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The Potential Impact of Probiotics Along with Prebiotic Against the Dermatic Pathogen *Staphylococcus aureus*: Isolation and Characterization

Nandini Sinhmar, Bindu Battan*, Surender Verma, Sulekha Chahal, Jitender Sharma

Department of Biotechnology, Kurukshetra University, Kurukshetra (136119), Haryana, India.
Department of Pharmaceuticals Science, Kurukshetra University, Kurukshetra (136119), Haryana, India.

***Corresponding Author**: bbattan@kuk.ac.in

Abstract

In recent years, probiotics and prebiotics are now well known for their expanded clinical applications beyond the gut microbiome to the skin microbiome by managing several skin disorders from acne to skin cancer. *Lactobacilli* and *Bifidobacterium* were extracted from non-dairy origins such as honey, tomato, and banana. The obtained isolates, recognized as *Lactiplantibacillus pentosus*, *Lactiplantibacillus plantarum*, and *Bifidobacterium animalis*, underwent detailed analysis to assess their probiotic qualities. This assessment involved various morphological and biochemical tests, including the catalase test, pH tolerance, temperature resistance, salt sensitivity, antibiotic susceptibility, and antimicrobial activity. All three isolates showed increased growth under skin-like conditions including higher growth at pH 4 to 5, at wide range of temperature and at various salt concentrations. This research paper deals with the isolation of *Lactobacilli* and *Bifidobacterium* from non-dairy sources and further characterization to evaluate their antibiotic sensitivity against Ampicillin, Penicillin, Gentamycin, Ciprofloxacin, and Tetracycline and to study their antimicrobial effect against the main skin's opportunistic pathogen *Staphylococcus aureus,* that causes approx. 80% of skin diseases. Furthermore, research was undertaken to formulate optimal synbiotics. This involved assessing the preferential growth of isolated probiotics in the presence of prebiotics, specifically inulin, following an evaluation of the isolates' activity scores both with and without prebiotic supplementation.

Keywords: Probiotics, Prebiotics, Skin microbiome, Skin diseases.

Introduction

Nowadays, food is not only consumed for their taste and for nutrition, but also to improve overall health and well-being of recipients in health sector due to their therapeutic effects in preventing and treatment of many diseases (1,2). Lactic acid bacteria (LAB) constitute a diverse category of gram-positive rods or cocci, devoid of spores, with a heterogeneous nature. They thrive in various environments, including the gastrointestinal tracts of humans and animals, as well as in plants and non-living components of the environment. Notably, they are non-pathogenic and exhibit anaerobic or facultative aerobic characteristics, while being catalase negative (3,4). Because of the considerable role in animals and human diets as supplements, strains belonging to *Lactobacilli* are usually referred to as probiotics and are commercially available for human consumption

(5,6). In 2014, experts from ISAPP (International Scientific Association for Probiotics and Prebiotics) termed it as, "probiotics are referring to many types of microorganisms which demonstrate health benefits for the host, while remaining alive" [7].

Probiotics have garnered significant attention for their beneficial influence on human health, emerging healthy alternative therapeutics to mitigate drug resistance associated with the extensive use of antibiotics in combating infections (8,9). Present globally, these microorganisms have the capacity to enhance the native microbiota, bolstering and fortifying the immune system, while also impeding the proliferation of detrimental pathogens through the production of bacteriocins [10]. They can prevent and mend many health diseases including general digestion problems, *Helicobacter pylori* infection, Lyme diseases, Fever blisters, lowering liver lipids concentrations, atopic dermatitis or eczema, cardiovascular diseases, diabetes, allergic diseases, and even cancer (11,12,13).

Skin is the largest external organ in humans, and is remarkably inhospitable environment of rich and diverse communities of microorganisms, approximately one billion microbes per square centimetres including species of bacteria, fungi, mites, archaea that provides a primary protective barrier against infection causing microbes, also exerting several roles in addition to homeostasis and thermoregulation, immune responses, metabolic functions etc (14). The resident skin microbiome varies according to their respective location on the surface and are controlled by several extrinsic and intrinsic factors including age, temperature, moisture, gender, and environmental factors (15,16). However, cleansing practices and ethnicity may also be the secondary factors which determine cutaneous microbial composition as it was already revealed that increased exposure to skin's microbiota may lead to several conditions or diseases from acute to chronic (17,18). Any sudden disturbance in the maintained skin ecosystem changes microflora from beneficial to pathogenic. The best example is *S. epidermidis* and *Staphylococcus aureus* as both are common and harmless members of the human skin ecosystem but when get disturbed can change phenotype and becomes pathogenic (19,20).

