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Inhibitory Effect of Probiotic Metabolites on Seborrheic Dermatitis and Acne-Related Pathogenic Bacteria

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Abstract: The topical application of probiotic metabolites has shown positive effects in the treatment of skin diseases; however, the effectiveness is strain dependent. Comparing the pathogen inhibitory effects of probiotic strains with different genetic backgrounds and analyzing their key metabolites can provide insights about the potential of applying probiotics for skincare. In this study, we investigated the fermentation growth inhibition of 18 commercial probiotic strains on the skin pathogens *Malassezia furfur* (*M. furfur*) and *Cutibacterium acnes* (*C. acnes*) in vitro. We found that most *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) and *Lacticaseibacillus rhamnosus* (*L. rhamnosus*) strains exhibited strong inhibition of *M. furfur* and *C. acnes*, which lasted up to 100 h. The main antibacterial metabolites observed were molecules below 10,000 Da in molecular weight, including peptides and organic acids (lactic acid, acetic acid, propionic acid, and butyric acid). The synergistic effect of organic acid combinations lowered the minimum inhibitory concentration (MIC). The composition of these antimicrobial metabolites varied among strains, which demonstrated the strain-dependent pathogenic inhibitory effects. This study provides insights into the application potential of using probiotic metabolites against seborrheic dermatitis and acne-related pathogenic bacteria.

Keywords: probiotics; skin microbiome; *Malassezia furfur*; *Propionibacterium acnes*; antibacterial; metabolites

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1. Introduction

The skin acts as a crucial barrier against external pathogens while supporting a complex microbial ecosystem, known as the skin microbiome. The skin microbiome comprises over 1000 different microorganisms, including bacteria, fungi, and viruses, with approximately 1 billion microorganisms per square meter of skin [1]. These microorganisms form a symbiotic relationship with the skin, and they can be classified as either resident or transient bacteria, depending on the duration of residence. Most microorganisms inhabit the skin surface and appendages, forming a stable microbial flora [2] to prevent harmful bacteria or pathogens from colonizing [3].

Recent studies suggest that the composition and distribution of different microorganisms maintain a dynamic balance. These microorganisms interact with the host skin and immune system through proliferation, secretion, and other processes [4–7]. Once this balance is disrupted, it may lead to dysbiosis and result in skin diseases such as acne, atopic dermatitis, and seborrheic dermatitis (SD) [2,8–22].

Unlike treatment with traditional antibiotics and immunological drugs, which can increase the risk of pathogenic bacteria drug resistance, probiotic therapy has emerged

as a promising approach for the prevention and treatment of skin diseases and wound protection without such risk [23]. Applications in human medicine and animal health have demonstrated that the topical or oral use of probiotics restores skin microbial homeostasis, improves the skin barrier, and promotes the synthesis of antimicrobial short peptides to treat skin inflammation [24]. Unfortunately, the topical use of probiotics on the resident microbial communities of the skin, which often plays important roles in opportunistic infections [9–11], has not been well studied [25–27]. The efficacy of different probiotics strains, dose, inhibition duration, and key functional metabolites remain unclear.

In this study, we conducted a comparative analysis of the pathogens' inhibitory effects exhibited by various probiotics. In order to explore the inhibitory potential probiotic strains against the opportunistic pathogenic microorganisms *M. furfur* and *C. acnes*, 18 selected probiotics were tested to identify the most effective probiotic strains and their key metabolites that could alleviate skin diseases such as seborrheic dermatitis and acne (As shown in Figure 1). We isolated four bacterial strains among them for further investigation of their antimicrobial activities, with a specific focus on two types of metabolites: peptides and organic acids. The results revealed that the combined effect of organic acid mixtures can effectively reduce the minimum inhibitory concentration (MIC).

