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Probiotic Research in Therapeutics

Volume 3: Probiotics and Gut Skin Axis–Inside Out and Outside In

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Foreword by J. V. Yakhmi

The saying attributed to Hippocrates, the Father of Medicine, that "Let food be thy medicine, and let medicine be thy food" never felt more valid than now when we are challenged by a variety of lifestyle diseases. The relevance of holistic healing has increasingly been related, in recent years, to the gut microbiome, composed of bacteria, archaea, viruses, and eukaryotic microbes, all of which reside in our gut, and together have a strong potential to impact our physiology, both in health and in disease. When faced with a variety of diseases, our present-day knowledge lays emphasis on the importance of a healthy microbiome, not only limited to gut health but also to metabolic disorders, cancers, immunity, brain health, and skin health. Can we manipulate the gut microbiota by probiotic intervention toward disease prevention and treatment? That is precisely what is receiving the attention of a large number of scientists engaged in research on human health. The growing market interest in health benefits of probiotics has intensified research and investments in this area. With an overwhelmingly large number of new products based on probiotics on the shelves of the supermarkets and pharmacies, it can be inferred that the research in this area is at a very exciting stage. Though the intricate mechanisms involved in the importance of gut flora may require some basic scientific expertise, surfing through scientific claims on usefulness of probiotic therapy can catch the fancy of even a general reader.

I have known Prof. Indu Pal Kaur, Chief Editor of this book series, for the past 12 years and have been closely following her research interests which essentially hover around being a formulation scientist, be it for small and large molecules, phytochemicals, and probiotics. I have noticed her deep interest in trying to complement the observational data compiled in the traditional system of medicine with scientific rationale from currently available information. I have myself discussed with her, several times, the human microbiome and its manipulations for useful therapeutic options. She has been active in the topic of probiotics for a long time and had, in fact, published her first review on Potential Pharmaceutical Applications of Probiotics way back in 2002, which has been cited over 500 times to date. Her passion to bring probiotics into mainstream therapeutics is not limited only to the ailments of the gut, *viz.* inflammation, ulcers, and cancers, but is also aimed to extend it to other lifestyle diseases, such as depression, chronic fatigue syndrome, vaginal candidiasis, wound healing, and skin health.

The present ebook series, comprising five volumes, brings latest information and key insights on application of probiotics in cancer and immunological disorders, gut inflammation and infection, skin ailments, neurodegenerative disorders, and metabolic disorders. The contributing authors are recognized experts which ensures that each chapter affords a critical insight into the topic covered, with a review of current research, and a discussion on future directions in order to stimulate interest. Each volume itself covers a broad theme in detail by including chapters disseminating basic information in the field in such a manner that it would attract the attention of even a stray reader or intending consumers. Of course, the whole series of five volumes is designed with care so as to not only ignite the minds of graduating students for future research but also boost the confidence of health professionals, physicians, dieticians, nutritionists, and those practicing naturopathy by underlining the integrity of the data documented in the chapters of these volumes from well-established labs and groups. All in all, a very thoughtful compendium of probiotics research in therapeutics!

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Foreword by Manpreet Randhawa

We as human beings have been evolving and have developed different physical and genetic characteristics in response to varying climates and lifestyles. As a matter of fact, we are sort of creating a clean bubble (that is essentially free of so many things including the microorganisms living on our skin) around us, to feel more secure and safe. However, during this transition we have completely forgotten about the ecosystem supporting us, until we see the continued rise of metabolic disorders such as obesity, diabetes as well as skin diseases such as eczema, psoriasis, and atopic dermatitis. Now, let us take a step back to reflect on these learnings and change our perception to support and enhance our ecosystem.

Thinking about our skin for a moment—it is the body's largest organ and offers the first level of defense—so taking good care of it is very essential. As a first line of protection, our skin is covered with a diverse set of microorganisms called the microbiome, which plays a very active role in maintaining healthy-looking skin. This microbiome tends to stay quite stable unless something drastic happens. Besides the skin, the microbiome tends to be a quintessential part of our gut as well. The gut microbiome maintains homeostasis throughout the body, but can majorly affect our other organs, especially our skin, if it becomes unbalanced. If we experience any issues with our gut, such as inflammation, leaky gut, or digestion problems, our skin is usually the first place we notice problems. This could be one of the reasons behind a well-known saying "whatever you eat will reflect on your skin"; hence, you may consider skin as a barometer of what is going on inside the body. So, I can say that the skin-gut axis is not something new, but something that has lately been explored at a scientific level to understand its mechanisms of action and the end results.

To further decipher the connection between the gut-skin axis, there are many studies that suggest that the intestinal microbiome's influence extends beyond the gut, and in fact contributes to the function (and dysfunction) of distant organ systems including the skin. These commensal bacteria help prime the gut immune system through specific interactions. However, altered gut flora may favor the production of inflammatory cells, thereby contributing to the development of skin disorders such as acne, atopic dermatitis, and eczema. Besides the microbiome itself, the metabolites (end products) secreted by the microbiota also play a critical role in maintaining homeostasis. Short chain fatty acids (SCFAs) are one of the products of dietary fibers fermented by components of the gut microbiome. Butyrate being one

of the SCFAs is known to suppress immune responses by inhibiting inflammatory cells' various functionalities. Other SCFAs are believed to play a pivotal role in determining the predominance of certain skin microbiomic profiles which subsequently influence cutaneous immune defense mechanisms. Therefore, one can predict that intestinal dysbiosis, a state of microbial imbalance, can negatively impact skin function. For example, free phenol and *p*-cresol, metabolic products of aromatic amino acids, represent disturbed gut milieu which can access the circulation, preferentially accumulate in the skin, and impair epidermal differentiation and skin barrier integrity. Indeed, high *p*-cresol serum levels are associated with reduced skin hydration and impaired keratinization. Interestingly, there is new evidence that the intestinal microbiome may impact cutaneous physiology, pathology, and immune response more directly, through the metastasis of gut microbiota and their metabolites to the skin. In cases of disturbed intestinal barriers, intestinal bacteria as well as intestinal microbiota metabolites have been reported to gain access to the bloodstream, accumulate in the skin, and disrupt skin homeostasis. These findings represent evidence of a more direct link between the gut microbiome and cutaneous homeostasis which has just begun to be explored.

Understanding the role of microbiome as an underlying issue for skin disorders has led to the use of probiotics both orally and topically. Several placebo-controlled clinical studies in human subjects suggest that oral supplementation of probiotics has significantly improved skin physiology through decreased skin sensitivity, stronger barrier integrity, and increased hydration. Apart from this the gut microbiome has also been shown to support restoration of skin homeostasis after ultraviolet (UV) radiation exposure. For example, *Lactobacillus johnsonii* La1 supplementation protected cutaneous immune homeostasis in healthy volunteers following UV radiation exposure.

To sum it up, one of the most important things you take toward achieving healthy skin is to keep what is living on and in your body—the microbial bacteria that make up your skin's and gut's microbiome—healthy. One way to improve gut and skin health is through the consumption of high-fiber, pre-, pro-, para-, or postbiotic diets as well as their respective topical applications to restore the diversity and metabolite makeup. In this book, you will discover the remarkable connections between your gut microbiome and your skin's ability to defend against unwanted conditions such as eczema, psoriasis, accelerated aging, acne, and wound healing, as well as crucial tips and advice on maintaining and improving the commensal bacteria.

Research & Innovation at Sytheon Ltd Manpreet Randhawa
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Preface

The first step to understanding the symbiotic dating of gut microbes with their host is to understand the traits of wholesome microbiota and their association with the disease. The Human Microbiome Project (HMP) undertaken by the National Institutes of Health in the United States in 2007 was the first serious effort to understand the know-how of microbial flora and its contribution to human health and disorder at primarily five body sites, out of which one was the skin. Skin microbiota plays an intricate role in the human immune system and helps to defend its host against invading bacterial pathogens. It is now scientifically proven that there exists a close and bidirectional association between the gut and skin in maintaining the homeostasis and allostasis of the skin and also gastrointestinal health. Skin is directly impacted by various circumstances that principally affect the intestine. Ability to manipulate the skin microbiota by probiotic therapy, orally or topically, is highly exciting and full of possibilities. It is said that the next era therapeutics would involve maneuvering the resident microbiome of human body to shift it from dysbiosis to symbiosis.

The third volume of the present ebook series entitled *Probiotic Research in Therapeutics: Probiotics and Gut Skin Axis–Inside Out and Outside In* is a very sincere effort from the editors to bring forth the compilation of scientific evidence which justifies the "Gut-Skin Axis" and discuss the purported benefits of probiotic therapy in establishing the gut and skin symbiosis. The volume comprises 11 chapters. The introductory chapter discusses the very origin of the concept of the Gut-Skin Axis and its role in the general well-being of individuals. Chapter 2 highlights the underlying mechanisms involved in gut-skin crosstalk. Chapter 3 reviews the nexus between the skin's microbiome and the barrier function of the epidermis and explores the possibility of exploiting this unique dialogue in developing innovative cosmetics and transdermal drugs in future, for well-being and beauty. Chapter 4 covers the health benefits of oral and topical probiotics, parabiotics, and postbiotics on the skin. The chapter addresses issues and challenges faced by the actives, via both the routes, along with in vitro and in vivo evidence highlighting their efficacy. Chapter 5 elaborates on the scope and challenges faced by the topical application of probiotics in skin conditions such as acute dermatitis, seborrheic dermatitis, allergic contact dermatitis, wound, acne, psoriasis, photoaging, and skin cancer. The chapter also focusses on the future and current research in probiotic

therapy for skin ailments. The next four chapters (Chapters 6–9) discuss the role of probiotics in prophylaxis and the management of various skin conditions, viz. acne, photodamage, wound, and atopic dermatitis. The authors have discussed the underlying mechanism of action of probiotics, while providing a consolidated overview of the preclinical and clinical status of probiotic therapy in the management and control of these indications. Next in line is the chapter highlighting the safety concerns involved, and regulatory guidelines governing the use of probiotics in the gut-brain-skin axis. The chapter also presents an elaborate picture of the current global probiotic market highlighting the major impacting factors and breakthroughs in the recent years. The last chapter gives the reader an interesting perspective of the recent advances and trends in the probiotic-based vaccine. The discussion highlights the most frequently applied strategies involving bacteria and yeast in the conception of probiotic vaccines, both preclinically and clinically.

With state-of-the-art commentaries on all aspects of probiotic research, from contributors across the globe, the ebook provides an authoritative and timely overview of the field. I hope this book will be a useful educational and scientific tool to academicians, health professionals, students, and pharma/biotech businessmen worldwide. As editors of the book, we express our sincere thanks to all the authors for their excellent contribution to the book.