In accordance to Gibson and Roberfroid in 1995 and Roberfroid in 2007, Prebiotics one the other hand, are "selectively fermented ingredients belong to oligosaccharides, polysaccharides, and oligofructose that allows specific alterations, both in activity and composition of resident microflora of host that accord benefits on health and well-being'' (21). The realm of prebiotics is multifaceted, comprising various oligosaccharides, polysaccharides, and inulin, specifically oligofructose, which can serve as a viable alternative or supplementary support to probiotics. In contrast to probiotics, which are live microorganisms, prebiotics function a kind of "fertilizers," fostering the selective growth and activity of probiotics while curbing the proliferation of harmful microbes. Presently, numerous scientists are delving into the significance of the microbiota as a pivotal tool in their respective research endeavours, aiming to develop novel biotherapeutics incorporating probiotics and prebiotics. These products hold promise for addressing skin disorders and diseases (22).

Researchers are starting to unravel the relationship between microbial communities and their associated diseases. Although understanding the skin's microbiome presents challenges, ample evidence already links dysbiosis in microbial composition to skin associated disorders from acute to chronic including acne, atopic dermatitis or eczema, seborrheic dermatitis, allergic inflammation, psoriasis, vitiligo, rosacea, UV-induced photodamage and photoaging, epidermolysis bullosa and skin cancer (23).

As our understanding of the influence of microbes on human health advances, there is a rapid emergence of probiotics-based dermal products for topical application. These products

may consist of probiotics alone, in the form of lysates or supernatants, or combined with prebiotics to form synbiotics. Clinical data suggests that the use of probiotics-based biotherapeutic products can rebalance the skin microbiome, offering protection against and prevention of various skin conditions such as acne, eczema, atopic dermatitis, hypersensitive skin, UV-induced photodamage, and wound healing. Furthermore, these products contribute to mitigating signs of aging, thereby promoting overall skin health (24,25). Previous studies primarily explored the potential of gut-focused probiotics to elicit beneficial effects on the skin. However, recent research is shifting towards the direct application of probiotic and prebiotic-based products onto the skin. While the concept of improving skin health internally is commendable, a more practical and logical approach may involve addressing skin conditions directly at the site of concern, namely the skin surface. Gram-positive bacterial species such as *Lactobacillus* and *Bifidobacterium*, commonly used as probiotics, offers significant benefits to the skin microbiota due to their lack of proinflammatory lipopolysaccharides. This characteristic enables them to release bioactive molecules into the skin tissue, triggering signaling pathways that mitigate skin cell dysfunction (26,27). Consequently, recent research efforts are directed towards formulating topical synbiotics tailored for maintaining skin health.

Thus, this research endeavour stands to enrich our understanding of the identification and prevalence of potential probiotic bacteria in non-dairy sources, along with their relevance in promoting skin health. Key criteria such as antimicrobial activity, antibiotic susceptibility, acid and salt tolerance, and temperature stability are vital features in screening the probiotic potential of isolated strains for therapeutic applications. The primary aim of this study was to assess the antagonistic impact of *Lactobacilli* and *Bifidobacterium* strains isolated from non-dairy products against the standard strain of *Staphylococcus aureus*, sourced from the Regional Centre of Biotechnology in Faridabad, known as a major contributor to skin-related ailments.

Materials and Methods

Sources were collected from nearby area and the edible part of tomato and banana while honey as such was used for the isolation of LAB. The primary medium chosen for the isolation and selection of lactic acid bacteria (LAB) was deMan, Rogosa, and Sharpe (MRS) broth and agar medium. Approximately 1ml of liquid extract from each of the three samples was combined with a PBS buffer or sterile saline and inoculated into 100 ml of MRS broth. The samples were then incubated for 24-48 hours at 37°C, and turbidity in the MRS broth containing sample extracts was observed. Subsequently, serial dilutions of all three samples were prepared up to 10^-7 and plated onto MRS agar plates. Colonies with similar morphologies were streaked onto sterile MRS agar plates under aseptic conditions to obtain isolated and pure colonies, which were then incubated for 24 hours at 37°C. The resulting pure colonies were either stored at 4°C for immediate use or preserved in a 20% glycerol solution for future applications (28).

Identification and characterization of bacteria

Lactobacilli strains were extracted from non-dairy origins, specifically honey, tomato, and banana, through the enrichment of MRS (De Man, Rogosa, and Sharpe) broth (Hi-Media Pvt Ltd., India). The strains were confirmed through microscopic examination using Gram staining and catalase testing. Only strains exhibiting gram-positive characteristics and negative catalase reactions were chosen for subsequent use, classifying them as Lactic acid bacteria, as strains of *Lactobacilli* are rod shaped, gram-positive and catalase negative.