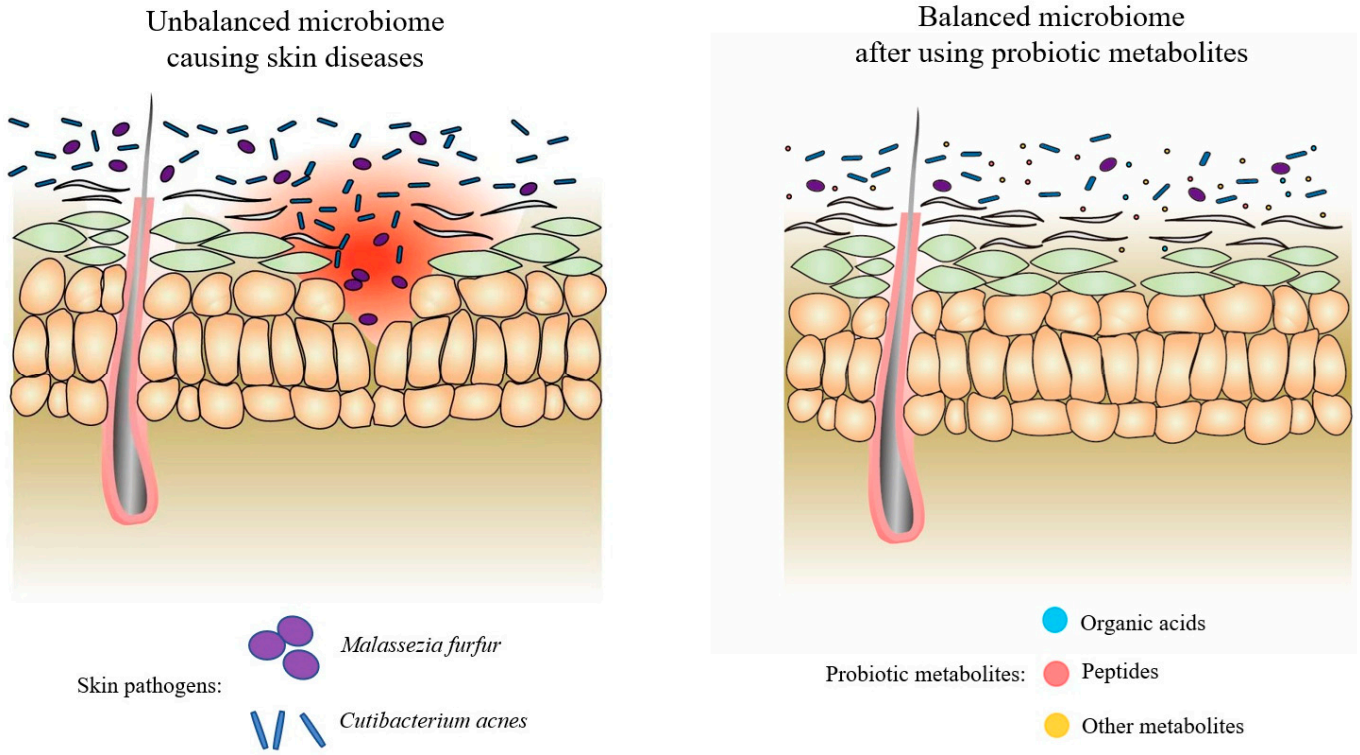


Figure 1. Schematic illustration of the study on the inhibitory effect of probiotic metabolites on seborrheic dermatitis and acne-associated pathogenic bacteria.

2. Materials and Methods

2.1. Preparation of Pathogenic Bacteria

M. furfur (BNCC 337308) and *C. acnes* (BNCC 186335) were purchased from BeNa Culture Collection. *M. furfur* was cultivated on modified Dixon glycine medium (0.6% peptone, 3.6% malt extract, 2% ox-bile, 1% Tween-40, 0.2% glycerol, 0.2% oleic acid, 7 mmol glycine, and 2% agar) at 30 °C for 48 h. *C. acnes* was cultivated on brain heart infusion 1053 medium at 37 °C for 48 h and in anaerobic jars (anaerobically).

2.2. Preparation of Probiotic Strains

Eighteen probiotic strains were obtained from Danisco (China) Holding Co., Ltd (Shanghai, China). Strains were cultivated in commercial MRS (DeMan, Rogosa and Sharpe) broth (Oxoid, UK) at 37 °C for 24 h. After fermentation, the culture broth was centrifuged at 5500 rpm for 5 min, and the supernatant was then filtered using a 0.22 μm filter (PALL) membrane to remove bacterial cells completely. The supernatant was finally stored at 4 °C until use.

