 $^{cde}$ | 1.40 ± 0.04 $^{a}$ |
| *L. helveticus* M10 | 2.1 ± 0.42 $^{e}$ | 1.41 ± 0.06 $^{a}$ |
| *L. rhamnosus* CCFM237 | 7.2 ± 0.14 $^{e}$ | 1.36 ± 0.03 $^{ab}$ |
| *L. reuteri* CCFM8631 | 16.0 ± 1.41 $^{de}$ | 1.30 ± 0.04 $^{abc}$ |
| *L. sakei* GD17-9 | 10.0 ± 1.41 $^{de}$ | 1.36 ± 0.06 $^{ab}$ |
| *L. casei* CCFM1073 | 45.0 ± 5.66 $^{c}$ | 1.23 ± 0.04 $^{bc}$ |
| *B. subtilis* CCFM1162 | 100.0 ± 14.14 $^{b}$ | 0.32 ± 0.01 $^{d}$ |
| *B. cellulosilyticus* FTJSI-E-2 | 130 ± 14.14 $^{a}$ | 1.24 ± 0.01 $^{bc}$ |
| *B. stercoris* FNMHLBEIK-4 | 35.0 ± 2.83 $^{cd}$ | 1.20 ±0.01 $^{c}$ |
| *S. cerevisiae* HN7-A5 | 3.4 ± 0.14 $^{e}$ | 1.42 ± 0.04 $^{a}$ |

Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

Moreover, the F-DOP yield after fermentation was determined and is illustrated in Table 2. Fermentation by the strains enhanced the F-DOP yield compared to that of DOPs. Strains in descending order of F-DOP yield were as follows: *S. cerevisiae* HN7-A5 (1.42 g/L), *L. helveticus* M10 (1.41 g/L), *L. plantarum* CCFM8661 (1.4 g/L), *L. rhamnosus* CCFM237 (1.36 g/L), *L. sakei* GD17-9 (1.36 g/L), and *L. reuteri* CCFM8631 (1.3 g/L). Based on the viable cell count and F-DOP yield, *L. plantarum* CCFM8661, *L. reuteri* CCFM8631, *L. casei* CCFM1073, *B. subtilis* CCFM1162, *B. cellulosilyticus* FTJSI-E-2, and *S. cerevisiae* HN7-A5 were considered suitable strains for fermenting *D. officinale*. These F-DOPs were selected to evaluate their skin care effects in subsequent experiments. Our results indicated that the ten strains could grow in medium containing *D. officinale* as the sole carbon source, albeit with varying degrees of specificity among the strains. Additionally, the fermentation of *D. officinale* by these strains resulted in an increased polysaccharide yield. Similarly, it was reported that the polysaccharide yield from *Astragalus membranaceus* was 2.3-fold higher with *Lactobacillus plantarum* fermentation than without it [24].

### *3.2. Skin Care Effects of F-DOPs In Vitro*

HaCaT cells maintain a normal keratinocyte shape and are commonly used to evaluate the skin protection of cosmetics, medicines, and food products [25]. Moreover, RAW 264.7 cells have a stable and mature adherent macrophage phenotype and are frequently employed to investigate the immune regulatory abilities and innate immune responses of samples [26].

SDS is an anionic surfactant widely used in household cleaning, cosmetics, and pharmaceutical products. However, prolonged exposure to SDS can disrupt cell membranes, leading to barrier disruption and skin irritation [27,28]. As presented in Figure 1, after treatment with 50 μg/mL SDS for 24 h, the percent survival of HaCaT cells was significantly decreased (69.04%) compared to that of the control group, suggesting that the cell injury model was successfully established. Both DOP and F-DOP treatments attenuated SDS-induced cell injury and remarkably increased cell viability. F-DOPs exhibited superior cyto-protection compared with DOPs. In particular, F-DOPs produced from *L. reuteri* CCFM8631 fermentation resulted in the highest cell viability (94.86%) among all groups.