16s RNA sequencing

Fresh culture in the exponential growth phase was used to isolate the genomic DNA.

The resulting pellets obtained was resuspended in Tris EDTA buffer after being cleaned with ethanol. For isolates by PCR amplification 5' GGATGAGCCCGCGGCCTA 3' was used as 16S forward primer and 5' CGGTGTGTACAAGGCCCGG 3'as the reverse primer. The result was integrated into sequencing programme at: http://blast.ncbi.nlm.nih.gov, where the isomers were determined and identified at a percentage > 90%. The identification and similarity of the strains was compared with the sequence of other *Lactobacilli* strains by using BLAST database. The strains were identified as *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus*, and *Bifidobacterium animalis*. The selected cultures were identified from was performed by Bio kart Pvt. Ltd, Bangalore. The identified strain from banana had 99.92% similarity with *Bifidobacterium animalis*, strain from honey had 99.92% similarity with *Lactiplantibacillus plantarum* and from tomato the identified strain had 92.69% similarity with *Lactiplantibacillus pentosus.* (Table1)

Table 1. Identification of the isolates from different sources.

S. No.	Sources and designation of the isolates	Identified Strain	Accession number (NCBI)	Base length	% of similarity
1	Tomato SUB13507764	Lactiplantibacillus pentosus	OR105181	1446 bp	92.69% similarity with Lactiplantibacillus pentosus strain 124-2 16S ribosomal RNA NR_029133.1
2	Honey SUB13507721	Lactiplantibacillus plantarum	OR105053	1249 bp	99.92% with Lactiplantibacillus plantarum strain JCM 1149 16S ribosomal RNA NR_115605.1
3	Banana SUB13507546	Bifidobacterium animalis	OR105051	1271 bp	99.92% with Bifidobacterium animalis subsp. lactis strain YIT 4121 16S ribosomal RNA NR_040867.1

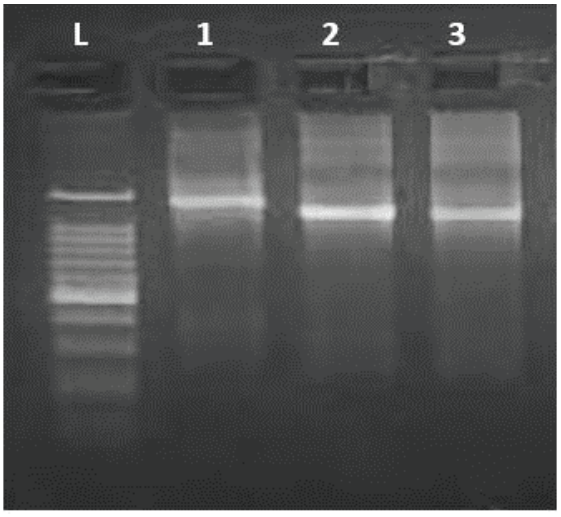

Figure 1. Gel image of PCR amplification.

Determination of optimal growth at pH

The optimal pH for the growth of all three isolated strains from non-dairy sources, namely *Lactiplantibacillus plantarum, Bifidobacterium animalis*, and *Lactiplantibacillus pentosus* was determined using MRS broth with pH values ranging from 2 to 7. Each broth was inoculated with 1% (v/v) of the respective isolates and then incubated at 37°C for 24-48 hours under anaerobic conditions. Bacterial growth was assessed by either measuring the optical density at 560 nm using a UV-visible spectrophotometer against a control or by spreading 0.1 ml of culture from the broth with varying pH onto MRS agar plates, with a control at pH 6.5, or observing colony formation after incubation at 37°C for 24 hours (29).

Assay for NaCl tolerance

The NaCl tolerance assay is useful to determine the optimal growth of isolated probiotics on varying salt concentration. For this 1% (v/v) of each sample were inoculated in 100 ml flasks containing MRS broth having varying concentration range of NaCl from 1% to 7% incubated at 37℃ for 24-48 h under anaerobic conditions. The growth was determined by optical density at 600 nm using UV-visible spectrophotometer (30).

Growth at various temperature

The temperature tolerance is done to determine both the refrigerated and shelf-stable varieties as many strains cannot tolerate certain range of temperature. The stability of various probiotic strains at different temperatures was measured by determining the viability of cells at different temperatures. For this, all isolates were inoculated in the flasks containing MRS broth and all the flasks were incubated at low to high range of temperature i.e., at 4℃, 25℃, 37℃, and 45℃ respectively. Growth was measured by taking optical density at 560 nm using UV-Spectrophotometer (31).

