

(RESEARCH ARTICLE)



Probiotics in dermatological therapy and skincare

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Abstract

Probiotics are characterised as “live microorganisms” and use of probiotics has shown promising effects on the prevention and treatment of different inflammatory skin disorders such as acne and atopic dermatitis. The *Lactobacillus* sp. were isolated using fermented rice water. Fermented rice water was serially diluted and plated on MRS Medium plates in which the bacterial isolates were observed to be round, flat, slightly mucoid, creamy in colour. The *Lactobacilli* was confirmed by Gram staining and the slide was observed to be gram positive rod shaped. Biochemical test was also performed in which TSI slant was observed with red slant, yellow butt without gas and H₂S formation. Screening was done for *Lactobacillus* sp. for probiotic properties. Optimal growth was determined at 560 nm at pH 5.5. NaCl concentration tolerance at 4% was observed to be optimal. The antimicrobial activity of the isolated *Lactobacillus* was identified by a well diffusion method and was diameter up to 0.8, 0.6 & 1.2 cm. Supernatants of each sample of *Lactobacillus* were monitored for antibacterial activity against indicator bacteria *Bacillus*, *Pseudomonas* inoculated on Nutrient agar and presence of clear zones of inhibition around the wells were observed. Antibiotics sensitivity test Disk diffusion method was performed and zones of inhibition around the disks were observed. Shea butter, raw honey and aloe vera gel along with that Pellets of *Lactobacillus* species were added and tea tree oil and essential oils were added drop by drop and this probiotic face serum was stored. The lab made face serum was further dissolved in sterilised distilled water and a loopful was inoculated to the Nutrient medium containing petri plates for determining the viability of *Lactobacillus* species after incubation.

Keywords: Probiotics; *Lactobacillus* sp; MRS medium; Fermented rice water

1 Introduction

Probiotics are characterized as “live microorganisms” when regulated insufficient sums, present a medical advantage on the host”. The use of probiotics has shown promising effects on the prevention and treatment of different inflammatory skin disorders such as acne and atopic dermatitis. Although probiotic bacteria have documented skin benefits, live cultures are generally not preferred in cosmetics. Rather than including live bacteria cultures, many of the probiotic skincare formulae use bacteria fragments or metabolites. There is not currently any science developed to support the idea that live cells are any more effective when applied to the skin than these fragments. In the near future, some brands using live bacteria might emerge. We will briefly review the science supporting the use of topical probiotics in dermatological therapy skincare products.

Nonetheless, such a large number of the items right now marketed as probiotics neglect to conform to the principal attributes. In 1900 Louis Pasteur first identified the microorganisms responsible for fermentation, finally bringing fitness to and promoting the sturdiness of Bulgarian rural human beings in the event that they consumed fermented ingredients which included yoghurt and the bacteria they contained. He cautioned that “lactobacilli may counteract the putrefactive outcomes of gastrointestinal metabolism that contributed to infection and getting older” where as E. Metchnikoff associated the enhanced longevity of Bulgarian rural people to the regular consumption of fermented

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dairy products such as yoghurt. He suggested that lactobacilli might counteract the putrefactive effects of gastrointestinal metabolism that contributed to illness and aging. Hippocrates declared, 2000 years earlier, that “death sits in the bowels.” Metchnikoff considered the lactobacilli as probiotics (“probios,”) conducive to life of the host as opposed to antibiotics); probiotics could have a positive influence on health and prevent aging. During the neolithic period of the age of the stone, the domestication of animals occurred and man began to get fermented food. Probably serendipitous contaminations in favorable environments played a major role. The corrective business has expanded the quantity of items delegated probiotics.