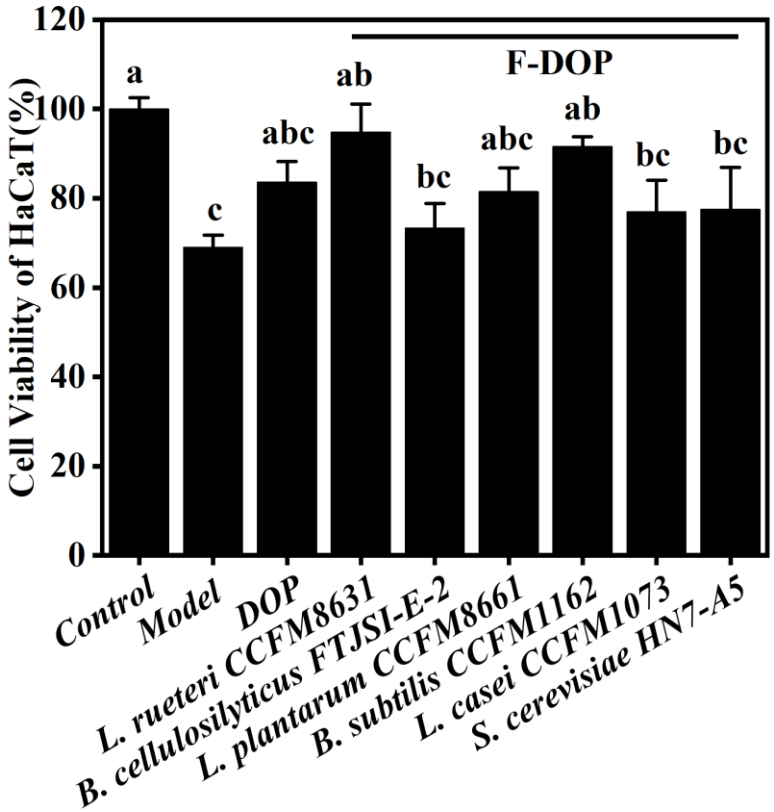


**Figure 1.** Effect of DOPs and fermented *Dendrobium officinale* polysaccharides (F-DOPs) on survival in sodium dodecyl sulfate (SDS)-injured HaCaT cells. Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

LPS is known to bind to Toll-like receptors and activate NF-κB through the MyD88-dependent signaling pathway, leading to the secretion of inflammatory mediators, such as TNF-α, IL-6, and NO [29]. As expected, after 24 h of LPS treatment in RAW264.7 cells, the NO content in the model group was 42.86 μM, which was significantly higher than that of the control group (13.42 μM) (Figure 2). However, this abnormal increase was inhibited by F-DOP supplementation. Specifically, F-DOPs after *L. reuteri* CCFM8631, *L. plantarum* CCFM8661, and *L. casei* CCFM1073 fermentation remarkably decreased the NO content to 22.56, 22.00, and 18.40 μM, respectively, indicating the outstanding anti-inflammatory activity of the F-DOPs. *L. reuteri* CCFM8631 was selected as the most suitable strain for the

fermentation of *D. officinale* based on the number of living bacteria, polysaccharide yield, and cell culture experiment results.

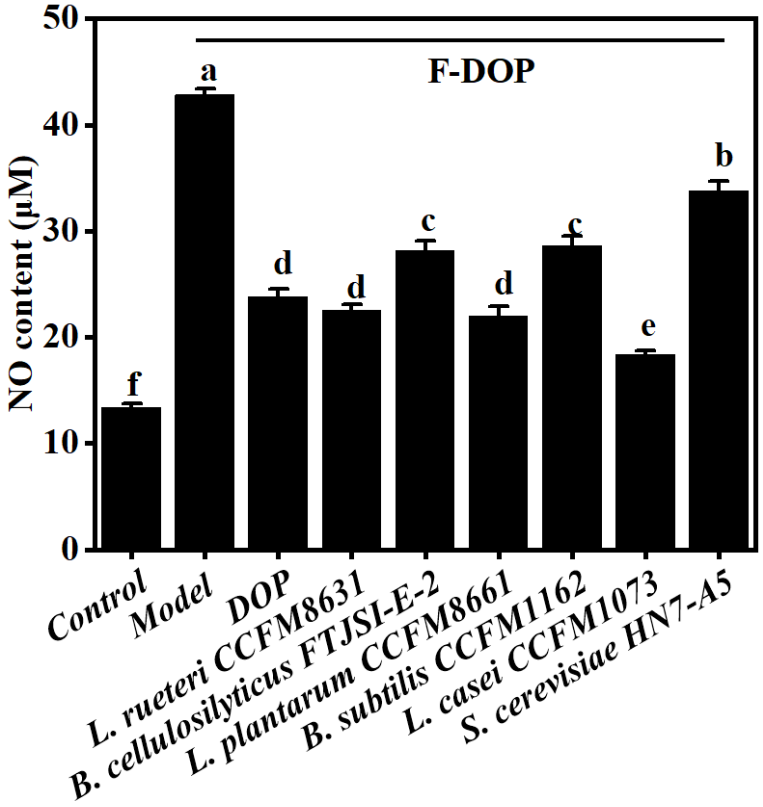


**Figure 2.** Effect of DOPs and F-DOPs on nitric oxide (NO) content in LPS-stimulated RAW 264.7 cells. Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