Antibiotic sensitivity test

To assess the antibiotic sensitivity of the bacteria, both disc diffusion and antibiotic strip diffusion methods were employed. Various antibiotics were tested on the isolated probiotic strains using Hi Comb™ MIC strips containing five different antibiotics. These MIC strips feature a comb-like structure with 15 extensions carrying porous material containing antibiotics of varying concentrations. Each strip encompasses 15 two-fold dilutions, ranging from the highest concentration at one end to the lowest at the other. Muller-Hinton agar (MHA) plates were prepared by pouring MHA media and allowing it to solidify at room temperature. Subsequently, 100 microliters of freshly grown cultures were spread onto the MHA plates, followed by the placement of appropriate antibiotic-impregnated strips over the surface. The zones of inhibition and minimum inhibitory concentration (MIC) values were then determined for each isolate. The antibiotic susceptibility pattern was evaluated using five antibiotic strips: Ampicillin (0.016-256 µg/ml), Penicillin (0.002-32 µg/ml), Ciprofloxacin (0.002-32 µg/ml), Gentamycin (0.016-256 µg/ml), and Tetracycline (0.016-256 µg/ml) for each of the three isolates obtained from banana, honey, and tomato pulp. When placed over the Muller-Hinton agar plates, these strips created a defined concentration gradient, allowing the antibiotics to diffuse into the porous agar bed. Consequently, zones of inhibition appeared in the form of ellipses. After 24 hours of incubation at 37°C, the zones of inhibition were examined to determine the sensitivity assay. Sensitivity was indicated by the presence of zones of inhibition, whereas resistance was characterized by the absence of such zones (32).

Antimicrobial activity

The antimicrobial activity against pathogen or conditional pathogen is one of the main requirements for probiotic strain as all probiotics show the strain-specific antimicrobial activity against pathogens. There is a necessity to examine the antimicrobial activity of each isolated strain against the selected pathogen i.e., *Staphylococcus aureus* via direct or indirect mechanisms of interactions. The existing methods belong to two major groups: *in- vitro* methods including well-diffusion method, disc-diffusion method, and co-culturing methods and *in- vivo* methods that directly involve animals or humans' trials. To assess the antimicrobial efficacy of the three isolates, the overnight cultures were centrifuged at 10,000 rpm at 4°C for 15-20 minutes. The resulting supernatants from each sample were then evaluated for their antibacterial properties against *Staphylococcus aureus*, which was inoculated onto Mannitol salt agar plates. Wells with a diameter of 8 mm were created in the agar plates, into which 50µl aliquots of each sample were added. Subsequently, the plates were incubated at 37°C for 24-48 hours, after which clear zones of inhibition surrounding the wells and discs were examined as indicators

of antimicrobial activity against the target microorganisms (33-35).

Effects of inulin as Prebiotic on growth of Probiotics

Human skin acts as shield to external environment and harbours millions of microbes that are involved in developing immunity. Probiotic strains are very sensitive in nature and their growth and survival depend on external environment including oxygen, pH, moisture, heat etc. The prebiotics are defined as "non-digestible oligosaccharides that beneficially effects host health by selectively supporting or stimulating the growth or activity of probiotics." Including prebiotics promotes the proliferation of lactic acid bacteria through the facilitation of lactic acid fermentation. Inulin has garnered significant attention due to its ability to modulate microbial composition, favouring probiotic growth. Inulin is a polymeric compound composed of fructose units ranging from 2 to over 200, with the specific composition influenced by factors such as plant species, age, and extraction method. While over 30,000 plant species serve as potential sources of inulin, chicory roots and dahlia are commonly utilized as commercial sources for extraction (36).

Inulin as prebiotics, serves as food or fuel utilized by probiotics to optimize their functions. Derived from chicory root, it is an excellent ingredient in skincare products, aiding in balancing the skin's microbiome and thereby supporting the preservation of its healthy appearance.

Inulin is a naturally occurring anti-oxidant and humectant that draws moisture from the surrounding environment to the skin and keeps it hydrated. It also acts as a skin-conditioning agent by forming a protective thin layer over the skin surface that reduces dryness, redness, and aging thus makes the skin smooth and supple. Combination of Inulin as prebiotic and probiotics forms synbiotics when applied topically, inulin helps the probiotics to thrive thus maintains skin youthful, and subsequently, repair and restore the skin barrier (37).