The term prebiotics was introduced by Gibson and RoberFroidin 1995 to describe food supplements that are non digestible by the host but are able to exert beneficial effects by selective stimulation of growth or activity of microorganisms that are present in the intestine. The phrase probiotic (from the Latin pro and the greek bio literally meaning “for existence”) was delivered by way of the German scientist Werner Kolathin 1953 to designate “energetic substances which are essential for a whole some development of existence.” In 1965, this time period was utilized by Lily and Stilwell in a special context to symbolize “materials secreted with the aid of one organism which stimulate the boom of any other.” More particularly, Fulerin 1992 defined probiotics as “alive microbial feed supplement which beneficially affects the host animal by way of improving its intestinal microbial stability.

While there are a few expected applications for probiotics in private consideration items, explicitly for oral, skin, and close consideration, legitimate guideline of the naming and advertising norms is as yet expected to ensure that shoppers are for sure buying a probiotic item. This audit investigates the ongoing business sector, administrative angles and likely uses of probiotics in the individual consideration industry.

Probiotics have various mechanisms of action though the exact manner in which they exert their effects is still not fully elucidated. These range from bacteriocin and short chain fatty acid production, lowering of gut pH, and nutrient competition to stimulation of mucosal barrier function and immune modulation. The later in particular has been the subject of numerous studies and there is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing Th1 responses, and attenuating the responses. The skin is the largest organ in the human body. Its primary function is to protect our bodies from external harm by acting as a Physical barrier, with additional roles that include regulation of the body.

1.1 Skin microbiome

Skin continuously undergoes self-renewal, so resident microbial cells are shed in the process. Most of the Microbes found on the skin are commensal organisms and harmless to healthy individuals; in fact, some are considered mutualistic. Organisms confer health benefits to the skin by secreting antibacterial substances, preventing pathogen colonization, and influencing host immune responses. Recent researches related to control of skin barrier functions prove close connection. Physical, immunological, and cell biological characteristics of skin and its bacterial population. Joshua Lederberg suggested using the term “human microbiome” to describe the collective genome of our indigenous Microorganisms (microflora) colonizing the whole body in starting from this time, microbiologists and dermatologists joined their efforts to identify and describe different microorganisms colonizing human skin to estimate a number of each population and to understand, which microbial variety can cause one or another dermatological condition. In general, the microbiome is defined as a collective genome of microorganisms. Thus, the skin microbiome is a genome of microorganisms present on skin, in which microorganisms support complex relations. Most microorganisms found on human skin are not dangerous for human health. Some of them are even necessary for skin health. They release antibacterial substances, prevent pathogenic colonization of skin and affect its immunity. According to held researches, three groups of microorganisms colonizing human skin are found, they are as follows:

- Group 1 Transitional Microorganisms Regularly, Occasionally appearing
- Group 2 Temporal Microorganisms Present on skin for a Short period.
- Group 3 Microorganisms Permanently Colonizing skin

There are various microflora i.e. bacteria and microscopic algae and fungi, especially those living in a particular site or habitat. The Skin Microflora in the context of bacteria, our skin can be viewed as a cultural environment composition of which is mainly a result of our genetics, diet, way of life and a region where we live. Therefore, human skin is unique and accordingly, the skin microbiome of each person is also unique. The skin microbiome is highly dependent on the micro environment of the sampled site, a reflection on the physiology of skin. Sebaceous sites such as the forehead have the lowest diversity, and Propionibacterium species are the dominant organisms. On the other hand, moist areas (e.g., armpits, navel, groin) constitute higher diversity of microbiota, with Staphylococcus and Corynebacterium species

as the predominant members [10,16]. Moreover, skin sites with greater bacterial diversity (e.g., Forearm, hand, buttock) can harbor diversity as high as or higher than that of the gut microbiome. The acidic condition resulting from sebum degradation discourages pathogens from invading and establishing in the skin. Personal hygiene is another environmental factor that has a direct effect on the skin's microbial flora. Soaps, makeup, and skincare products (e.g., moisturizers) alter skin conditions that in turn may influence the types of microbes residing on the skin. On the contrary, in dry areas, skin microbiomes include β -Proteobacteria and Flavobacteriales. Various factors affect the microbial flora of the skin and they can be generally categorized into host and environmental factors.