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About the Editors

Indu Pal Kaur is a Professor of Pharmaceutics and currently the Chairperson at the University Institute of Pharmaceutical Sciences (UIPS), Panjab University, Chandigarh, India, with more than 25 years of teaching and research experience. Her research forte is enhancing bioperformance of drugs including small and large biomolecules viz. probiotics using active-tailored delivery systems. Emphasis of her work lies on industrial and clinical translation of her work as evidenced by four technologies transferred by her group to the industry. She has been granted ten (10) patents and has filed 19 more patent applications. She has 145 high-impact publications, 21 book chapters, four books, and four journal special issues to her credit. She has delivered 50 invited talks, and more than 150 presentations have been made by her research group, with 30 being adjudged as best presentations. She is on the editorial and review boards of more than 50 journals. She has received funding to the tune of 75 million INR from government agencies and has a couple of industrial consultancies amounting to 15 million INR. Prof. Kaur was awarded, the very prestigious, "US Fulbright Fellowship (FNAPE)" in 2017 and she was a visiting faculty at Rutgers State University, New Jersey, in 2017–2018. Organizers of Pharmaceutical Producers of India (OPPI) bestowed upon her "The Best Women Scientist Award-2018." Her novel ideas and technologies received BRIC Technology Exposition Award, consecutively in 2019 and 2020.

Kavita Beri is a Board-Certified Physician and Scientist in the field of Regenerative Medicine. Dr. Beri is the Founder of BE MIND BODY SKIN, a Next Generation Integrative Aesthetics and Wellness Spa in Ocean, New Jersey. She is also the founder of a newly launched all-natural cosmetic line, KYVTA, that is based on the concept of "Vibrational Cosmetics." She has published several scientific articles and presented her research work both nationally and internationally. She is also a visiting scientist at the Center for Dermal research and an Adjunct Professor of Biomedical Engineering, Rutgers State University, USA. Dr. Beri is an Editorial Advisor and Dermatology Social Media Editor for the Future Science Journal. She is a member of the scientific advisory board for TecknoScience Publisher HPC Today Magazine and a reviewer for several other journals in regenerative medicine, biotechnology, dermatology, and cosmetics. Her passion is in mind, body, and skin regeneration and its connection through the microbiome. She is a certified yoga

teacher, training new yoga instructors on philosophy of Yoga and the Mind Body and Energy connection. She has a deep interest in applications of Vibrational Medicine and how it can be connected to Anti-Aging Aesthetics by connecting the concept of microbiome-host symbiosis.

Parneet Kaur Deol is presently working as Assistant Professor at G.H.G. Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana, Punjab, India. She has more than 10 years of experience in probiotic research and has published her work in highly reputed peer-reviewed international journals. She has 21 international publications to her credit with cumulative impact of more than 50. She has filed one Indian patent application. She has co-edited a special issue for "Current Pharmaceutical Design" with Prof. Indu Pal Kaur in the year 2019. Dr. Deol has presented her work at various national and international platforms. She was awarded with the "Dr. Harpal Singh Buttar and Mrs. Harinder Kaur Buttar Award of Excellence in Pharmaceutical Sciences" in the year 2016 and Mekaster Young Scientist Award in 2018 for her research work. Recently she fetched two research projects from the Department of Science and Technology-Science and Engineering Research Board (DST-SERB), New Delhi, worth 65 lakh.

Simarjot Kaur Sandhu is presently working as analytical chemist at Taro Pharmaceutical Industries Ltd., Brampton, Ontario, Canada. She has done her doctorate from the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India. Her area of research lies in improving the bioprofile of phytopharmaceuticals and probiotics by encapsulating them within nanoparticles. She has published six international publications and has filed two India patent applications, one European, and one US patent application out of her PhD work.

Gut-Skin Axis: Role in Health and Disease

1

Alok Malaviya, K. Vamsi Krishna, Shruti Malviya, and T. Nimisha Das

Abstract

The human microbiome includes microorganisms and their cumulative genetic details that reside in the human body. Skin, the body's most external organ and exposed to the external environment, is an ecosystem with 1.8 m^2 area. It has a varying epidermal thickness, folds, and appendages in different areas including along with varying moisture and temperature level on the skin surface. Microbial colonization on the skin surface starts from the time of birth. The mode of delivery affects the colonization process to a considerable extent. The group of microbes colonizing the skin surface is determined by physical and chemical features of it, which applies to microbes inhabiting the gut and other ecological niches in the body as well. There is several common important characteristics shared commonly by gut and skin, where both are (1) heavily vascularized, (2) richly perfused, (3) densely innervated, (4) integrated to the immune system, (5) highly associated with the endocrine system, (6) extensively colonized with recognizable microbiota, and (7) both helps our body to communicate with its external environment. It has variously been reported that a close and bidirectional association within the gut and skin in maintaining the homeostasis and allostasis

Alok Malaviya and K. Vamsi Krishna contributed equally to this work.

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of skin and also gastrointestinal (GI) health. Therefore, numerous intestinal pathologies have been linked to skin comorbidities. It has been found that skin is directly impacted by the various circumstances that principally affect the intestine. Similarly, various gastrointestinal disorders could be linked to distinct dermatological entities. In the same context, a growing body of proof proposes an association of intestinal dysbiosis with many regular inflammatory skin pathologies including atopic dermatitis (AD), psoriasis, rosacea, and acne vulgaris. And the realization of this interconnected association between skin and gut has resulted in a new concept of the "Gut-Skin Axis." An intimate bidirectional engagement between the gut and the skin has been well established by growing research evidence in this domain. Recent reports have indicated that the administration of specific Lactobacilli strains to mice can significantly alter the overall skin phenotype. Despite increasing research efforts in this domain, a systematic investigation of the "Gut-Skin Axis" remains ill explored by both gastroenterology as well as dermatology researchers. And in this context, here we are discussing various aspects of the Gut-Skin Axis and its role in the general well-being of individuals.

Keywords

Dysbiosis · Microbiome · Gut-Skin Axis · Probiotics · Dermatitis

1.1 Introduction

The total number of microorganisms present in an environment is termed as "microbiome" or "microbiota" (Adamczyk et al. 2018; Kim and Kim 2019). Microorganisms along with their collective genetic information present in the human body are comprised of the human microbiome. And hence, the genetic information stored in the microbiome is considered as the human genome's counterpart and represents the compilation of entire genetic information in an individual (Mańkowska-Wierzbicka et al. 2015). The human body acts as a microbial ecosystem inhabited by microorganisms from different species. Some of the areas in the human body right from the skin to the oral cavity, digestive tract, airways, and genitourinary system are different sites colonized by microbiota (Kong and Segre 2012; Schommer and Gallo 2013; Adamczyk et al. 2018; Ellis et al. 2019; Lee et al. 2019).

1.1.1 Skin

Skin is the most external and exposed organ of the human body. It covers an area of approximately 1.8 m^2, which represents an ecosystem of microorganisms inhabiting

it. The varying epidermal thickness, folds, attachments, and different levels of humidity and skin surface temperature have a noteworthy influence on the composition and quantitative distribution of microbial species present on the skin surface (Grice and Segre 2013; Adamczyk et al. 2018). Some of the organisms inhabiting the skin include bacteria, fungi, viruses, and mites. In most cases, the constituents of skin microbiota are innocuous and maintain a symbiotic association with the skin cells including mutualism, parasitism, and commensalism (Adamczyk et al. 2018). The main role of the skin includes (1) to protect the bodies from various possible assaults by external organisms or different types of harmful materials, (2) to counterattack a wide variety of challenges, and (3) to respond aptly to all-pervading dangers.

Although the skin is completely exposed to the external environment, only selected sets of microorganisms are predominantly present on the skin surface due to their adaptation to the skin's physical and chemical compositions. Factors contributing to specific habitats of the skin include (1) its thickness, (2) number of folds, (3) the density of hair follicles, and (4) different glands present on it. In general, it is cool, acidic, and desiccated. The epidermis represents a physical barrier present on the skin surface that resists the entry of microbes and latent toxins inside the body, while holding moisture and nutrients inside the body. Terminally differentiated, enucleated keratinocytes makes up the topmost layer of the epidermis, also known as the stratum corneum. These are also known as "squames," which are composed of keratin fibrils along with cross-linked, cornified envelopes embedded in bilayers of lipid, thus forming the "bricks and mortar" of the epidermis. These "squames" are continually shed from the skin surface making the skin a continuously self-renewing organ.

The various structures on the skin such as cutaneous invaginations and associated attachments along with eccrine and apocrine sweat glands, sebaceous glands, and follicles of hair are known to have their specific exclusive microbiota. Among these, eccrine glands are virtually seen on all skin surfaces which help in (1) continuously bathing the surface of the skin with a secretion made up water and salt, thus helping in thermoregulation of the human body; (2) water and electrolyte excretion; and (3) skin acidity, preventing microbial growth and colonization. Apocrine glands are specific in their location and are present in the axillary vault (armpit), nipple, and genital regions, and they produce a milky, viscous, and odorless "secretion" in response to the hormone adrenaline, which contains pheromones triggering specific behaviors such as sexual or alarm, in the receipt person. Bacterial processing and use of these apocrine gland secretions end up in the stereotypical odor connected with sweat. Sebum, a lipid-rich hydrophobic covering that defends and lubricates the skin and hair and thus providing an antibacterial shield, is produced by the pilosebaceous unit of sebaceous glands (Schommer and Gallo 2013; Grice and Segre 2013; Dréno et al. 2016; Balato et al. 2019; Ellis et al. 2019).

1.1.2 Development of Skin Microbiome

Skin colonization by microorganisms starts at the time of birth. The mode of delivery affects the colonization process to a considerable extent (Fig. 1.1). In the case of vaginally delivered neonates through the vaginal canal, maternal microflora like *Lactobacillus* spp., *Prevotella* spp., etc. colonizes the neonatal skin. In contrast to this, the skin surface of neonates delivered through cesarean mode is primarily exposed to the microbes living on the mother and medical workers' skin as well as the microflora present in the hospital environment from the very beginning. And, therefore the microflora of such newborns will be completely different than the previous one, primarily colonized by bacterial species like *Staphylococcus* spp., *Corynebacterium* spp., *Propionibacterium* spp., etc. The fungal colonization progression on the skin is correspondingly dynamic. In 89–100% of the newborns, the fungal genus *Malassezia* are noticeable from the first day on the skin surface. During 3 years of age, changes in skin microbiota could be seen due to the changes in skin physiology, diet, locomotion, etc. The skin of the children is colonized by *Streptococcus* and various types of *Proteobacteria* such as *Betaproteobacteria* and *Gammaproteobacteria* (Fig. 1.1). Both anatomical and physiological factors contribute to the microbial composition in adults and resulting in a difference in skin microbiome between women and men. Growth of the skin microflora is highly impacted by factors such as the amount and composition of sebum, sweat, and hormonal secretions (Kong and Segre 2012; Adamczyk et al. 2018).