2.3. Growth Inhibition Test

The probiotic supernatant was added to the medium of *M. furfur* or *C. acnes* at different concentrations, followed by adding 1% of the fresh pathogen cultures, while the same amount of sterile water instead of probiotic supernatant was employed as the control. The growth of *M. furfur* and *C. acnes* was measured every 24 h at OD600 (nm) using a Anthos2010 microplate reader (Anthos Labtec Instruments GmbH, Wals-siezenheim, Austria).

2.4. Identification of Active Metabolites

Probiotic strains that showed growth inhibition in the previous study (2.3 Growth Inhibition Test) were then isolated to further investigate their antimicrobial activities, with a specific focus on two types of metabolites: peptides and organic acids.

To obtain the antimicrobial peptides, the supernatants of the probiotic strains were sterilized via microfiltration, followed by precipitation using an ammonium sulfate solution (70% to 80% saturation) at 4 °C. The precipitate and supernatant were then separated via centrifugation. After that, the precipitate was dissolved in sodium phosphate buffer at pH 6.0 and dialyzed (dialysis bag, 10,000/200 Da molecular weight cut-off (MWCO)) to obtain crude protein extract containing proteins larger than 10,000 Da. The supernatant was dialyzed the same way. The crude extracts obtained from the precipitate and supernatant after dialysis were used for the growth inhibition assay.

To obtain the antimicrobial organic acids, the supernatants of the four bacterial strains were neutralized to pH 6.5 with 0.1 M NaOH and then used for the growth inhibition assay. The contents of lactic acid, acetic acid, propionic acid, and butyric acid in the supernatants of the four bacterial strains were determined using HPLC analysis. Based on the organic acid content of each sample, a solution containing only organic acids and deionized water was prepared, sterilized, and used as a control for the growth inhibition assay.

3. Results

3.1. Inhibitory Effects of 18 Probiotic Strains on Skin Pathogens

The growth inhibitory results of the supernatant from the fermentation of 18 probiotic strains presented in Table 1 demonstrate that three strains of *B. lactis* and three strains of *L. rhamnosus* exhibited significant growth inhibition of *M. furfur* and *C. acnes*, with an inhibition rate exceeding 90% within 48 h. *B. lactis* maintained a high inhibition rate over 72 h, while the inhibitory effect of *L. rhamnosus* decreased to approximately 60%. *L. plantarum* and *B. animalis* performed well in the initial 48 h, but their antibacterial effects decreased to 60% or less after 72 h. Most strains exhibited prolonged inhibitory effects on *C. acnes* growth, especially *B. lactis* and *L. rhamnosus*. *L. salivarius* and *L. plantarum* also demonstrated relatively high inhibitory effects. The minimum inhibitory concentrations (MICs) of the probiotic supernatants required for inhibiting the growth of *M. furfur* and *C. acnes* were 20–25% and 8–12%, respectively.

Out of the eighteen bacterial strains evaluated for their antimicrobial performances based on their inhibition effectiveness and duration, HN019 exhibited the highest efficacy,

with an inhibition rate of over 90% against both pathogenic bacteria after 72 h. Bi07 and B420 showed the highest long-term inhibition rates against *M. furfur*, with a slightly lower long-term inhibition rate against *C. acnes* compared to HN019. Conversely, Lp115 exhibited the opposite pattern, with a slightly lower long-term inhibition rate against *M. furfur* and the highest long-term inhibition rate against *C. acnes*. Therefore, these four strains were selected for further investigation regarding their antimicrobial properties.

Table 1. The growth inhibition results of 18 probiotic strains on *M. furfur* and *C. acnes*. ++ indicates that the inhibition rate exceeds 90%. + indicates that the inhibition rate is between 40% and 90%. − indicates that the inhibition rate is less than 40%.