Skin barrier and microbiota act like a shield protecting the organism against harmful effects of the outdoor environment. There is well-balanced interaction between permanent and temporary populations on skin. This balance continuously depends on internal and external (including environmental) factors that change composition of microbial populations on skin and a function of the host skin barrier. Change of this balance is characterized as dysbacteriosis occurrence of which can worsen such chronic skin diseases as atopic dermatitis and psoriasis, or acne. However, dysbacteriosis can be observed not only among bacteria, imbalance between bacteria and commensal fungus strain on head skin is observed in cases of patients disposed to dandruff. Cosmetics for skin: The increase in products termed probiotic on the market does not necessarily equate with areas onto celebrate the successful translation of science to commerce and consumers. Too many products fail to comply with the characteristics required to be called probiotic. Many false claims and rampant misuse of the term has resulted in mainstream consumer channels providing incorrect information to consumers. Probiotics are not inside us, not in fermented food, not necessarily better if there are more species or a higher viable count. Formulations are being concocted not based on research evidence but on marketing and what might appeal to consumers.

1.2 Probiotics and skin microflora

The skin is able to act as a physical barrier exerting several functions such as fluid homeostasis, thermoregulation, immune responses, neurosensory functions, metabolic functions, and primary protection against infection. The skin microflora plays a significant role in competitive exclusion of pathogens that are aggressive and provoke infection in the skin and in the processing of skin proteins, free fatty acids (FFAs), and sebum. Interestingly the resident microbiota may be regarded as "beneficial" to the normal, healthy host, but may become dangerous to the host with disturbed skin integrity. Microorganisms may have a role even in atopic dermatitis (AD), eczema, rosacea, psoriasis, and acne. Even if there are only very few studies pursuing a probiotic approach for microflora-related skin disorders, it is intriguing to suppose that topical probiotic application can be beneficial either for preventing or for treating altered microflora-associated skin diseases.

1.3 Antimicrobial substances

The potential topical use of probiotic strains capable of producing potent antimicrobials toxins (i.e., bacteriocin, bacteriocin-like substances, organic acids, and H₂O₂) has received increasing attention to successfully prevent pathogen adhesion and out-compete undesired species (O'heta.2006; Giloretal.2008). Topical compositions containing probiotic bacteria, spores, and extracellular product and uses thereof represented the basis of the invention of Farmer (2005) suitable for topical application to the skin which can be utilized to inhibit the growth of bacteria, yeasts, fungi, viruses, and combinations thereof. The invention also uses of Probiotics for Dermal Application disclosed methods of treatment and therapeutic systems for inhibiting the growth of pathogens and combinations thereof, by topical application of therapeutic compositions. The probiotic microorganisms may be bacteria, yeast, or mold. Suitable probiotics should be selected according to one or more particular properties, being the preferred properties of their competitive exclusion of pathogenic organisms from the surface to which they are applied, adherence to human tissue, sensitivity to antibiotics, antimicrobial activity, acid tolerance, and a high oxygen tolerance. In particular, the method consists of different application modalities (i.e., lotions, spraying, wipe paper) of one or more probiotic microorganisms to a wide variety of surfaces, such as human skin and hospital equipment and fixtures, in an amount effective to, at least partly, prevent their contamination, colonization, growth, and cross-contamination by the pathogenic bacteria.

2 Material and methods

2.1 Isolation of Bacteria

The medium which was selected for the lactic acid bacteria was deMan, Rogosa, and Sharpe (MRS) agar medium. Rice was cooked and excess water was drained. It was allowed to cool at room temperature. It was soaked fully in water and stored in a container. It was covered and left overnight at room temperature. Fermented rice water was serially diluted and plated. A loopful of inoculum was taken from the plate and streaked on a MRS and incubated at 37°C for 24 hrs. A loopful of samples was streaked on the sterile MRS agar Petri Plate by quadrant streaking method, under aseptic

conditions. After streaking all the Petri Plates, they were incubated at 37°C for 24 to 48hr. After the incubation, colonies were restreaked on the MRS agar Petri Plate for the formation of isolated colonies. Then from these plates isolated colonies were restreaked on MRS agar slants and stored at 4°C.