1.1.3 Factors Affecting the Skin Microbiome

In essence, microflora composition and distribution is a result of everything that individual touch, bathes in, breath, eat, and drink. Factors precise to the host like age,

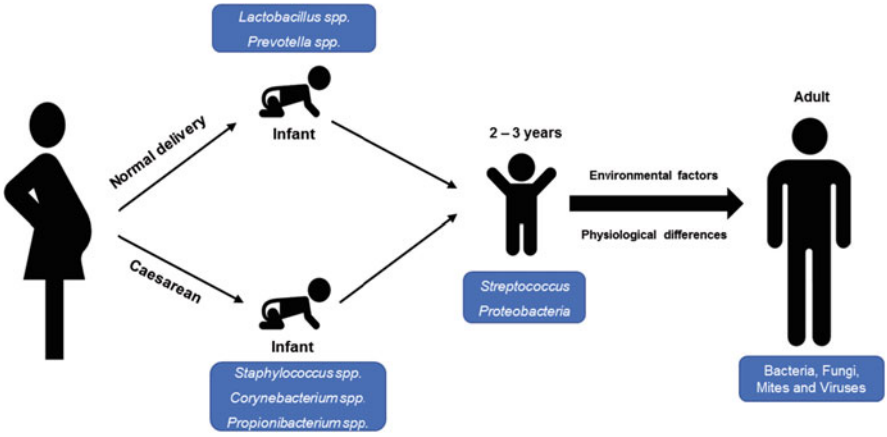

Fig. 1.1 Effect of two different modes of delivery on skin microbiome development

place, and gender impact their skin microbial flora. As we mentioned earlier, the fetal skin is germ-free, and colonization starts straightaway after the delivery and the composition differs based on the type of delivery, either by vaginal delivery or by cesarean. Additionally, different cutaneous environments such as sweat, sebum, and hormone production also cause microbial composition differences among men and women.

Similarly, various other factors that might be specific to an individual may also alter the colonization of skin microbiota. Some of these factors include (1) occupation, (2) clothing choice, (3) antibiotic usage, (4) cosmetic usage, (5) soap usage, (6) usage of hygienic products, and moisturizers, etc. However, these products modify the skin barrier conditions and their impact on skin microflora remains uncertain. Family and household contacts and pets in the home that carry microorganisms also have an important influence on the microbial population. Some other factors include (1) sunlight (ultraviolet light), (2) the ambient temperature, (3) environmental contact, (4) air quality, (5) humidity, (6) ventilation, and (7) co-occupancy. (Kong and Segre 2012; Grice and Segre 2013; Prescott et al. 2017; Ellis et al. 2019).

1.1.4 Skin Microbiome: Composition and Distribution

Skin microbiota composition and their diversity have not been completely elucidated as the majority of the microbes cannot be grown by standard culture methods, and they represent only a tiny proportion of the culturable microbes that can be seen on the surface (Grice and Segre 2013; Adamczyk et al. 2018). New DNA-based molecular biology tools for the analysis and recognition of 16s ribosomal RNA genes have tried to recognize the variety of microbes within our bodies. Even then, degenerate PCR primers cannot amplify all 16S ribosomal RNA genes with similar efficacy. Whole-genome shotgun metagenomic sequencing of bacteria can identify the complete genetic diversity and make it probable to predict the gene roles related to the skin microflora. Nevertheless, WGS metagenomic exploration of skin microbiota has not been documented.

1.1.4.1 Bacteria

As characterized by 16S rRNA metagenomic sequencing, no less than 19 phyla can be observed in the skin microflora, and among them, the major is Actinobacteria (51.8% *Corynebacterium* spp., *Propionibacterium* spp., *Microbacterium* spp., *Micrococcus* spp.), *Firmicutes* (24.4% *Staphylococcus* spp., *Clostridium* spp., *Streptococcus* spp., *Enterococcus* spp.), *Proteobacteria* (16.5% *Janthinobacterium* spp., *Serratia* spp., *Halomonas* spp., *Delftia* spp., and *Comamonas* spp.) and *Bacteroidetes* (6.3% *Sphingobacterium* spp., *Chryseobacterium* spp.) (Table 1.1).

The majority of the skin surface microbiota belongs to the phyla *Corynebacterium*, *Propionibacterium*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes,* and *Proteobacteria*. Both the skin and gut microbiota shows noticeably a little variety at the phylum level, while the variety is high at the species level. Also, they

Table 1.1 Microbial distribution on various skin regions (Schommer and Gallo 2013; Grice and Segre 2013; Dréno et al. 2016; Adamczyk et al. 2018)

Microbe	Phyla	Site of distribution
Bacteria	*Actinobacteria* (*Micrococcus* spp. and *Corynebacterium* spp.)	Moist areas—Axilla, inner elbow, or inguinal fold
	Firmicutes (*Staphylococcus* spp.)	
	Propionibacterium spp.	Sebaceous areas—Forehead, alar crease, retroauricular crease, and the back
	Actinobacteria	Dry areas—Forearm, buttock, and various parts of the hand
	Proteobacteria	
	Bacteroidetes	
	Firmicutes	
Fungi	*Malassezia* spp.	Sebaceous areas—Forehead, alar crease, retroauricular crease and the back
	Aspergillus	Foot skin
	Candida albicans	
	Cryptococcus	
Mites	*Demodex folliculorum*	Hair follicles and sebaceous glands
	Demodex brevis	Sebaceous and Meibomian glands of eyelids

demonstrate several variations based on the skin location. The upper part of the hair follicle inhabits nearly 50% of all the bacteria that belong to *Staphylococcus epidermidis*. The remaining bacterial species belong to the same genera (*Staphylococcus saprophyticus*, *S. hominis*, *S. warneri*, *S. haemolyticus,* and *S. capitis*) and to *Micrococcus* genera (*M. luteus*, *M. varians*, *M. lylae*, *M. sedentarius*, *M. roseus*, *M. kristinae*, and *M. nishinomiyaensis*). In general, sebaceous sites with low phylo-type richness tend to have the lowest bacterial diversity and include the forehead, the area behind the ear (retro-auricular crease), and side of the nostrils. The most prevailing organisms in the above sites and other sebaceous locations are *Propionibacterium* spp. Similarly, moist areas of the body including the navel (umbilicus), axillary vault, side of the groin (the inguinal crease), uppermost area of the fold in the middle of the buttocks (gluteal crease), the sole of the foot, behind the knee (popliteal fossa), and the inner elbow (antecubital fossa) are mainly colonized by the *Staphylococcu*s and *Corynebacterium* spp. The dry areas such as forearm, buttock, and different parts of the hand are the most varied skin sites with mixed representation from the four major phyla. *Streptococcus aureus, S. pyogenes* (group A *Streptococci*), *Corynebacterium* spp., and *P. aeruginosa* (Gram-negative bacilli) are some of the pathogenic bacteria that can be found on the skin. Different skin infections can be caused by these pathogenic bacteria (Table 1.1).

1.1.4.2 Fungi

Maximum skin microbiome investigations aim at understanding the bacterial composition; however, fungi, viruses, and mites also constitute as key members of skin microbiota apart from the bacterial members. It was revealed that fungi are present in most of the body sites by the shotgun sequencing studies, and it constitutes lesser

than 1% of total microbiota and however, for the area surrounding the ears and forehead, they are abundant. *Malassezia* spp. (*M. restricta*, *M. globosa,* and *M. sympodialis*) is the main fungi (almost 53–80%) found in all of the skin regions. *Malassezia* species are lipophilic microbes and are often connected with skin's sebum-rich areas. Other genera of fungi such as *Penicillium* (*P. chrysogenum*, *P. lanosum*), *Aspergillus* (*A. candidus*, *A. terreus*, *A. versicolor*), *Alternaria*, *Candida* (*C. tropicalis*, *C. parapsilosis*, *C. orthopsilosis*), *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Mucor*, *Debaryomyces*, *Cryptococcus* (*C. flavus*, *C. dimmennae*, *C. diffluent*), *Trichophyton*, and *Rhodotorula* can be seen in the skin microbiota.

C. albicans and *Cryptococcus* can colonize the skin, causing both Candidiasis and Cryptococcosis during favorable conditions that include injured epidermal surface, raised humidity, and temperature levels. Because of the limited knowledge available on fungal organisms on the surface of the skin, further studies of human skin fungi with improved methods for fungal sequence recognition and analysis are essential.

1.1.4.3 Mites

In addition, *Demodex folliculorum* and *Demodex brevis* which are demodex mites, tiny arthropods that are typically linked with rosacea, and also causative agents of a number of other skin disorders are commonly found in pilosebaceous facial units and are counted as part of actual skin microbiota.

1.1.4.4 Viruses

The maximum of the skin viruses appears to be consisting of bacteriophages that target bacteria (*Propionibacterium* and *Staphylococcus*). The genetic makeup (DNA and RNA) of viruses rapidly evolving makes it more difficult to produce genomic libraries, so it is a difficult challenge to identify and classify viruses located on the surface of the skin. Viruses are considered to be not only infective agents and also factors that lead to the preservation of homeostasis of the skin. *Polyomaviridae* and *Papillomaviridae* are double-stranded DNA (dsDNA) viruses that can be seen as part of a skin microbiota (Kong and Segre 2012; Schommer and Gallo 2013; Grice and Segre 2013; Dréno et al. 2016; Prescott et al. 2017; Adamczyk et al. 2018; Balato et al. 2019; Ellis et al. 2019) (Table 1.1).