Results and Discussion

Scientists are making sustained efforts to substitute chemical-based pharmaceutical drugs with natural biotherapeutic products. Recently, there has been renewed interest and investigation into the potential benefits of both probiotics and prebiotics. Hence, employing probiotics and prebiotics is believed to positively influence the normal functions of healthy skin and play a significant role in preventing and treating various skin conditions, such as acne, atopic dermatitis, psoriasis, photoaging, and wound healing. Collectively, they are believed to support skin health by hydrating, nourishing, and reducing inflammation, thus mitigating the risk of skin diseases. This paper aims to isolate and characterize probiotics sourced from non-dairy origins to assess their impact on the target bacteria *S. aureus,* which is implicated in dermatological conditions. Overall, this research advocates for the utilization of probiotics and prebiotics as a viable strategy for preventing and managing skin issues.

This study discovered that probiotics from the *Lactobacillus* and *Bifidobacterium* genera, sourced from non-dairy origins like fermented goods, fruits, and vegetables, exhibited resilience to acidity and salt, demonstrated resistance to antibiotics, and displayed antagonistic properties against pathogens associated with skin conditions. Numerous *Lactobacilli* species naturally inhabit the human body, including the gastrointestinal tract (GIT), oral cavity, and skin.

Hence, the objective of this study was to isolate and evaluate LAB strains from various environments, aiming to identify new probiotic candidates through in-vitro characterization. All three isolates underwent characterization for probiotic traits through morphological assessment via Gram staining and biochemical analyses, encompassing tests for catalase activity, pH tolerance, temperature resilience, salt tolerance, antibiotic susceptibility, and antimicrobial

efficacy. These attributes are crucial considerations when selecting probiotic strains for synbiotic formulations, offering an advantage in antimicrobial activity against harmful pathogens.

The majority of *Lactobacillus* and *Bifidobacterium* species identified from various origins have extensive records of safety for human consumption and have been designated as GRAS (38). Both strains have been extensively utilized as food supplements or in pharmaceuticals, or in both capacities. However, the primary criterion for selecting probiotics involves examining the behaviour of the isolated strains under conditions that mimic the skin environment and assessing their ability to endure harsh conditions, thereby establishing, and proliferating within the prevailing nutritional and ecological parameters.

Isolation and identification

Ample research has been conducted in the identification and isolation of probiotics from various biotic and abiotic sources including humans, fermented foods, dairy sources, air, and soil, but recent research has focused on isolates from varieties of non-dairy sources. As probiotics nowadays are not only used as diet supplements but also as therapeutic products, it is necessary to administer inflexible screening assays for the identification of new probiotic strains to discover their functional properties by different biochemical processes at varying pH, temperature, salt concentration, and safety properties such as antibiotic resistance, and antimicrobial activity for survival over skin surface under harsh conditions (39). The molecular identification of promising probiotic strains was conducted through 16S rDNA sequence analysis, following the 16S rRNA gene sequence and DNA-DNA hybridization analysis protocols. Strains obtained from honey, tomato, and banana were determined to be *Lactiplantibacillus plantarum, Lactiplantibacillus pentosus*, and *Bifidobacterium animalis*, respectively (40). The bacterial isolates were further characterized and screened for the confirmation of *Lactobacillus* species. The creamy colour gram-positive, rod-shaped bacterial isolates were observed by Gram-staining, and there was no bubble formation with hydrogen peroxide indicating that they were catalase negative.

pH tolerance

The acid tolerance of the isolates is a crucial trait, particularly given the acidic pH of the skin where many commensal organisms thrive. Hence, it is imperative for the isolated probiotic strains to endure acidic conditions and thrive within the pH range of 4-5, which is conducive to skin application. This study aimed to pinpoint probiotic isolates from non-dairy origins that exhibit stability within the skin's pH range of approximately 4 to 5. The viability assays demonstrated the pH stability of all three isolates *Lactiplantibacillus plantarum, Lactiplantibacillus pentosus* and *Bifidobacterium animalis*. It was observed that all three survived well at pH 4-5 after incubation at 37°C for 24 h, which is suitable for skin pH.