Polysaccharides have attracted considerable attention owing to their low toxicity and diverse pharmacological activities [30]. A previous review summarized that DOPs decrease free radicals (2,2-diphenyl-1-picrylhydrazyl and hydroxyl), enhance antioxidant systems (superoxide dismutase, catalase, and glutathione peroxidase), inhibit the NF-κB pathway, and downregulate inflammatory responses, having potential applications in the field of skin care and cosmetics [7]. Consistent with previous findings, our study also demonstrated that pretreatment with DOPs protected HaCaT cells from SDS-induced damage and decreased NO production in RAW 264.7 cells in LPS-induced inflammatory model. Interestingly, fermentation increased the polysaccharide yield of *D. officinale* and improved its antioxidant and anti-inflammatory activities. Yang et al. reported that *Polygonatum kingianum* polysaccharides fermented by *Lactobacillus casei* resulted in superior anti-aging effects on *Caenorhabditis elegans*, with a 10.09% increase in lifespan compared with that resulting from the original polysaccharides. This improvement could be attributed to a decrease in molecular weight distribution, a change in chemical and monosaccharide composition, and the smoothness of the microtopography [8]. The beneficial effects on skin health of DOPs and F-DOPs (fermented by *L. reuteri* CCFM8631) were investigated in a DNFB-induced injury model in vivo, which is described in Section 3.3.

### *3.3. Skin Care Effects of F-DOPs In Vivo*

#### 3.3.1. Apparent Skin Changes and H&E Staining

Representative skin images of the different groups are shown in Figure 3A. Repeated application of DNFB to the dorsal surface resulted in severe skin lesions characterized

by redness, swelling, crust formation, dryness, and incrustation, indicating the successful establishment of the skin damage model (MC group). However, these symptoms in the MC group were relieved by pretreatment with the DOP and F-DOP ointments. Notably, the CCFM8631 group showed better attenuation of skin damage than the DOP group, similar to that of the PC group.

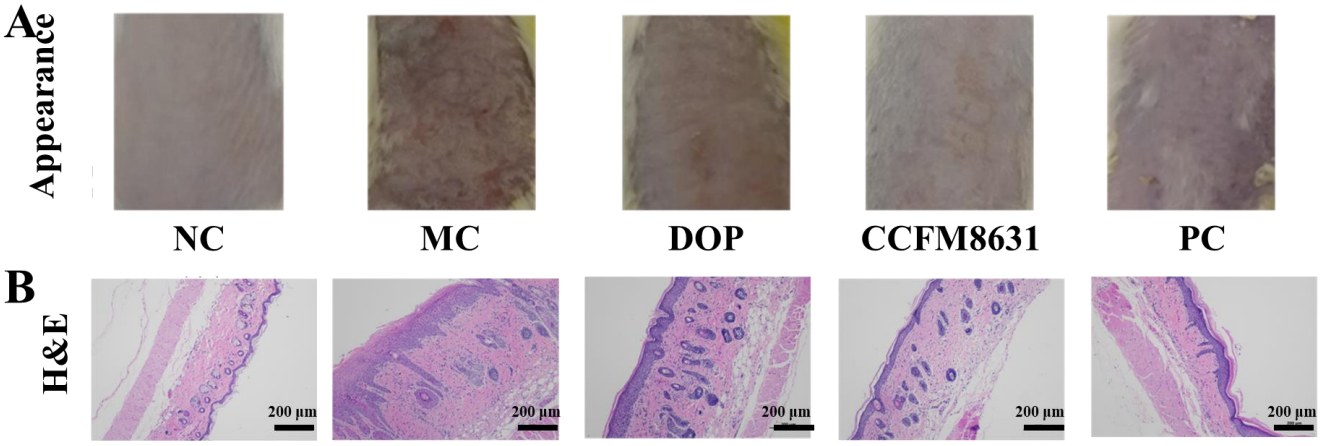


**Figure 3.** Effects of samples on (**A**) skin appearance and (**B**) hematoxylin and eosin staining in the DNFB-damaged model in vivo.