1.2 Gut-Skin Axis

The human gut microbiome is a conglomeration of bacteria, viruses, fungi, and also protozoa that exceeds the host cells by ten times as much as the skin microbiome. Advances in metagenomics and the development of high-throughput DNA sequencing technologies have strengthened our understanding of the intestinal microbiome and its diverse impact on human health and pathology. The microbiome of the gut is responsible for the host vital metabolic and immune benefits. Both the gastrointestinal tract and skin are exclusively interconnected in purpose and function and are compactly vascularized with richly innervated organs with critical immune and

neuroendocrine roles. Both these organs are important in maintaining physiologic homeostasis. Few cumulative shreds of evidence have established a close and bidirectional relation between the gut and skin concerning gastrointestinal (GI) health, homeostasis, and allostasis of skin. Different GI abnormalities are occasionally associated with cutaneous manifestations, and the intestinal microflora seems to be involved in the pathophysiology of various inflammatory ailments. In the breakdown of indigestible complex polysaccharides, the gut microbiome assists the process and is essential for the generation of nutritional elements for instance vitamin K. The impact of the intestinal microflora on the immune system of the host is immense, and the association is intricately managed to allow respectively dietary and environmental antigen immune tolerance and offer defense against latent pathogens. Consequently, intestinal microflora alterations cause the onset of autoimmune and inflammatory diseases in the skin which is far-off from the intestine. Evidently, atopic dermatitis (AD), psoriasis, rosacea, and acne vulgaris are common inflammatory skin pathologies, a state of microbial imbalance called intestinal dysbiosis can be invariably observed. This revelation gave rise to the new "*Gut-Skin Axis*" concept. It is, after all, not a new one.

Dermatologists, John H. Stokes and Donald M. Pillsbury, during the 1930s, established an innovative theory linking the relation linking gut microbiota and systemic and skin inflammatory conditions (Stokes and Pillsbury 1930) that were later brought together as the "Gut-Brain Axis" (Arck et al. 2010) and the "Gut-Brain-Skin Axis" (Bowe and Logan 2011). An intricate association linking intestinal dysbiosis and dermatological conditions is presently proposed by different clinical evidence. The mechanisms behind such interpretations, however, have yet to be long established. A multifactorial interrelationship among the nervous, immune, and endocrine systems and furthermore aspects such as food habits and medications are probable to be involved in the association among the gut and the skin. It is well-known that psychosocial strain is involved in both the worsening and the commencement of a variety of skin ailments in the "Gut-Brain-Skin Axis" concept. It is reasonable that neurotransmitters can be produced in reaction to stress and other stimuli by the microflora which can modulate the skin functions via neural pathways (Vaughn et al. 2017; Salem et al. 2018; Szántó et al. 2019).

1.3 Microbial Association with "*Gut-Skin Axis*"

The skin serves different purposes effectively like shielding, regulating temperature, and retaining water and keeps homeostasis. The gut microbiota influences the signaling pathways which coordinate the skin homeostasis and impact the integumentary health (Fig. 1.2). However, the mechanisms are not yet completely explored. Gut microbes *Bacteroides vulgilis* and *Faecalibacterium prausnitzii* produce a variety of metabolites like retinoic acid, polysaccharide A, etc. Likewise, *Clostridium* cluster IV and XI bacteria stimulate the amassing of regulatory T cells and lymphocytes that also assist anti-inflammatory responses in the body.

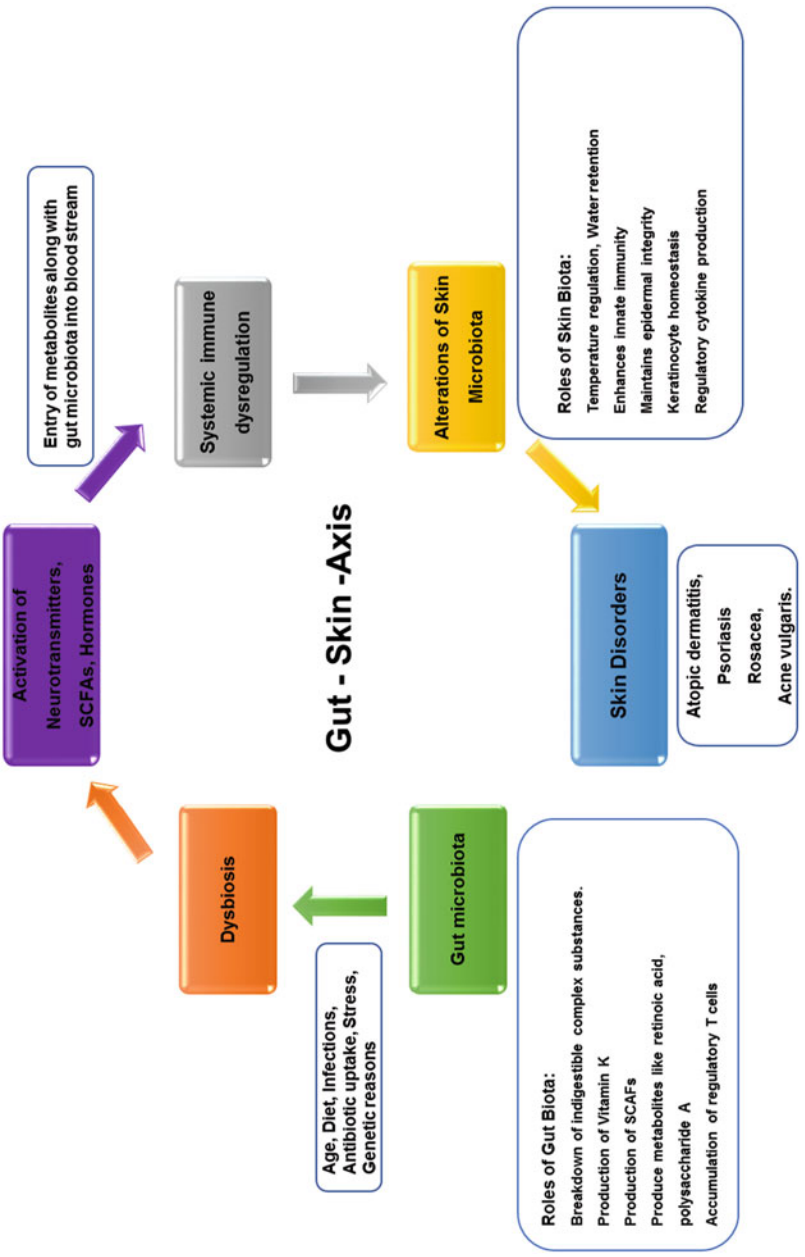

Fig. 1.2 "Gut-Skin Axis" communication and role in the development of various skin conditions

The aggregation of Th17 and Th1 pro-inflammatory cells are stimulated by some of the filamentous bacteria. Some gut commensal species secrete neurotransmitters like norepinephrine, serotonin, and acetylcholine or may also induce the release of neuropeptides from neighboring enteroendocrine cells where they enter the gut epithelium bloodstream and can lead to systemic effects. Propionic acid, butyric acid, acetic acid, and lactic acid, the short-chain fatty acids (SCFAs), like generated via intestinal microbial fermentation (Michael de Vrese 2008), enter the bloodstream and influence the skin (O'Neill et al. 2016). Butyrate hinders immune responses by subduing the propagation, relocation, adhesion, and cytokine development of inflammatory cells. Also, it prevents histone deacetylase and inactivates the pathways to NF-κB, thereby facilitating the propagation of regulatory cells engaged in different physiological cutaneous roles comprising hair follicle control, differentiation of stem cells, and wound cure (Meijer et al. 2010; Loser and Beissert 2012).

SCFAs control immune cell activity as well as apoptosis. The prevalence of certain skin microbiome profiles influencing dermal immune response systems was considered to be a significant role played by SCFAs. *Propionibacterium*, for example, may develop a propionic acid that can show an effective antimicrobial effect on *Staphylococcus aureus*, a community-acquired methicillin-resistant microbe (Shu et al. 2013). Bacterial species such as *S. epidermidis* and *P. acnes* can withstand larger SCFAs variations than other bacterial members. All these findings completely support an interconnected mechanism between the gut and the skin (Shu et al. 2013; Schwarz et al. 2017) (Fig. 1.2).

Various rodent and human studies have documented different beneficial effects on skin health. In research by Levkovich et al. (2013), mice consuming supplementation of *Lactobacillus reuteri* encountered better dermal thickness, increased folliculogenesis, and an improvement in the development of sebocytes which were more smooth and shiny fur (Levkovich et al. 2013). Horii et al. (2014) performed a trial demonstrated an improved release of the serotonin by intestinal enterochromaffin cells in the rats by intake of *Lactobacillus brevis* SBC8803, and the resulting activation of parasympathic pathways led to a reduction of the cutaneous sympathetic arterial nerve signal (CASNA), which also enhanced skin blood circulation. Transepidermal water loss (TEWL) also declined dramatically and also enhanced corneal hydration in human clinical studies, after 12 weeks of consumption of the same organism (Horii et al. 2014).

In a separate experiment, the intake of probiotic *Lactobacillus paracasei* NCC2461 in different placebo-controlled volunteers for 2 months resulted in reduced skin sensitivity and TEWL, which is due to a measurable upsurge in the circulating transforming growth factor-beta (TGF-β) (Gueniche et al. 2014). In another analysis by Baba et al. (2006), the mRNA expression of keratin 10 and involucrine, early and late differentiation markers, respectively, enhanced when fermented milk with *Lactobacillus helveticus* was given to human epidermal keratinocytic cultures, thereby suggesting that the bacterial involvement in promoting the epidermal differentiation. In comparison, a rise of profilaggrine, a protein which performs the keratinocytes terminal differentiation, indicates a possible moisturizing gain for this bacterium. This finally produces filaggrin, a protein that assists usual epidermal flexibility and hydration (Baba et al. 2006).

1.4 "*Gut-Skin Axis*" and Associated Disease

The skin microflora is very crucial in maintaining healthy skin. The skin microflora interacts with the host with the help of their metabolites which is very important in the establishment and maintaining the host homeostasis. But different factors like food habits, antibiotic consumption, sanitation, diseases caused by pathogens, etc. can alter their composition which creates an imbalance. This state of imbalance is termed as *dysbiosis* which can contribute to the development or possible pathogens and reduction of beneficial bacteria. This in turn is a consequence in abnormal activation of immune cells, affecting the epithelial barrier structure, leading to adjustments in the reactivity of the immune system and successively to inflammatory disease development and finally leading to induction and/or exacerbation of the disease. It is well-established that there is a constant cross-talk among the gut, skin, and brain through the "*Gut-Brain-Skin Axis*." Via different studies, it was understood that the alterations in the gut microbiota composition can affect local immune responses that modify not only the immunity of the host but also inflammation in body parts distal from the gut. There is convincing proof demonstrating the connection between gut dysbiosis and numerous inflammatory and autoimmune diseases, including dermatological diseases/skin inflammatory disorders such as atopic dermatitis (AD), psoriasis, acne, etc. (Fig. 1.2) (Grice and Segre 2013; Grice 2014; Mańkowska-Wierzbicka et al. 2015; Vaughn et al. 2017; Salem et al. 2018). Table 1.2 shows some of the skin diseases and microbes associated with them. In the following section, we will discuss about some of these skin-associated diseases.