2.2 Identification Of Bacteria

Lactobacilli confirmation can be done microscopically by performing gram staining. The colour, shape and motility was also observed

2.3 Screening of isolated *Lactobacillus* spp. For probiotic properties:

2.3.1 Determination of optimal growth and pH

For the determination of optimal pH and growth, the overnight culture of the *Lactobacillus* in the MRS broth media with a varying pH ranging from 2.5 to 8.5, using HCl or NaOH were inoculated with 1%(v/v) and incubated in anaerobic condition for 24hr at 37°C in the presence of 10% CO₂. Bacterial growth was monitored by the determination of optical density at 560 nm using a spectrophotometer against the uninoculated broth.

2.3.2 Assay for NaCl tolerance

In order to determine the NaCl tolerance, isolates from each sample were incubated with 1%(v/v) overnight culture of *Lactobacillus* in the test tubes containing MRS broth adjusted with different concentrations (1-10%) of NaCl. The inoculated medium was incubated in anaerobic condition for 24hr at 37°C. The bacterial growth was determined using a spectrophotometer reading the optimal density at 560 nm.

2.3.3 Determination of bile salt tolerance:

Bile salt tolerance of the isolated bacteria was examined by inoculating the freshly cultured isolates at 1% (v/v) into MRS broth containing Bile salts different concentrations (0,0.1,0.3,0.5 and 1%(w/v)). The medium was then incubated at 37°C for 24 hours in an anaerobic condition. The growth of the isolates was monitored at 0, 3, 5 and 24hr by measuring the absorbance of the culture broth at 620 nm using spectrophotometer.

2.3.4 Antimicrobial activity

The antimicrobial activity of the isolated *Lactobacillus* was identified by well diffusion method, with slight modification. The overnight culture of *Lactobacillus* was centrifuged (10,000rpm for 20 min at 4°C) and the supernatant was adjusted to pH 7.0 with NaOH to exclude the antimicrobial effect of H⁺. Supernatant of each sample of *Lactobacillus* were monitored for antibacterial activity against indicator bacteria (*Bacillus*, *Pseudomonas*) inoculated on Nutrient agar. Wells of 5mm in dia meter were punctured into the agar plate and aliquots of 50µl from each solution samples were placed into the wells. The plates were incubated for bacterial growth and examined for the presence of clear zones of inhibition around the wells.

2.3.5 Antibiotics sensitivity

For the observation of antibiotics sensitivity test Disk diffusion method was performed. Different antibiotics were tested over the isolates. The appropriate antimicrobial-impregnated disks were placed on the surface of the MH agar plate inoculated with each isolate. The zones of inhibition around the disks were observed and the diameters were measured.

2.3.6 Synthesis of probiotic bacteria:

Isolated lactobacilli species was in addition inoculated in nutrient agar broth. Inoculated broth was incubated for 48hrs. For acquiring better concentration of lactobacillus species. The broth became then centrifuged at 5000 rpm for 10min for pelleting down the classy cells. The pellet became washed three times with autoclaved distilled water 3 instances after which it was weighed for determining the quantity of mobile way of life received after incubation.

2.4 Inoculation of *Lactobacillus* spp in skincare

Shea butter is melted to liquid form. By double boiler over low heat on the stove top. Once it liquefies, heat is removed and allowed to cool for 2 minutes then stir in the raw honey and aloe vera gel, the mixture is allowed to cool off in the refrigerator for about 15 minutes. Pellets of *Lactobacillus* species were added and tea tree oil and essential oils were added drop by drop. this probiotic face serum is transferred in sterilized glass jar and stored in cool dry place The lab made face serum was further dissolved in sterilized distilled water and a loopful was inoculated to the Nutrient medium containing petri plates for determining the viability of lactobacillus species after incubation.