H&E staining was performed to examine structural changes in the skin tissue of each group. In the NC group, a distinct stratum corneum, thin stratified squamous epithelium, and well-organized and tightly arranged cells in the epidermis, with no apparent pathological alterations were observed (Figure 3B). After modeling with DNFB, the skin tissue structure was disrupted and was characterized by a thickened stratified squamous epithelium, disordered cell arrangement, and infiltration of inflammatory cells. Compared with the MC group, treatment with CCFM8631-FDOP, like the PC group, mitigated dorsal swelling and inflammatory infiltrating, which exhibited superior effects than the DOP group. Moreover, the epidermal thickness of the mouse skin was measured to further evaluate the skin protection of the samples. As shown in Figure 4, the epidermal thickness was significantly increased from 20 μm in the NC group to 199 μm in the MC group. Both DOP and CCFM8631-FDOP treatment decreased the epidermal thickness compared to that in the control group. In the CCFM8631 group, the epidermal thickness was 47 μm, which was better than that of the PC group (60 μm). These results indicate that the F-DOPs produced by CCFM8631 fermentation have the potential to alleviate DNFB-induced skin damage.

### 3.3.2. Change in TEWL

TEWL is an indirect measure of skin barrier integrity because it reflects the rate of water evaporation from the skin surface. Normally, the skin maintains a constant range of water loss. However, when its barrier function is compromised, water loss increases [31,32]. As shown in Figure 5, TEWL was significantly higher in the MC group (40.13%) than in the NC group (14.60%), indicating skin barrier damage. In the DOP, CCFM8631, and PC groups, TEWL values decreased to 28.63%, 27.68%, and 18.68%, respectively, suggesting that the samples could help restore skin barrier function.