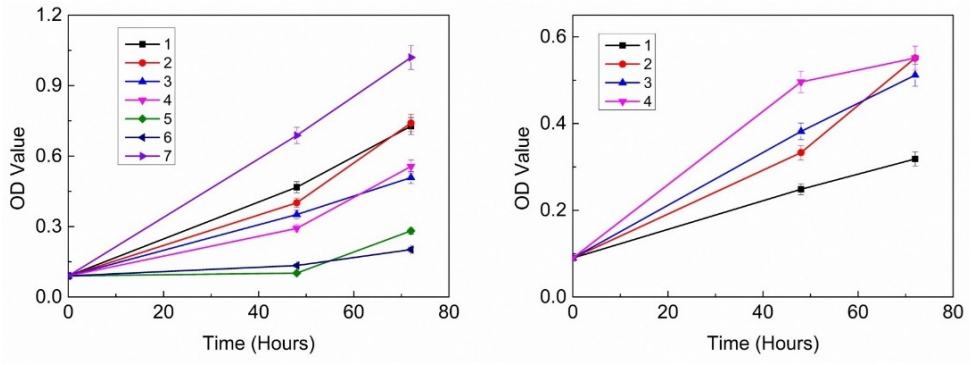
Name	Strain	Growth Inhibition of *M. furfur*			Growth Inhibition of *C. acnes*		
		48 h	72 h	96 h	48 h	72 h	96 h
Bifidobacterium lactis	HN019	++	++	++	++	++	++
Bifidobacterium lactis	Bl-04	−	−	−	+	+	+
Bifidobacterium lactis	B420	++	++	++	++	+	+
Bifidobacterium lactis	Bi-07	++	++	++	++	+	+
Bifidobacterium animalis	Bb-12	++	−	−	++	+	+
Lactobacillus brevis	Lbr-35	−	−	−	+	+	+
Lactobacillus acidophilus	NCFM	+	−	−	+	+	+
Lactobacillus plantarum	Lp-115	++	+	−	++	++	++
Lactobacillus paracasei	Lpc-37	+	−	−	+	+	−
Lactobacillus rhamnosus	HN001	++	+	−	++	+	+
Lactobacillus acidophilus	La-14	−	−	−	++	−	−
Lactobacillus casei	Lc-11	+	−	−	++	+	−
Lactobacillus rhamnosus	GG	++	−	−	++	+	+
Lactobacillus salivarius	Ls-33	+	−	−	++	+	+
Lactobacillus rhamnosus	Lr-32	++	−	−	++	+	+
Lactobacillus reuteri	1E1	+	−	−	+	−	−
Lactobacillus fermentum	SBS-1	−	−	−	+	+	+
Lactobacillus bulgaricus	Lb-87	−	−	−	++	+	−

3.2. Antibacterial Proteins and Peptides

The dialyzed samples (10,000 MWCO) of B420, Bi07, HN019, and Lp115 showed no inhibition on *M. furfur* and *C. acnes*. The crude extract of B420 protein even promoted the growth of *C. acnes*. However, the dialyzed samples (200 Da MWCO) exhibited antibacterial effects, and the results are shown in Figure 2.

For the growth inhibition test of *M. furfur*, the crude supernatant extracts of B420, Bi07, HN019, and Lp115 demonstrated a significant inhibitory effect. The growth inhibition rate of the crude supernatant extracts of HN019 and Lp115 reached 90% in 48 h and above, while those of B420 and Bi-07 were approximately 60%. In the case of the crude protein extract, Bi07 and HN019 also exhibited an inhibitory effect at approximately 40% to 50%. However, the inhibition rate of all samples declined at 72 h, ranging from 1% to 20%.

In the growth inhibition test of *C. acnes*, the crude protein extract of B420 showed an inhibition rate of 61% at 48 h, while no inhibition was observed in the crude protein extracts of other strains. The 48 h inhibition rate of the supernatant from the Bi07 crude protein extracts was 40%, and that of HN019 was 28%. By 72 h, the inhibition rate of these samples decreased to 10% to 40%.