From the result obtained, it was interpreted that at pH 2 all showed less growth, at pH 3 the all isolates showed moderate growth, but at pH 4 and 5, all isolates showed the highest growth, which is the normal range of skin pH that supports microbial growth. (Table 2)

The pH of the skin plays a crucial role in maintaining homeostasis, ensuring proper barrier function, and preserving the integrity and cohesion of the stratum corneum. Additionally, it serves as a key component of the skin's antimicrobial defence system against external environmental factors. Typically, the skin maintains a slightly acidic pH, known as the acid mantle, ranging from 4 to 5. This pH varies according to the needs of specific skin regions, aiding in the regulation of the cutaneous microflora ecosystem. This balance helps safeguard the skin against harmful pathogens, contributing to overall skin health and well-being (41,42). There is a positive relationship between lower pH values ranging from 4 to 5 and preservation of the skin microbiome.

Table 2. Screening of all isolates at different pH levels.

S. No.	pH	Lactiplantibacillus pentosus	Lactiplantibacillus plantarum	Bifidobacterium animalis
Control	6.5	-	-	-
1	2	-	-	-
2	3	+	+	+
3	4	++	++	++
4	5	+++	+++	+++

Table symbols: '-' represents no growth, '+' less growth, '++' moderate growth, '+++' high growth.

Temperature tolerance

The surface temperature of the skin is an essential physiological indicator, reflecting the dynamics of heat exchange between the human body and its surroundings (43). Temperature is also a major factor that determines the stability and viability of probiotics when they are subjected to various harsh conditions during further refining methods, freeze-drying, nanoparticles, hydrogels or bio gels, and other pharmaceutical processes.

According to the results of the temperature tolerance assay indicated that, every isolate could withstand a broad range of temperatures and flourished at room temperature, both above and below it, and at higher temperatures relative to lower temperature. Isolated strains *Lactiplantibacillus plantarum* showed higher stability, as it grows well at different range of temperature followed by *Lactiplantibacillus pentosus* and *Bifidobacterium animalis.* (Figure 2)

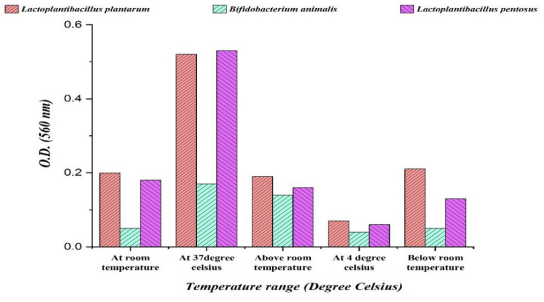

Figure 2. Effect of different temperature on *L. pentosus, L. plantarum*, and *B. bifidum*.

Salt tolerance

Sweating diminishes bacterial load on healthy skin, and its generation directly impacts the salt levels within the skin. Research indicates that an excess of salt can influence the innate immune system by altering T cell responses (44,45).

The results of the assay showed that the viability of the isolates was reduced by high salt concentration. Tolerance to NaCl is required for controlling the skin's innate immune system. Species of *Lactobacilli* can thrive in conditions where the concentration of salt varies from 2% to 7%. The results of the assay showed that the viability of the isolates was reduced by high salt concentration. From the graph it was interpreted that *Lactiplantibacillus pentosus* was highly stable at 2% and 3% and least stable at 6% whereas, *Lactiplantibacillus plantarum* showed less stability at varying salt range as it was most stable at 2% and least stable at 6% and 7%. *Bifidobacterium animalis* was highly stable at 3% and 5% salt concentration. (Figure 3)

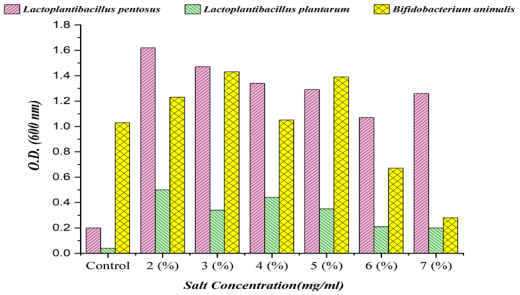

Figure 3. Effect of different Salt concentration on *L. pentosus, L. plantarum*, and *B. bifidum*.

Antimicrobial assay

Antimicrobial efficacy stands as a vital criterion in probiotic culture selection, serving as natural adversaries against potentially harmful pathogens. Consequently, two *Lactobacillus* strains and one *Bifidobacterium* strain were assessed for their activity against *Staphylococcus aureus*, a primary culprit in skin infections. On agar plates, wells measuring 8 mm in diameter were created, and 50-100 µl of cell-free supernatant was introduced into these wells. The antimicrobial activity was gauged by the diameter of the inhibition zones surrounding the wells after an overnight incubation period at 37℃. Zones of inhibition measuring ≥10 mm were deemed positive. Varied sensitivities among the isolated probiotics resulted in distinct zones of inhibition against the target pathogens within the pH range of 4-5.