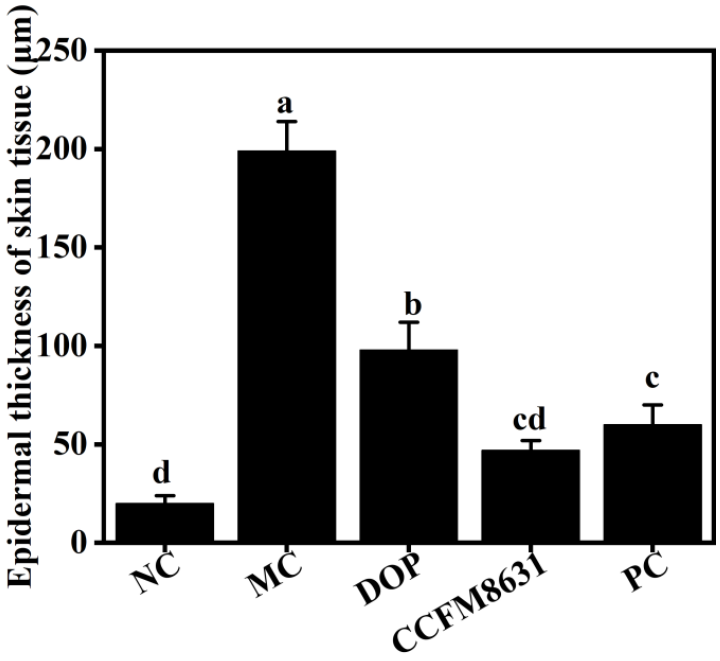


**Figure 4.** Changes in epidermal thickness of skin tissue. Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

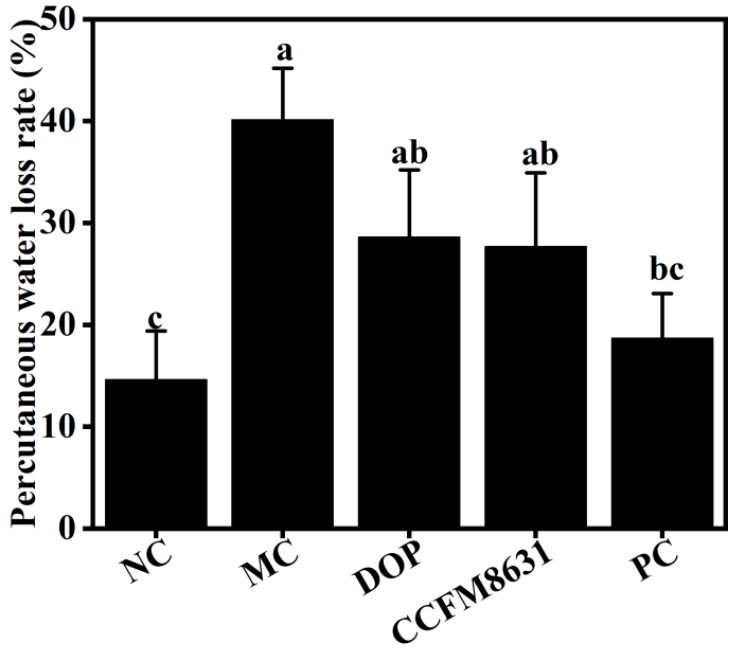


**Figure 5.** Effects of samples on TEWL values in DNFB-damaged model in vivo. Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

#### 3.3.3. FLG Content

FLG is a protein synthesized by keratinocytes that plays a crucial role in maintaining the integrity of the epidermal skin barrier. It aggregates keratin into filaments, which contribute to the structural stability of the skin barrier [33]. Moreover, the degradation products of FLG, known as natural moisturizing factors, regulate skin hydration [34]. As shown in Figure 6, the FLG content was significantly lower in the MC group (96.08 pg/mg)

than in the NC group (168.93 pg/mg), suggesting impairment of the skin barrier. However, this decrease in FLG content was ameliorated by treatment with DOPs or CCFM8631-FDOPs. The FLG level in the CCFM8631 group was 221.75 pg/mL, remarkably higher than that in the PC group (102.08 pg/mL). This result indicates that CCFM8631-FDOPs could improve the expression of FLG and repair DNFB-injured barrier function.

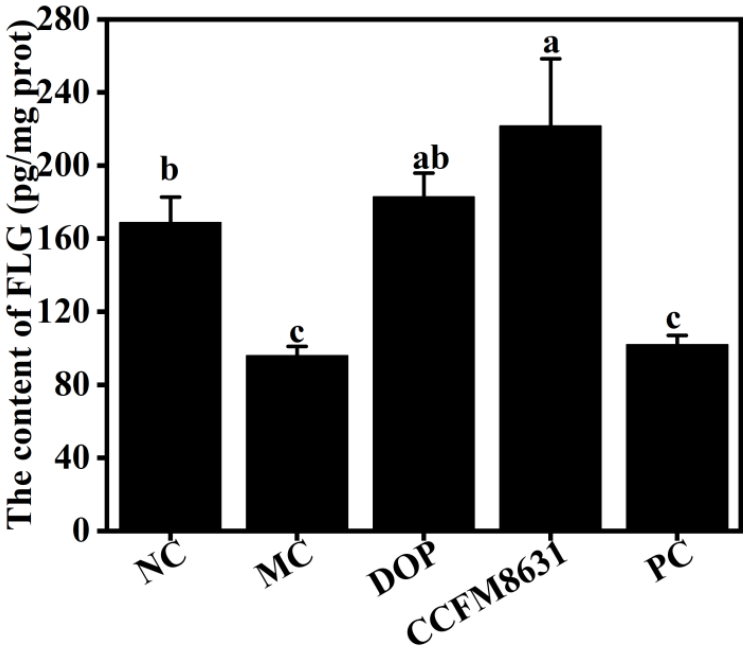


**Figure 6.** Effects of samples on FLG content in DNFB-damaged model in vivo. Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

#### 3.3.4. IL-6 Level

IL-6 is an important inflammatory cytokine produced by T cells and macrophages in response to infection and tissue damage. However, excessive IL-6 promotes the activation and aggregation of neutrophils, thereby amplifying injury [35]. Figure 7 shows the IL-6 levels in the skin tissue of the experimental mice. Compared to the NC group, DNFB treatment stimulated a strong inflammatory response, as evidenced by a significant increase in IL-6 levels. The administration of DOPs and CCFM8631-FDOP decreased the IL-6 content by 2.08% and 24.60%, respectively, compared to that in the MC group. Notably, the IL-6 level in the CCFM8631 group was 5.82 pg/mL, which was not significantly different from that in the PC group (5.59 pg/mL), suggesting an excellent anti-inflammatory effect.