(**a**) (**b**)

Figure 2. (**a**) The 0–72 h growth inhibition of the six samples on *M. furfur*. Sample 1: crude protein extract of Bi-07; Sample 2: crude protein extract of HN019; Sample 3: crude supernatant extract of B420; Sample 4: crude supernatant extract of Bi-07; Sample 5: crude supernatant extract of HN019; Sample 6: crude supernatant extract of Lp-115; Sample 7: positive control. (**b**) The 0–72 h growth inhibition of six samples on *C. acnes*. Sample 1: crude protein extract of B420; Sample 2: crude supernatant extract of Bi-07; Sample 3: crude supernatant extract of HN019; Sample 4: positive control.

3.3. Inhibitory Effects of Organic Acids

Further investigation of the organic acids produced by strains B420, Bi07, HN019, and Lp115 revealed that the supernatants of the four bacterial strains, neutralized to pH 6.5 with 0.1 M NaOH, exhibited partial inhibition. The inhibition rates ranged from 40% to 80% at 48 h and 0% to 50% at 72 h.

HPLC analysis of the fermentation broth of the four probiotics presented (Table 2) showed that lactic acid and acetic acid were the most abundant organic acids, accounting for 88% to 94% of the total content. Propionic acid accounted for 5% to 10%, while butyric acid comprised only 0.2% to 1%. The contents of organic acids varied significantly among the strains. For example, the lactic acid content in Lp115 was four times higher than that in B420, and the acetic acid content in B420 was over two times higher than that in Lp115.

Table 2. The concentrations of lactic acid, acetic acid, propionic acid, and butyric acid in the supernatants of the four selected strains via HPLC analysis.

No.	Strain	Lactic Acid (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)
1	B420	5.64	9.26	1.82	0.2
2	Bi-07	17.39	6.25	1.65	0.17
3	HN019	10.82	8.85	1.06	0.05
4	Lp-115	19.88	4.04	1.80	0.06

The results of the growth inhibition test of *M. furfur* and *C. acnes* using the prepared organic acid mixture are presented in Figure 3.

The organic acid mixtures derived from the four selected strains demonstrated significant inhibition effects, with inhibition rates ranging from 53% to 90% at 72 h. Sample 2 exhibited the highest inhibition rate at 90%, while Samples 1 and 3 exhibited inhibition rates around 70%.

The organic acid mixture of the four samples exhibited notable inhibition effects, with inhibition rates ranging from 90% to 95% at 72 h. Sample 1 exhibited the highest inhibition rate at 96%.

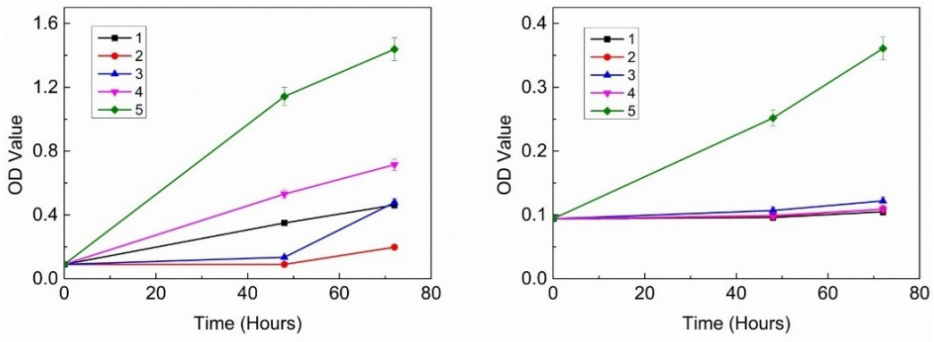


Figure 3. (**a**) The 0–72 h growth inhibition of *M. furfur*. (**b**) The 0–72 h growth inhibition of *C. acnes*. Samples 1 to 4 contained only the organic acids found in the supernatants of the four strains 1-B420, 2-Bi07, 3-HN019, and 4-Lp115, respectively, as determined via HPLC analysis. Sample 5 was the assay control.