Table 1.2 Microbes involved in the onset of the various skin diseases

Disease	Microbes involved	References
Atopic dermatitis	Increase in *Staphylococcus* spp. (*S. aureus*, *S. epidermidis*, *S. hominis,* and *S. haemolyticus*)	Ellis et al. (2019), Kim and Kim (2019)
	Increase in *Clostridium difficile*, *Escherichia coli* and *Faecalibacterium prausnitzii*	
	Decrease in *Cutibacterium*, *Streptococcus*, *Acinetobacter*, *Corynebacterium,* and *Prevotella*	
Psoriasis	Increase in *Firmicutes*, *Corynebacterium*, *Propionibacterium*, *Staphylococcus*, *Streptococcus* and *Malassezia ovalis* (Fungi)	Benhadou et al. (2018), Ellis et al. (2019)
	Decrease in *Actinobacteria*	
Acne vulgaris	*Cutibacterium acnes* (formerly *Propionibacterium acnes*)	Ellis et al. (2019), Lee et al. (2019)
Rosacea	*Helicobacter pylori*	Buechner (2005), Mańkowska-Wierzbicka et al. (2015)
	Increase in *Demodex folliculorum* (mite), *Bacillus oleronius*, *Staphylococcus epidermidis* and *Chlamydophila pneumonia*	

1.4.1 Atopic Dermatitis

Atopic dermatitis (AD) or eczema, a chronic inflammatory cutaneous ailment commonly occurs in the first 5 years of age. Almost 15–20% of the kids and 2–10% of adults are infected by this across different countries around the world. The patients with this ailment show a noteworthy barrier dysfunction that is a consequence of either a genetical mutation of a gene coding a structural protein called filaggrin which maintains the epidermal homeostasis by assisting with water retention or environmental factors like the use of hygiene products. The pH of the skin surface elevates due to the use of hygiene products like soaps and detergents, and it is detrimental for the epidermal barrier function, and thus it was observed that the skin surface pH of patients with AD is higher than the others with regular skin. However, it is not yet clarified that the elevation of skin pH causes the cutaneous dysbiosis of AD patients (Rather et al. 2016). But, the topical application of *Lactobacillus johnsonii* NCC 533 (HT La1), for 3 weeks resulted in reducing the pathogenic bacteria *Staphylococcus aureus* colonization and reduction of skin pH by metabolites produced by the same (Blanchet-Réthoré et al. 2017).

The microbial contact starts at the birth time and the infant's gut and skin microbiota are very much dependent on the type of delivery. Microbes belonging to the *Lactobacillus* and *Prevotella* genus are the dominant ones in the infants delivered through the vagina, and they resemble their mother vaginal microbiota, whereas *Staphylococcus, Corynebacterium, and Propionibacterium* spp. are dominant in the infants born through caesarean section and resembles their mother's skin surface (Li et al. 2014).

The immune disorders, namely, asthma, allergy, and inflammatory bowel diseases are believed to be associated with caesarean section delivery. While there is no link with the caesarean section in the case of AD, the vaginal-derived *Lactobacilli* bacteria have a defensive responsibility to prepare the naive immune system of infants against *Staphylococcus aureus*, a pathogen that could have significance in different skin conditions. A few studies emphasize the significance of the proper development of a diverse gut microbial population in early life to help prevent AD. Intestinal microbial composition is potentially a crucial factor in the development of this condition. Different metabolites generated by the intestinal microflora travel through the entire body and affect the distant sites of the organism. During the condition called "leaky gut syndrome" where the integrity of the epithelial barrier deranged, that causes an inclination in the penetrability of the gut, higher levels of various immunogenic molecules, food antigens, microbial toxins, and harmful bacteria penetrating into the body and accumulating in the skin. This perhaps agitates the epidermal wall and leads to inflammation and uninterrupted immune responses. Bacterial metabolites such as free phenol and p-cresol are produced in response to the infectivity by a pathogen *Clostridium difficile* subsequent to antibiotic treatment (O'Neill et al. 2016). They gain entry to the blood circulation, concentrate in the skin, and weaken the consistency of the epidermal membrane by lowering the keratin 10 expression of keratinocytes. Regular supplementation of probiotic *Bifidobacterium breve* jointly with a prebiotic galactooligosaccharides increased

skin hydration in healthy adult women by decreasing average serum phenol levels. Some colon bacterial metabolites, like secondary bile acids comprising lithocholic acid and deoxycholic acid, also affect the physiology of the skin. Lithocholic acid affects the adaptive immune response by influencing the initiation of the Th1 cells (Miyazaki et al. 2014).

The SCFAs like butyrate, propionate, acetate, and lactate are identified to assist the gut epithelial barrier integrity and exert anti-inflammatory outcomes. In a few studies, analysis of fecal samples showed a clear reduction of SCFAs in the AD patients. It is therefore appealing to hypothesize that any product capable of influencing the intestinal microflora and SCFA production can affect inflammatory responses and therefore influence the condition of the skin as well. Probiotics usage restores the gut with SCFAs secreting bacteria in the AD patients and thus helps in promoting the epithelial barrier integrity. Gluten sensitivity is known to harm the intestinal barrier (de Sousa Moraes et al. 2014) causing a leaky gut and thus associated with severe cutaneous manifestations resembling the AD (Szántó et al. 2019). The usage of probiotics in gluten sensitivity-related AD as adjuvant therapies seems to be a fascinating strategy since certain gluten polypeptides may be hydrolyzed by such probiotics. Concentrations of vitamin D also correlate with the AD's severity. In a research, deficiency of vitamin D is connected with shifts in the microbe's composition in a study of cystic fibrosis patients, which may stimulate inflammation (Baek et al. 2014; Kanhere et al. 2018). Probiotics help in increasing the vitamin D levels in serum along with the expression of vitamin D receptor which helps in defending the gastrointestinal inflammation (Shang and Sun 2017). All these suggest many-sided communications among gut microbiota and AD, and thus modulation of the intestinal microflora with probiotics may be a good option for adjuvant therapy in management of AD. For this cause, it is necessary to characterize the gut flora and its metabolites, along with the probiotic strains involved in the prebiotic composition, the duration and extent of the probiotic treatment, and vigilantly planned clinical trials.

1.4.2 Psoriasis

Psoriasis, triggered by a multitude of external causes like a bacterial infection, treatments with antibiotics or profound dietary changes, and internal genetic aspects, is immune-intervened and retrieving inflammatory skin ailment. It is characterized by keratinocyte hyperproliferation called "acanthosis," with the resultant hyperplasia of keratinocytes and differentiation of dysregulated keratinocytes called "parakeratosis".: The disease affects about 2–4% of the population of the world (Bata-Csorgo et al. 1995; Salem et al. 2018; Balato et al. 2019; Szántó et al. 2019). Another gastrointestinal disorder, like inflammatory bowel disease (IBD), which is closely associated with gut dysbiosis, is also followed by the onset of psoriasis. The dysbiosis pattern found in cases of patients with IBD shares a close trend with the bacterial variety found in psoriatic patients (Vaughn et al. 2017; Salem et al. 2018; Ellis et al. 2019; Szántó et al. 2019). In both cases, it was observed that depletion of

symbiotic bacteria such as *Bifidobacteria, Lactobacilli* and *Faecalibacterium prausnitzii,* and augmentation in the of pathogens like such as *Salmonella, Escherichia coli, Helicobacter, Campylobacter, Mycobacterium,* and *Alcaligenes* were observed (Scher et al. 2015). This reduction of the beneficial bacteria may translate into reduced control of the gut immune responses and influence the distant organ systems. There is a lot of data suggesting that there is a vital role in the cytokine network of Th17 cells, a new subgroup of T cells, as a prime member in the pathogenesis of the disease. The Th17 cytokines improve the IL-10 cytokine family's expression, particularly the cytokines, IL-20, and IL-22 that facilitate keratinocyte hyperproliferation (Ma et al. 2008; Nograles et al. 2008). The production and functioning of Th17 cells are also potentially controlled by SCFAs. Psoriasis is associated with the loss of *Faecalibacterium prausnitzii*, a significant source of defensive SCFAs in the intestine, indicating a correlation among intestinal dysbiosis, SCFAs, and Th17-mediated inflammation in the disease's pathomechanism (Eppinga et al. 2016). All this data suggests that the implication of the "*Gut-Skin Axis*" in the pathophysiology of psoriasis and raises the significance of the application of oral probiotics in disease management. Till today, only three oral administrations of probiotic studies have been studied with three different probiotic species influencing distinct pathways of the pathomechanism of psoriasis (Szántó et al. 2019). An improvement is seen in the course of disease, but the accessible information is very less and heterogeneous, thus making it tricky to propose an appropriate supplementation procedure with probiotics in psoriasis affected patients (Vaughn et al. 2017; Salem et al. 2018; Balato et al. 2019; Szántó et al. 2019).

1.4.3 Acne Vulgaris

Acne vulgaris is a recurrent pilosebaceous disease (3D complex structure exists on the mammalian skin surface consisted of hair follicles, hair shafts, and sebaceous glands), which is clinically expressed as non-inflammatory comedones or inflammatory papules, pustules, and nodules (Salem et al. 2018; Szántó et al. 2019). The acne pathogenesis is defined by three factors: over secretion of sebum, follicular hyperkeratinization, and enhanced secretion of pro-inflammatory cytokines interceded by *Propionibacterium acnes*. This acne condition affects roughly 70–80% of teenagers and young adults amongst the ages of 12 and 25 and is the eighth most prevalent medical disorder globally (Bowe et al. 2014; Balato et al. 2019; Salem et al. 2018). Acne is very much predominant in the western countries where their diet typically has a lot of carbohydrates. The involvement of a commensal skin bacterium, *Propionibacterium acnesium*, or also called as *Cutibacterium acnes* (*C. acnes*) in the pathogenesis of acne, is one of most studied subjects in acne research, and however, it is still not wholly explained. *C. acnes* is a principal species of skin microbiota and produces SCFAs on the skin and maintains skin homeostasis. It is believed that the *C. acnes* proliferates because of elevated sebum and production of fatty acid in the hair follicles and accompanying sebaceous glands, thus inducing the generation of inflammatory mediators (Salem et al. 2018; Balato et al. 2019;

Szántó et al. 2019). In a recent investigation, no quantitative difference of this bacterium was found among patients with acne and healthy individuals suggesting that presence of this bacterium determines the onset of the disease rather than the proliferation of the same (Barnard et al. 2016; Dréno et al. 2018).