DNFB is a hapten that interacts with various skin proteins to form covalent conjugates, resulting in an enhanced immune response. Multiple applications of DNFB disrupt the skin barrier and induce skin lesions [36]. Our results revealed that fermentation with *L. reuteri* CCFM8631 improved the ability of DOPs to alleviate DNFB-induced skin damage. This benefit is closely related to the combined effects of skin barrier repair and anti-inflammatory properties. In another study, *Punica granatum* L. polysaccharides suppressed the secretion of pro-inflammatory cytokines by inhibiting the NF-κB and STAT3 signaling pathways and enhanced skin barrier protection by increasing aquaporin-3 and FLG expression, which then ameliorated imiquimod-elicited psoriasis [37]. Notably, the CCFM8631-FDOP was a mixture, containing fermented DOPs and microbial exopolysaccharides. It is not clear whether the increased activity of F-DOPs was caused by the structural change of DOPs during fermentation or the synergistic action of DOPs and CCFM8631 polysaccharides. Thus, in the future, our research will focus on the structure-activity relationship of CCFM8631-FDOP.

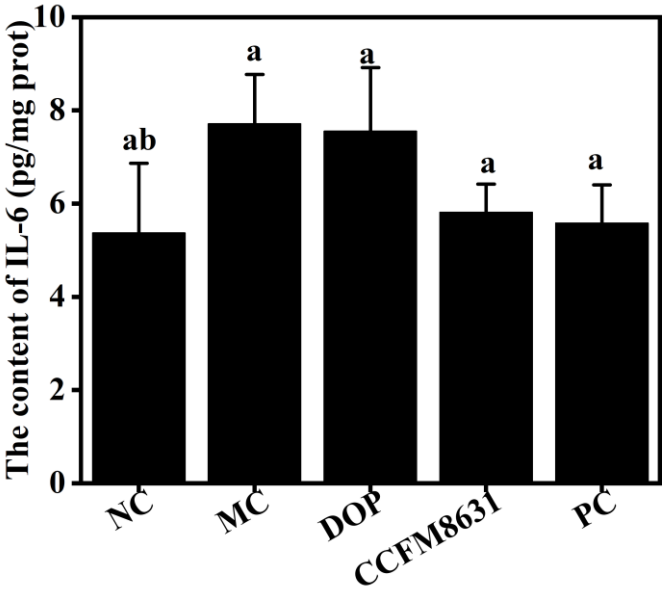


**Figure 7.** Effects of samples on IL-6 levels in DNFB-damaged model in vivo. Statistical analysis with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison analysis with the Tukey test. Different letters indicate significant differences among different groups ($p < 0.05$).

## 4. Conclusions

This study aimed to screen suitable strains for fermenting *D. officinale* to produce polysaccharides with excellent skin care properties. Supplementation with *D. officinale* increased the proliferation of all ten microbial strains. Moreover, fermentation with *S. cerevisiae* HN7-A5, *L. helveticus* M10, *L. plantarum* CCFM8661, *L. rhamnosus* CCFM237, *L. sakei* GD17-9, and *L. reuteri* CCFM8631 resulted in significantly increased F-DOP yields compared with those of the unfermented group. Therefore, *L. plantarum* CCFM8661, *L. reuteri* CCFM8631, *L. casei* CCFM1073, *B. subtilis* CCFM1162, *B. cellulosilyticus* FTJSI-E-2, and *S. cerevisiae* HN7-A5 with high viable cell counts and polysaccharide yields were selected to evaluate skin care effects in vitro and in vivo. The results indicated that F-DOPs, especially after *L. reuteri* CCFM8631 fermentation, protected HaCaT cells from SDS-induced injury, decreased LPS-induced NO secretion in RAW 264.7 cells, and alleviated the DNFB-triggered skin damage in a mouse model with reduced inflammatory response and epidermal thickness and improved TEWL and skin barrier integrity. Summarily, in this study, CCFM8631 fermentation enhanced the yield of polysaccharides. More important, CCFM8631-FDOP exhibited better skin care ability than DOPs both of in cells and animal model in vitro and in vivo.

**Author Contributions:** X.T. and B.W., formal analysis and data curation and writing-original draft; B.M. and J.Z., writing—review and editing; J.Z. and G.L., supervision and project investigation; K.Y. and S.C., funding and supervision. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the National Natural Science Foundation of China (Grant No. 32172173), and Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province.

**Institutional Review Board Statement:** License number of mice experiments was SYXK (Guangdong) 2018-0186.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data are contained within the article.

**Conflicts of Interest:** The authors declare no conflict of interest.

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