4. Discussion

The personal care industry is increasingly utilizing probiotics and their metabolites as bioactive ingredients to enhance skin health via topical application, *Lactobacillus* is the most commonly used genus of probiotics [28–30]. In this study, *B. lactis* strains demonstrated greater effectiveness in inhibiting the growth of *M. furfur* and *C. acnes* compared to *L. rhamnosus*, indicating the potential of being used as probiotics for skin care.

Previous studies have confirmed that the antibacterial substance compounds of the found probiotics in probiotic metabolites include organic acids, bacteriocins, ethyl acetate, and phenyllactic acid [28–32]. This study further suggests that these antibacterial compounds are likely to be complex mixtures with varied antibacterial functions, differing in types and proportions across strains (Table 3). These compounds may primarily consist of peptides and organic acids. Additionally, it was observed that the metabolites with inhibitory effects on *M. furfur* and *C. acnes* were predominantly concentrated in substances with a molecular weight below 10,000 Da. It is speculated that these substances primarily disrupt the cell membrane structure and intracellular environment homeostasis, ultimately leading to cell death [33].

Table 3. The growth inhibition of *M. furfur* of the active metabolites of the four strains.

No.	Strain	Growth Inhibition of *M. furfur*		
		Crude Protein Extract	Crude Supernatant Extracts	Organic Acid Mixture
1	B420	-	55%	72%
2	Bi-07	31%	50%	92%
3	HN019	30%	79%	71%
4	Lp-115	-	88%	54%

The primary discovery of this study was the diverse effects resulting from different combinations of organic acids in the antimicrobial tests. The combined impact of organic acid mixtures notably reduced the minimum inhibitory concentration (MIC). In the antimicrobial test against *M. furfur*, the antibacterial effect of the sample (B420) with the lowest organic acid mixture is better than that of the sample (Lp115) with the highest content. Moreover, the antimicrobial effects of the two samples (Bi07 and Lp115) with the highest total organic acid contents differed by 40%, while the sample with the lowest total organic acid content exhibited intermediate antimicrobial effect. This test also revealed

that a single component is not the sole determinant of antimicrobial efficacy. For instance, although sample B420 had the lowest lactic acid content, its antimicrobial effect reached 70%. Conversely, sample Lp115, despite having a slightly higher lactic acid content than Bi07, yielded a 40% lower antimicrobial effect, the lowest among the four samples at 54%. A similar trend was observed for acetic acid. despite its acetic acid content being only 65% of that in B420, it exhibited the most potent antimicrobial effect, indirectly indicating the synergistic antimicrobial effects of several organic acids. Joana Salomskiene's research revealed that *L. Lactis* 140/2 showcased the highest antimicrobial activity by producing a complex of organic acids [34], including lactic acid, citric acid, benzoic acid, and tartaric acid. This suggests that organic acids produced by strains such as Bi07 may also have complex antimicrobial effects, necessitating further research.

To date, most studies measured the antibacterial effect over a 48 h period. The extended measurement period in our study allowed for a better understanding of the dosage and treatment time. Notably, the sample of *B. lactis* HN019 had a prolonged inhibitory effect on both *M. furfur* and *C. acnes*, achieving a 100% inhibition rate for 72 h. Therefore, *B. lactis* HN019 could be the potential candidate for further probiotic application studies in skin microbiome modulation.

5. Conclusions

In this study, 10 strains among 18 tested probiotics showed inhibitory effects on *M. furfur* and *C. acnes*. Our findings indicated that the fermented supernatant, containing a mixture of organic acids, peptides, and other substances, exhibited inhibitory effects on *M. furfur* and *C. acnes*. The main active substances responsible for these effects were found to have a molecular weight smaller than 10,000 Da. Different probiotics produced antimicrobial substances in varying quantities. The inhibition activity of the fermentates lasted between 0 to 100 h, and it decreased gradually throughout the treatment. To obtain the active substances from probiotic metabolites, a membrane filtration method with a 10,000 Da MWCO (molecular weight cut-off) membrane can be used during production. This process not only yields a product with bacteriostatic properties but also results in reduced color and taste compared to conventional methods, making it highly versatile and applicable in various settings.

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