Many studies have accepted that *Lactobacilli* are bio conservatives as they exhibit a broad antimicrobial spectrum against diverse pathogens belonging to Gram-negative and Gram- positive microorganisms, including foodborne pathogens *E. coli, Pseudomonas*, and dermal pathogens including *Staphylococcus aureus*, the main culprit responsible for approx. 80% of skin diseases. Both lyophilized and concentrated cell-free substrates derived from the cultivation of isolated and selected *Lactobacilli* and *Bifidobacterium* in MRS broth underwent testing for antimicrobial activity (46). The primary cause of antimicrobial activity in *Lactobacilli* stems from the generation of organic acids, such as lactic and acetic acids, as well as the production of microbial metabolites, hydrogen peroxide, and various low molecular weight antimicrobial peptides like bacteriocins. Additionally, there is a decrease in pH because of competition with pathogenic bacteria (47,48,49). The antimicrobial impact is also attributed to the undissociated acid form and its ability to lower the intracellular pH, thereby inhibiting vital cell functions of pathogens (50,51).

The antimicrobial activity of the isolated *Lactobacillus* and *Bifidobacterium* strains was determined using the well diffusion method, which resulted in inhibition zones ranging from 0.9 to 1.5 cm or 9 to 15 mm in diameter against Staphylococcus aureus, the primary pathogen linked to various skin issues, particularly atopic dermatitis. From the antimicrobial assay performed, it was observed that the supernatants from all isolates showed activity against the pathogenic strain *Staphylococcus aureus.* Based on the findings, it was determined that *Lactiplantibacillus pentosus* had the largest inhibitory zone of 15mm, followed by *Lactiplantibacillus plantarum* of 13mm, and finally *Bifidobacterium animalis* of 9mm. It was determined that *Lactiplantibacillus plantarum*, *Bifidobacterium animalis*, and *Lactiplantibacillus pentosus* had the strongest antagonistic activity against *S. aureus.* (Table 3)

Table 3. Zone of inhibition of all isolates against *Staphylococcus aureus.*

S. No.	Name of the strain	Zones of inhibition against *Staphylococcus aureus* in mm.
1	*Lactiplantibacillus pentosus*	15 mm
2	*Lactiplantibacillus plantarum*	13 mm
3	*Bifidobacterium animalis*	9 mm

Antibiotic sensitivity assay

Antibiotic sensitivity assays for determining the sensitivity and resistivity against various antibiotics are of great importance in the human and veterinary fields. The safety assessment of strains intended for probiotic use necessitates an evaluation of their antibiotic susceptibility profiles and the presence of antibiotic-resistant genes. All three isolates displayed

comparable antibiotic susceptibility patterns with minor exceptions. None of them exhibited susceptibility to Penicillin and Ampicillin, while they demonstrated susceptibility to Ciprofloxacin, Gentamycin, and Tetracycline. The resistance of probiotic strains to certain antibiotics could serve both preventive and therapeutic purposes in combating skin-related ailments. This investigation unveiled that the intake of certain antibiotics, such as penicillin and ampicillin, would not affect the growth of the *Lactobacilli* population, whereas other antibiotics could significantly diminish *Lactobacillus* spp. populations. The antibiotic resistance data indicate differences among the isolates in terms of their antibiotic sensitivity patterns (52).

The tolerance of all three isolates toward ampicillin, tetracycline, ciprofloxacin, gentamycin, and penicillin was determined using antibiotic strips of different antibiotics with different ranges. We concluded that all the tested *Lactobacillus* and *Bifidobacterium* strains were resistant toward ampicillin and penicillin. *Lactiplantibacillus pentosus is s*ensitive to gentamicin and ciprofloxacin with MICs of 4.0 and 0.25, respectively. *Lactiplantibacillus plantarum* is sensitive to tetracycline, gentamicin, and ciprofloxacin with MICs of 1.0, 2.0 and 0.12, respectively. *Bifidobacterium animalis* is sensitive to Tetracycline, gentamicin, and ciprofloxacin with MICs of 32.0, 16.0 and 0.25, respectively. Generally, they are sensitive to broad-spectrum antibiotics including tetracycline and resistant to the beta-lactam antibiotic ampicillin and cell wall inhibitor antibiotic like penicillin. (Table 4)

Table 4. Antibiotic susceptibility profiles of the three probiotic bacteria to the selected antibiotics of Tetracycline, Ampicillin, Ciprofloxacin, Penicillin, and Gentamycin

Antibiotics	*Lactiplantibacillus pentosus*	*Lactiplantibacillus plantarum*	*Bifidobacterium animalis*
Tetracycline	R	S (MIC: 1.0)	S (MIC: 32.0)
Gentamycin	S (MIC: 4.0)	S (MIC: 2.0)	S (MIC: 16.0)
Ampicillin	R	R	R
Penicillin	R	R	R
Ciprofloxacin	S (MIC: 0.25)	S (MIC: 0.12)	S (MIC: 0.25)

MIC: minimum inhibitory concentration (µg/ml), R: resistant, S: susceptibility.