The acne prevalence is significantly high in developing countries because of high glycemic or western style diet habits (Melnik 2013). The high glycemic load food facilitates the insulin production and IGF-1 (insulin like growth factor-1), thereby inducing the intensified cytoplasmic expression of the metabolic FoxO1 (fork-head box transcription factor), a cell nutrition state sensor. Ultimately, FoxO1 activates mTORC1 (mammalian target of rapamycin complex 1), a metabolism and cell proliferation administrator, to mediate sebaceous gland hyperproliferation, lipogenesis, and acroinfundibular keratinocyte hyperplasia, thus leading to the acne production (Melnik 2015; Agamia et al. 2016).

Very few researches have focused their investigation on the role of intestinal microflora in acne patients. Stokes and Pillsbury (1930) found that a high proportion of acne patients had hypochlorhydria (condition of having low levels of stomach acid) (Stokes and Pillsbury 1930) that causes the colon bacteria to drift to distal sections of the small intestine, causing a condition of gut dysbiosis and SIBO (small intestinal bacterial overgrowth) (Salem et al. 2018; Balato et al. 2019). For the nutrients, the larger bacterial species contends and impairs the fats, proteins, carbohydrates, and vitamins absorption. Folic acid, zinc, chromium, selenium, and ω-3 fatty acids have shown to impact one's psychological health and have been involved in acne pathophysiology. These bacteria also allow toxic metabolites to be produced that can damage enterocytes, proliferate intestinal penetrability, and ultimately trigger the systemic inflammation. Strickler et al. established an improved reactivity to stool-separated *Coliforms* in 66% of acne patients with relevant to controls (Salem et al. 2018). Similarly, other researchers, Juhlin and Michaëlsson (1983), observed the incidence of *E. coli*'s lipopolysaccharide endotoxins in acne patients which suggests that intestinal microflora may improve the occurrence of circulating endotoxins in the blood of acne vulgaris patients in contrast to healthy controls. Certainly, acne appears to have a likely gut-skin connection that may be caused by intestinal microflora alterations (Juhlin and Michaëlsson 1983). Loveman et al. (1955) observed that the *Bacteroides* spp. were more frequently accompanying with acne patients (Loveman et al. 1955). Correspondingly, it was detected that 54% of patients with acne have variances in their gut microflora as compared to healthy control by Russian investigators Volkova et al. in 2001 (Volkova et al. 2001). In a recent investigation, the ratio of the two major phyla—*Bacteroidetes* to *Firmicutes*—improved in the gut bacteria of acne patients which is reliable with the enterotype of western food habits (Deng et al. 2018).

C. acnes is said to activate the skin's IGF-1/IGF-1 receptor mechanism, indicating a simultaneous stimulation of the gut and skin bacterial IGF-1/IGF-1 receptor pathway, which leads to acne pathophysiology (Isard et al. 2011). However, further investigation is needed to understand whether these mechanisms are related or not. The IGF-1 receptor system can be caused by gut dysbiosis that can cause a alter in the amount and/or content of lipid-rich sebum, which allows phylotypes of

C. acnes to colonize the pilosebaceous unit thereby disrupting the strong balance of skin microbiota members (Smith et al. 2006). This mechanism is thought to be feasible for the probable inter connectedness of intestine and skin microbiota. Lipopolysaccharides (LPS) are additional cause for the gut's microbial dysfunction and inflammatory acne. In acne patients, the LPS bio-synthesis pathways have been shown to be upregulated, that might be a consequence of the improved abundance of *Bacteroidetes* species in the gut that generates the LPS (Deng et al. 2018). In a study by Fabbrocini et al. (2016), the continuous intake of the probiotic *Lactobacillus rhamnosus* SP1 for 12 weeks caused in lessened IGF-1 expression in the skin and enhanced acne symptoms, indicating a new notion that IGF-1 and LPS pathways may not be distinct from each other and that intestinal dysbiosis leads to acne pathogenesis (Fabbrocini et al. 2016). The pathophysiology of acne is too influenced by intestinal microflora by cross-talk amid intestinal commensal bacteria and the mTOR pathway. Different metabolites produced by intestinal microflora have been observed to control various physiological functions such as cell proliferation, lipid metabolism, and other mTOR-mediated metabolic functions that in turn influence the composition of intestinal microbiota by controlling the intestinal barrier. This relationship functions as a pathway via which the gut microbiota may impact the pathophysiology of acne (Noureldein and Eid 2018). Stokes and Pillsbury (1930) postulated an idea which states the intricate linking in amid acne and GI dysfunction facilitated by the brain. Anxiety and depression, the psychological comorbidities—along with GI distress are associated with acne. These different stresses makes the gut microbiota to also produce several different neurotransmitters, namely, serotonin, norepinephrine, and acetylcholine or generating the neuropeptides by activating the nearby enteroendocrine cells which increase the intestinal penetrability that causes the intestinal and systemic inflammation, resulting in systemic effects by circulating through the compromised intestinal barrier (Zhang et al. 2008; Do et al. 2009; Ramrakha et al. 2012; Duman et al. 2016; Prakash et al. 2016).

1.4.4 Other Skin Diseases

Rosacea, a chronic inflammatory cutaneous ailment characterized primarily by erythema (redness of the skin) and telangiectasia (a disorder in which narrow blood vessels produce thread-like red lines or markings on the skin) predominantly on the face (Buechner 2005). The role of gastrointestinal bacteria *Helicobacter pylori* infection has been connected to the onset of this disease (Rebora et al. 1995). However, it is still a debatable issue about the infective aspect of intestinal dysbiosis in rosacea. Rosacea, like psoriasis, has been correlated with IBD (Egeberg et al. 2017). In a recent metagenomic study conducted in Korea involving 12 Korean women with rosacea, an alteration in intestinal microbial was found to be associated with this disease. In another case study, it was reported that a blend of orally administered doxycycline and probiotics served as an efficacious usage for rosacea patients. This illustrates the promise of probiotics for the treatment of rosacea disease management (Nam et al. 2018).

Other less common skin disorders but serious skin pathologies, like Hidradenitis suppurativa (HS), Erythema nodosum (EN), and Pyoderma Gangrenosum (PG), are commonly related to intestinal inflammation (Fleisher et al. 2018; Szántó et al. 2019). However their etiology is complex, and the relation among these diseases and gut dysbiosis has not yet been established, except that these diseases are associated with IBD. A lot of research needs to be done so as to know the role of intestinal microflora in the pathogenesis of these diseases.

1.5 "*Gut-Skin Axis*" and Therapeutic Implications

The advancement of scientific knowledge over the past few years made us realize that commensal microorganisms have a key role in host physiology, adaptive-immune responses, and metabolism in addition to in health and disease. Thus, various research investigations are going on to manipulate the human microbiome and improve human health. Advancement in latest technologies like next generation sequencing, metagenomics etc., have transformed the techniques to describe the microbiome. Skin microbiota, an effortlessly reachable target for therapeutic intervention and hence it is an attractive area for research along with the gut microbiome. Modifications that aimed to reestablish and preserve the activity of gut microorganisms through diet by probiotic and prebiotic supplements, fecal microbial transplantation, and antibiotics have been demonstrated to be beneficial for the enhancement of host health illnesses.

1.5.1 Diet: Probiotics and Prebiotics

It is known that the gut microbiome can be greatly influenced by diet. Bacterial composition can be modulated by the long-term or short-term dietary habitats. As the gut microbiome influences the inflammatory diseases, the modifications of their composition give an opportunity for therapeutic purposes. The gut microbiome can be positively revised by probiotics (ingestion of live beneficial organisms) along with prebiotics (compounds that promote the growth of commensal organisms in the gut) (Michael de Vrese 2008).

1.5.1.1 Atopic Dermatitis

Probiotics can be used in the prevention of allergic disorders like atopic dermatitis (AD) through microbial, epithelial, and immune effects (Table 1.3). Probiotics competitively bind to the epithelial cells and thus prevent the pathogens invasion and also suppress the development of pathogens by releasing a compound called bacteriocin (Salem et al. 2018). Through raising the expression of tight junction proteins and SCFAs, probiotics help in restoration of the impaired barrier function. They help in obstruction of pro-inflammatory cytokines like IL-4, INFγ, IL-17, etc.; promote anti-inflammatory cytokines like IL-10, TGF-β, etc.; upsurge the quantity of regulatory T cells that assists in the repression of the cutaneous expression of

Table 1.3 Effects of probiotics in different in vitro and animal model studies

	Probiotic	Study group	Outcomes	References
Atopic dermatitis	*Lactobacillus plantarum* CJLP133	Children—1–2 years old	↑ SCORAD	Han et al. (2012)
			↓ Interferon γ, eosinophil, and IL-4 count	
	Lactobacillus rhamnosus HN001	High-risk birth cohort	↓ Cumulative incidence of AD at 4 years	Wickens et al. (2013)
			No change in SCORAD	
	Lactobacillus rhamnosus Lcr35	AD mouse model	Regularization of CD4+ CD25+ Foxp3+ regulatory T cells	Kim et al. (2012)
			Down-regulation of interleukin-4 and thymic stromal lymphopoietin	
	Lactobacillus plantarum CJLP55, CJLP133, and CJLP136	AD mouse model	↑ Development of IL-10	Won et al. (2011)
			Alteration of the Th1/Th2 equilibrium	
	Lactobacillus rhamnosus IDCC 3201	AD mouse model	↓ Suppression of mast cell mediated inflammation	Lee et al. (2016)
	Bifidobacterium breve M-16V and *Bifidobacterium longum* BB5	Mothers and infants	Preventive effects of AD	Enomoto et al. (2014)
	Lactobacillus salivarius LS01 and *Bifidobacterium breve* BR03	AD adult patients	↑ Severity, life quality and ratio of Th17/Treg cells	Drago et al. (2012), Iemoli et al. (2014)
			↓ Immune activation and microbial translocation	
Acne vulgaris	Lactinex tablets—*Lactobacillus acidophilus* and *Lactobacillus bulgaricus*	300 patients suffering with acne	80% of patients experienced clinical improvement	Siver (1961)
	Lactobacillus and *Bifidobacterium* with antibiotics	40 patients	↓ Acne lesion count	Marchetti et al. (1987)
	Lactobacillus rhamnosus SP1	20 adults with acne	↓ Expression of IGF-1 expression, ↑ FoXO1	Fabbrocini et al. (2016)
	Bifidobacterium spp.	Obese diabetic mice	↑ GLP-2 → Improved tight junction integrity; ↓ Intestinal permeability	Cani et al. (2009)

(continued)

Table 1.3 (continued)