3 Results and discussion

MRS Medium plates were observed to have isolated bacterial sample from fermented rice water. The bacterial sample was further tested for confirmation of lactobacillus species. The bacterial isolates were observed to be round, flat, slightly mucoïd, creamy in color. Gram staining was performed and observed to be gram positive bacteria and observed to be rod shaped. Biochemical test was also performed in which TSI slant was observed with red slant, yellow butt without gas and H₂S formation.

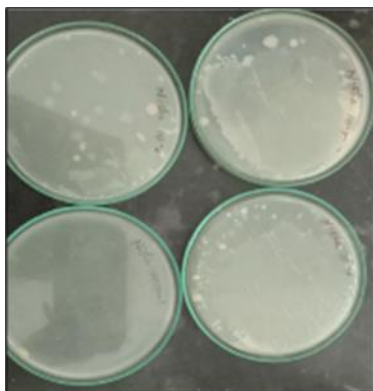


Figure 1 Spread Plate

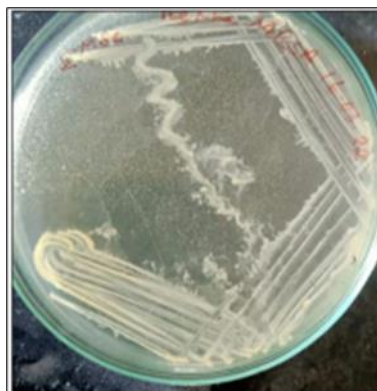


Figure 2 Streak Plate

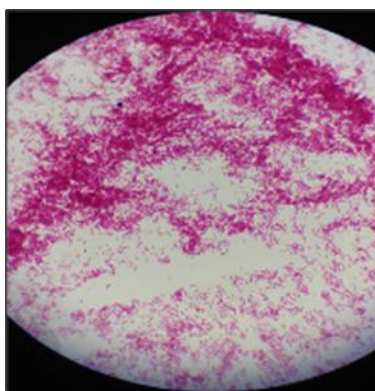


Figure 3 Gram staining

3.1 Antimicrobial Activity

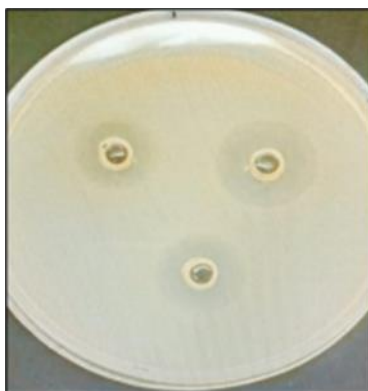


Figure 4 Antimicrobial Activity

The antimicrobial activity of the isolated *Lactobacillus* was identified by well diffusion method, where the zone of inhibition was up to diameter 0.8, 0.6 & 1.2 cm against *Bacillus sp.*, *Pseudomonas sp.*, and *Escherichia coli.* Antibiotic sensitivity was performed by well diffusion method and the zone of inhibition was formed around the antibiotic discs. The zone for *Bacillus sp.*, *Pseudomonas sp.*, was found to be higher zone of inhibition. Finally the Shea butter was melted to liquid form along with that raw honey and aloe vera gel was added and refrigerated for 15 minutes and then *Lactobacillus spp.* pellets and tea tree oil & essential oils were added drop by drop and were kept in cool dry place.

3.1.1 Antibiotics sensitivity

For the observation of antibiotics sensitivity test Disk diffusion method was performed. Different antibiotics were tested over the isolates. The appropriate antimicrobial-impregnated disks were placed on the surface of the MH agar plate inoculated with each isolate. The zones of inhibition around the disks were observed and the diameters were measured.