Prebiotics and their respective activity scores

Prebiotics function as promoters of probiotic growth, and their efficacy can be assessed through the calculation of a probiotic score, which evaluates the ability of prebiotics to support probiotic growth (53). The prebiotic score is determined by measuring the growth of bacterial cells from each isolate in the presence and absence of prebiotics and other saccharides, such as sucrose, using spread plate, pour plate, and optical density measurements at 560 nm. The impact of prebiotics on the growth of all three isolated probiotic strains was evaluated through the prebiotic activity score, represented as a percentage. A higher prebiotic score for inulin compared to sucrose indicates that the selected prebiotic molecule effectively supports the growth of the isolated probiotic bacterial strains.

Activity score of prebiotics on probiotics (%) = OD at 560 nm in absence of prebiotic/OD at 560 nm in presence of inulin.

Activity score of sucrose on probiotics (%) = OD at 560 nm in absence of sucrose/ OD at 560 nm in presence of sucrose.

Prebiotics, which are oligosaccharides resistant to digestion, stimulate the growth of probiotics when consumed in suitable quantities. Previous research has indicated that probiotics and prebiotics contribute positively to

skin health by enhancing skin moisture, elasticity, and radiance, as well as by improving skin barrier function and follicular structure through the regulation of keratinocyte differentiation. From the results, it was concluded that the inulin taken as prebiotic showed maximum activity score for *Lactiplantibacillus plantarum* isolated from honey followed by *Bifidobacterium animalis* from banana *and Lactiplantibacillus pentosus* from tomato as compared to sucrose taken as control carbohydrate as growth promoter in place of inulin. Also, inulin taken as prebiotic, showed higher activity than sucrose as a carbohydrate for promoting growth of isolated probiotics strains. (Table 5)

Table 5. Activity score of probiotics in presence and absence of prebiotics (inulin).

S. No.	Probiotic strain	Optical density at 560 nm in the absence of prebiotics	Optical density at 560nm in the presence of prebiotic (Inulin)	Optical density at 560 nm in the presence of control carbohydrate (Sucrose)	Activity score of probiotics in the presence and absence of Inulin	Activity score of probiotics in the presence and absence of Sucrose
1	*Lactiplantibacillus pentosus*	1.049	1.175	1.109	89.2%	73.9%
2	*Bifidobacterium animalis*	0.878	1.447	1.042	60.6%	59.16%
3	*Lactiplantibacillus plantarum*	1.083	1.112	1.663	97%	65.12%

Under in-vitro conditions, the isolates underwent assessment for their probiotic attributes, which encompassed tolerance to salt, acid, and temperature, as well as antagonistic activity against specific pathogens and antibiotic sensitivity. The isolated strains exhibit promising probiotic traits, such as tolerance to temperature, acidity, and salt, irrespective of their diverse origins. These favourable characteristics render the isolated strains viable for topical applications on the skin surface.

Conclusion

In recent times, there has been a surge in the exploration of probiotics, not only as supplements but also as therapeutic agents. Probiotics, which are live microorganisms that confer health benefits when consumed in adequate quantities, have gained considerable attention. Among the most widely utilized probiotics are species belonging to *Lactobacilli* and *Bifidobacterium*, which have been employed for many years. The skin cells and skin ‘s microbiome works synergistically to maintain homeostasis in daily routine. Employing probiotics and prebiotics, either independently or in an appropriate combination, could represent a fresh and efficient strategy for addressing a variety of skin disorders, spanning from acne to eczema. The sensitivity and resistance of numerous probiotic strains to commonly prescribed antibiotics render them safe for the formulation of diverse products for both animal and human use. Data analyse have already indicated that probiotics’ antimicrobial properties against *Staphylococcus aureus* are beneficial for averting and managing skin conditions such as acne, inflammation, atopic dermatitis. In addition, it has been observed that the use of probiotic cultures and their lysates either alone or with prebiotics as cosmetic products and their ingredients moisturizes and exfoliates the skin, thus maintaining good skin health.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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