	Probiotic	Study group	Outcomes	References
Psoriasis	*Lactobacillus pentosus* GMNL-77	Imiquimod-induced psoriasis mouse model	↓ Expression of TNF-α, IL-6, IL-23/IL-17	Chen et al. (2017)
			Less scaling, erythema, and epidermal thickening	
	Lactobacillus sporogenes	A 47 years old female	Patient experienced—after 4 weeks—clinical improvement	Vijayashankar and Raghunath (2012)
	Bifidobacteria infantis 35624	26 psoriatic patients	↓ Inflammatory biomarker and plasma cytokine levels	Groeger et al. (2013)

thymic stromal lymphopoietin engaged in dendritic cells stimulation; and then averts the variation of immature T cells into subtypes (Th2 and Th17). This exerts a therapeutic role as the regulatory T cells can drift to the skin and restrain the Th2 and Th17 responses (Kim et al. 2013; McCusker and Sidbury 2016). The most studied and tested probiotic species are *Lactobacillus* and *Bifidobacterium* (Michael de Vrese 2008). However, probiotics were found to be ineffective in AD treatment in first meta-analysis performed in 2008 that involved ten randomized trials with 781 kids (Boyle et al. 2018) and in the other meta-analysis involved ten trials with 1898 kids (Lee et al. 2008), established that probiotics can be more effective in avoiding AD than healing it. The outcomes of a probiotic *Lactobacillus plantarum* CJLP133 strain was investigated in a double-blinded randomized placebo-controlled trial done among kids of age group 1–12 years old for 12 weeks. This study demonstrated a progress in scoring atopic dermatitis (SCORAD), with a concomitant reduction in interferon-γ, eosinophil, and IL-4 number (Han et al. 2012). Supplementation with *Lactobacillus rhamnosus* HN001 in high-risk birth cohort, from 35 weeks to 6 months gestation if breastfeeding and child supplemented with probiotic from birth to 2 years decreased the cumulative incidence of AD at 4 years, yet there was no considerable decline in SCORAD (Wickens et al. 2013). In one of the studies, regularization of CD4+, CD25+, and Foxp3+ regulatory T cells and the downregulation of interleukin-4 and thymic stromal lymphopoietin were observed in an AD mouse model when supplemented with orally with *Lactobacillus rhamnosus* Lcr35 (Kim et al. 2012). Similarly, suppression of dermatitis stimulated by dust mites was reported through improved development of IL-10, and alteration of the Th1/Th2 equilibrium was observed in the AD mouse model, when supplemented with probiotics strains *Lactobacillus plantarum* CJLP55, CJLP133, and CJLP136 (Won et al. 2011). In the same mouse model, mast cell-mediated inflammation was suppressed when supplemented with *Lactobacillus rhamnosus* IDCC 3201 (Lee et al. 2016). In a study by Enomoto et al. (2014), preventive effects

of probiotics in AD were observed when mothers and infants were supplemented with *Bifidobacterium breve* M-16V and *Bifidobacterium longum* BB5 (Enomoto et al. 2014). In a study, AD adult patients, when orally administered with *Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03 for a duration of 12 weeks, exhibited increased severity, life quality, and the ratio of Th17/Treg cells that reduced the immune activation and microbial translocation (Drago et al. 2012; Iemoli et al. 2014). Handful of data can be found on the probiotics and prebiotics role in the management of AD in adults. Reasonable use of probiotics and prebiotics (synbiotics) in kids may have dramatically changed outcomes. Quite a few processes such as suppression of Th2 and induction of Th1 response, Treg cells' upregulation, and skin and mucosal barrier structure's enhancement, increased intestinal microbiota variety, and suppression of *S. aureus* binding are the results of probiotics and prebiotics (Rather et al. 2016).

1.5.1.2 Acne

The ingestion of probiotic tablets that contain *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* strains as a supplement to 300 patients suffering from acne, 80% of subjects, particularly in subjects with inflammatory lesions are improved (Siver 1961). By producing bacteriocins, probiotic strains *Streptococcus salivarius* and *Lactococcus* HY449 hinder the development of *P. acnes* (Bowe and Logan 2011; Bowe et al. 2014; Kober and Bowe 2015). A greater decline in acne lesion count was observed in the patients who were given probiotics *Lactobacillus* and *Bifidobacterium* species orally in combination with oral antibiotics relative to a control group who had only oral antibiotics (Marchetti et al. 1987). In an in vitro study, a probiotic strain *Streptococcus salivarius* downregulated genes linked with bacterial adhesion to epidermal surfaces by the suppression of IL-8 secretion and inhibition of the NF-κB pathway (Cosseau et al. 2008). Probiotics also helped in lowering the glycemic load and reduces the IGF-1 signaling. This reduces the proliferation of keratinocytes and hyperplasia in sebaceous glands. In another study, reduction in oxidative stress markers has been reported in patients fed with *Lactobacillus rhamnosus* for a duration of 12 weeks (Fabbrocini et al. 2016). Similarly in another study, *Bifidobacterium* spp. was administered in obese diabetic mice which resulted in suppression of high-fat diet-induced endotoxemia and inflammation through a GLP-2 (glucagon-like peptide 2)-reliant mechanism where the elevated GLP-2 resulted in improving the tight junction integrity and thus reducing the intestinal penetrability (Cani et al. 2009). Various experiments and trials have shown that oral consumption of prebiotics and probiotics reduces the systemic markers of inflammation and oxidative stress occurring in acne disease by working synergistically in treating inflammatory acne (Findley and Grice 2014; Dréno et al. 2018; Ellis et al. 2019).

1.5.1.3 Psoriasis

Unlike AD and acne, very limited but promising data is available on the probiotic supplementation for the treatment of psoriasis. In one of the studies, the expression of TNF-α, IL-6, and pro-inflammatory cytokines in the IL-23/IL-17 cytokine axis

was suppressed by the oral administration of a probiotic *Lactobacillus pentosus* GMNL-77. In the same study, when the same strain was used on an imiquimod-induced psoriasis mouse model, compared to the untreated mice, the probiotic treated mice have minimal scaling, erythema, and epidermal thickening (Chen et al. 2017). In an experimental trial that involved patient suffering with pustular psoriasis, insensitive to steroids, dapsone, and methotrexate, in 2 weeks of commencing the probiotic administration of *Lactobacillus sporogenes* three times a day, clinical improvement was observed and after 4 weeks; complete resolution was achieved (Vijayashankar and Raghunath 2012). Significant reduction of inflammatory biomarker and plasma cytokine levels were observed in a double-blind, placebo-controlled trial involving 26 psoriatic patients when *Bifidobacteria infantis* 35624 for was administered orally for 6–8 weeks (Groeger et al. 2013). A brief summary of the effects of probiotics in different in vitro and animal model studies for atopic dermatitis, acne vulgaris, and psoriasis has been presented in Table 1.3.

1.6 Conclusion and Future Perspective

Both the gut and the skin are dynamic immune and neuroendocrine organs, and the physiology of their local ecosystem is regulated by their respective microbiome. This close association between the intestine and the skin is irrefutable. Together the gut bacteria themselves and their metabolic by-products impact the skin's physiology and engages with the skin in several ways, such as (1) nutrient absorption with a direct influence on the skin, (2) nutrient absorption that can induce hormonal changes that influence the skin, (3) impact of the gut microbiome on the immune system, and (4) regulation of the local microbiome that liberates metabolites that can have distant effects on the skin. This gut-skin cross-talk is also evident by the reports which show that the pathology of the gastrointestinal tract and diet influences the skin health. Different skin disorders were being associated with gastrointestinal inflammation, namely, atopic dermatitis, rosacea, psoriasis, and acne. Skin lesions may also arise in combination with gastrointestinal disorders, like inflammatory bowel disease (IBD) and celiac disease (CD).

Various skin diseases have variously been linked with imbalanced gut microbiome; hence, it has been proposed that various skin conditions could be improved by the modulation of the gut microbiota. Incidentally, oral probiotics could be an easy, effective, and inexpensive method in the therapeutic management of skin inflammation. There are numerous lines of research supporting the probiotics use for skin disorders. Probiotics can have outcomes on distant organ systems via the immune system when consumed. By communicating with lymphoid tissue, probiotics can control the release of inflammatory cytokines that are frequently augmented under different skin conditions. Amid these positive findings, several concerns remain.

A potentially new era of research with broad implications on various skin conditions could be envisaged by the involvement of the gut microbiome. In this direction, a superior understanding of the roles of "gut-skin microbiome" in shaping

the skin's immune, metabolic, and pharmacologic microenvironment might be crucial toward achieving better treatment responsiveness of probiotics for the skin conditions. Although the detection of the most beneficial microbiota combination in the clinical situations will necessitate a very wide-ranging human database, it is hypothesized that once the most beneficial microbiota combination of each clinical condition has been recognized, the patient's microbiota could be altered to develop a personalized microbial solution. The individuals' microbiota composition could be used as a biomarker, a diagnostic tool and possibly a therapeutic target due to its resilience, stability, and responsiveness to physiological, pathological, and environmental alterations. It is expected that targeted interventions on "gut-skin microbiome" by prebiotic or probiotic might be used as an effective healthcare solution for addressing various skin conditions. Application of appropriate oral probiotic strain along with prebiotics may aid in the management of different skin disorders. Hence, different clinical trials must be carried out in order to optimize the best formulation of efficient probiotic strain or strains, duration of the supplement or treatment to better exploit the probiotics' potential in dermatology.

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Mechanistic Role of Probiotics in Improving Skin Health

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Abstract

Skin is an ecosystem of massive microorganisms, and its dynamic interaction with this flora confers it with barrier characteristics on it. Today's stressors, such as pollution, dietary choices, and other immunological and hormonal changes, can still tip the scales in favour of disease, including infections. Steroids and/or antibiotics are generally used to treat the latter. However, the cost of therapy, the resulting side effects, and the prevalence of antibiotic resistance necessitate the use of less expensive and natural alternatives. Topical probiotic treatment can help control illness and regulate skin flora, according to clinical and experimental studies. The gut–skin axis, now a well-established fact, suggests that a healthy gut flora is important for a normal and healthy skin condition and that oral probiotic supplementation might help control inflammatory skin disorders like acne. Direct modification of skin flora by topical probiotic treatment, on the other hand, might have far-reaching consequences. They have a variety of functions, including the generation of antimicrobial compounds, the prevention of pathogen adherence, and the activation of the immune system, as well as the ability to colonise the skin surface. This chapter discusses various mechanisms through which probiotics elicit their beneficial effects along with their implications in various skin disorders. Delivery of these beneficial microorganisms to skin is highly challenging. Maintenance of probiotic viability during manufacturing and storage; their retention on skin for sufficiently long periods; and their germination on skin surface after application are the major formulation challenges which need redressal.