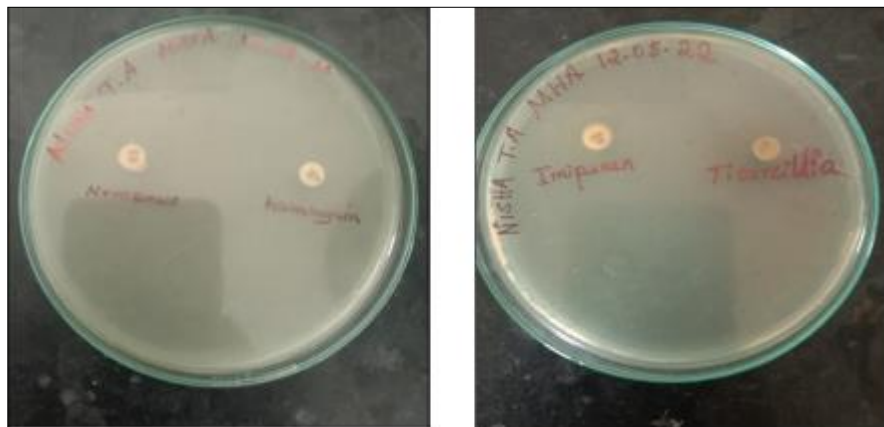


Figure 5 Antibiotic sensitivity

3.1.2 Synthesis of probiotic bacteria:

Isolated lactobacilli species containing broth was centrifuged at 5000 rpm for 10min for pelleting down the classy cells. The pellet was weighed 1.59gm for determining the quantity of mobile way of life received after incubation. Finally the *Lactobacillus spp.* pellets were collected for the preparation of the probiotic face cream.



Figure 6 Pellets of *Lactobacillus* species

3.2 Preparation of Probiotic Face serum

Finally the Shea butter was melted to liquid form along with that raw honey and aloe vera gel was added and refrigerated for 15 minutes and then Lactobacillus spp. pellets and tea tree oil & essential oils were added drop by drop and were kept in cool dry place. Now the probiotic face cream was ready.



Figure 7 Preparation of Probiotic Face serum

Table 1 Biochemical tests of Lactobacillus spp

Basic Characteristics	Properties (Lactobacillus spp.)
Capsule	Negative(-ve)
Catalase	Negative(-ve)
Citrate	Negative(-ve)
MR	Negative(-ve)
VP	Negative(-ve)
TSI	Red Slant, Yellow butt, No Gas, No H ₂ S
Gram staining	Gram positive
Gas Production	Negative(-ve)
Indole	Negative(-ve)
Motility	Mostly Negative(-ve)
Oxidase	Negative(-ve)

Table 2 Determination of optimal growth and pH

pH Value	Sample (OD 560 nm)
2.5	0.212
3.5	0.268
4.5	0.642
5.5	1.045
6.5	1.014

7.5	1.011
8.5	0.246

Table 3 Assay for NaCl Tolerance

NaCl Concentration	Sample (OD 560)
1%	1.258
2%	1.183
3%	1.096
4%	1.074
5%	0.948
6%	0.917
8.5	0.346

Table 4 Determination of Bile Salt Tolerance

Time in Hour	0.10%	0.30%	0.50%	1%
0	0.218	0.218	0.219	0.210
3	0.211	0.261	0.203	0.126
5	0.374	0.354	0.116	0.152
24	0.923	0.571	0.096	0.086

4 Conclusion

This probiotic face serum is further tested to reduce inflammation, Acne, And Atopic dermatitis People with atopic dermatitis (AD), or atopic eczema, have a higher amount of the bacteria *Staphylococcus aureus* in their skin the probiotic Lactobacillus Spp. in the skin works on it. Analysis of the data indicated that it reduced the number of *S. aureus* and decreased AD symptoms. If it has anti acne and antimicrobial properties It prevents skin from u radiation lactic acid is also Been described as Exfoliating and moisturising agent probiotics will continue to expand in applications and market share, and this exponential growth is not likely to subside. It has been observed that existing cosmetic products and their ingredients make use of viable cultures of probiotics and/or their lysates for their production to maintain good skin health. Their incorporation in certain cosmetics have been proposed in order to achieve more radiant looking skin.

Compliance with ethical standards

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Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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