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Gut-skin axis · Homeostasis · Topical probiotics · Chronic skin diseases · Inflammation

2.1 Introduction

Skin, the largest and most complicated organ of our body, is unique in many ways. It forms a protective layer that not only holds everything in place but safeguard them from external stimuli like air, water, heat, pathogenic microorganisms, and other harmful substances. It also helps in haemostasis, immune-defence, perception, and production of many essential compounds including vitamin, collagen, ceramide, and mucin.

Furthermore, the skin is also considered to provide diverse habitats to a surfeit of microorganisms (bacteria, fungi, viruses, and arthropods) that forms the human skin microbiome (Rosenthal et al. 2011; Grice and Segre 2011). In any individual, the skin microbial composition is highly heterogeneous and depends on the local microenvironment of the specific skin site. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors, and exogenous environmental factors (Grice and Segre 2011). The normal microbial flora of the skin is relatively stable, with specific genera (Witting et al. 2015) that may aid the host, harm them, or exist as commensals. However, a delicate balance co-exists between the host (skin) and the microbial population. Disruptions in the balance on either side of the equation can result in skin disorders or infections (Chiller et al. 2001). Factors like pollution, stress, dietary habits, immunology, and hormonal alterations can tilt the balance towards diseases or infection of varying intensity.

2.2 Probiotics as Elixir for Skin Ailments

Skin forms a perceptual interface between the body and the environment. Recent research suggests that the skin and its microflora interact closely at immunological, biological, and physical levels. This ultimately controls the functions of the skin barrier (Amara and Shibl 2015). Augmenting skin barrier is important in various skin related ailments such as atopic dermatitis, dry skin, or aging. It has been already reported that the skin health is directly related with the gut homeostasis. Characteristics of gut microflora are primarily dependent upon the individual's diet variation. Modern lifestyle is causing deleterious impact on gut ecosystem and thus on gut microflora. This nonlinear change in gut microflora is one of the main reasons for increased susceptibility to the skin diseases (Singh et al. 2019).

Probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit to the host." Probiotics are emerging as potential candidates in the area of skin health. They are used to maintain the general skin

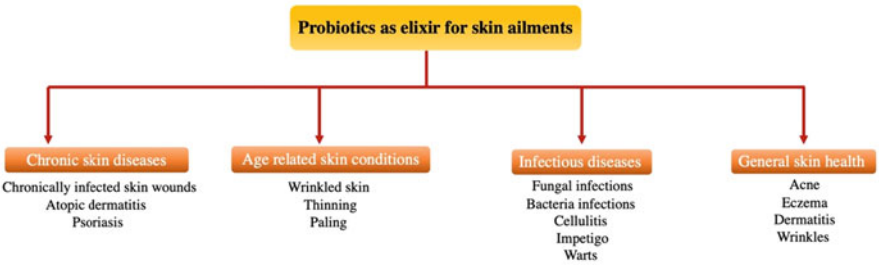

Fig. 2.1 Promising role played by probiotic in various skin ailments

health, for treatment of different skin diseases and for their antiaging benefits, and are highly efficient without causing any side effect to the patient (Roudsari et al. 2015). Figure 2.1 enlists the skin conditions in which probiotics offer promising role.

2.2.1 Chronic Skin Diseases

Chronic skin diseases are typically incurable or very hard to cure and influence the quality of patient's life. Patients with these ailments have lower levels of psychological and social well-being. Most discussed chronic skin diseases are chronically infected skin wounds, atopic dermatitis (AD), and psoriasis. These illnesses are accompanied by various physical and psychological restrictions in everyday life (Evers et al. 2008).

- Chronically infected skin wounds are substantial reason of morbidity and mortality as they show delayed wound healing. They majorly affect patients' quality of life and result in massive healthcare overheads. The main reason of delayed wound healing is attributed to ineffectiveness of antibiotics due to resistant infections. The Organisation for Economic Cooperation and Development stated the promising role of probiotics as alternative therapy for chronic skin wounds over antibiotic administration. Probiotics release bioactive molecules that inhibit pathogen growth and interfere with the pathogens' quorum-sensing system (Fijan et al. 2019).
- Atopic dermatitis (AD) is a chronic allergic skin disease often accompanied with other allergic diseases such as asthma and allergic rhinitis. The manifestation of this are dry, red, and pruritic skin in a typical distribution with frequent flares (Kapoor et al. 2008). Probiotics are reported as potential and efficient therapy for the treatment of AD. Probiotics result in the reduction of inflammation by suppressing the levels of INF-γ, IL-4, and Th17 cells in splenic CD4 T-cells and increasing the expression of IL-10 and T-regulated-related cytokines in mesenteric lymph nodes. Probiotics also inhibit the maturation of dendritic cells and thus inhibit naive T-cells from differentiating into Th2 cells, which are responsible for initiating the inflammation in the skin (Rather et al. 2016).

- Psoriasis is one of the major discussed chronic inflammatory skin diseases. It is associated with epidermal keratinocyte hyperplasia and epidermal immune cell over-activation. The severity of disease and modulation of inflammation are directly linked with the local skin and gut microflora (Hsu et al. 2020). Probiotics strengthen the barrier function of the epithelium and regulate innate and adaptive immune responses of the host. *Lactobacillus* in combination with biotin has been successfully used in the treatment of pustular psoriasis (Vijayashankar and Raghunath 2012).
- Rosacea is a chronic, relapsing inflammatory skin disease. It is associated with abnormal activation of immune responses, vascular dysfunction, and prominent permeability barrier alterations. In this condition, the skin barrier gets impaired, and symptoms improve when the skin barrier is strengthened. Probiotic are found to improve the skin barrier and affect skin hydration and transepidermal water loss. Probiotic increases the serum concentration of TGF-β and thus has potential to play a significant role in skin integrity (Kober and Bowe 2015).

2.2.2 Age-Related Skin Conditions

The most obvious indicator of ageing is the quality of skin. Skin undergoes several evident changes on ageing like thinning, paling, and more wrinkled skin. There may be appearance of dry skin patches, pigmented spots, bruising, and bleeding under the skin. There is general psychology prevalent in our society for appreciating the young and healthy-looking skin. There also exists a common assumption that older people do not bother about their skin appearance. But observed that skin condition is a significant issue for any age group. More appropriately it can be referred to as the "look-good, feel-good" factor (Cowdell 2010; Akazaki et al. 2002; Al-Nuaimi et al. 2014).

Reasons assigned to skin ageing are change in skin microflora, change in skin pH, altered stratum corneum lipid composition, oxidative stress, altered immune response, and collagen level reduction. Probiotics are reported to address all of these issues and can even modulate the systemic immune response by regulating the release of cytokines which may positively influence the skin homeostasis (Farage et al. 2010).

Certain probiotics are reported to increase ceramide level in stratum corneum of healthy subjects and in atopic dermatitis patients. Ceramide is chief water-holding molecule for horny layer of skin. Increased ceramide levels improve reduction in dryness, water loss, tone, and fullness of skin. This justified potential of probiotics in management of pathophysiological skin alterations, including ageing (Jensen et al. 2005).

Acne vulgaris is one of the most prevalent skin diseases majorly affecting teenagers. The main factors responsible for this ailment are hyperkeratinisation, blockade of sebaceous follicles, microbial colonization of pilosebaceous gland, and stimulation of sebaceous gland secretions by androgens. Different strains of *Lactobacillus* were reported to have potential for treating acne vulgaris. Probiotics

have been reported to directly obstruct *P. acnes* by producing antibacterial proteins (Kober and Bowe 2015).

2.2.3 Infectious Diseases

Any infection of skin which is caused by any microorganism is considered as a type of skin infectious disease. On the scale of severity, they can be benign to life-threatening based upon the type of pathogen involved. The type of pathogen, the layer of skin infected, and the medical condition of the patient forms the overall clinical representation of the disease. Some of the common but most prevalent infectious diseases are cellulitis, impetigo, warts and fungal infections. The selection of treatment is primarily dependent upon the offending pathogen. A variety of virulent pathogens are responsible for skin infectious diseases, but methicillin-resistant *Staphylococcus aureus* (MRSA) was reported as the prime pathogen (Dawson et al. 2012).

Several *Lactobacillus* and *Bifidobacterium* strains are reported to inhibit the in vitro growth of *S. aureus* and MRSA clinical isolates. The effects were reported to be mediated by secretion of acids or bacteriocin-like inhibitors and by direct cell competitive exclusion (Sikorska and Smoragiewicz 2013).

Even the exact mechanisms of probiotics in the field of infectious diseases are not clear, but there are so many reported research points. Probiotics produces lactases and antimicrobial compounds and thus antagonize pathogens; they also compete with pathogens for nutrients and growth factor and for pathogen binding and receptor sites as well. Some act by stimulating the immunomodulatory cells. The major point of attraction is that they are very less susceptible for developing resistance by microbial strains. So overall probiotics are promising candidates for the treatment of infectious skin diseases (Amara and Shibl 2015).

2.2.4 General Skin Health

Skin undergoes a variety of physical, chemical, and environmental stress every day. Some of the main factors affecting the skin on regular basis are pollution, UV radiation, hot and cold temperatures, humidity levels, psychological stress, and/or dietary deficiencies. These altogether affects the microbiota present over the skin and thus the quality of the skin. The skin becomes dull and less attractive in social terms. If these factors continue to affect the skin for a long term, several skin ailments like acne, eczema, dermatitis, wrinkles may appear. Once any chronic disease becomes prevalent over the skin layer, it is very hard to get rid of that particular condition. So it is always advised to take care of the skin health (Roudsari et al. 2015). It is already reported in many research reports that the skin status is directly related with the gut homeostasis. So probiotics becomes potential targets to maintain skin health as they are prime candidates for managing the gut homeostasis (Singh et al. 2019). Probiotics or microbial community is reported to have huge impact on overall

human health. Their consumption maintains the normal skin and natural gut microflora and also prevents the invasion or growth of pathogenic microflora (Amara and Shibl 2015). In diseased condition, the skin microbial ecology is changed. Probiotics helps in restoring the same and thus maintains a healthy skin (Bustamante et al. 2020).

2.3 Mechanism of Action

2.3.1 Indirect Effects Generated via Oral Administration (Gut-Skin Axis and Gut-Brain-Skin Axis)

2.3.1.1 Gut-Skin Cross-Talk (Alteration in Gut Bacteria Can Have Effects on Skin)

The gut dysbiosis has a crucial role in manifestation of a number of skin disorders, viz. psoriasis, atopic dermatitis, dandruff, rosacea, acne vulgaris, and skin cancers (Pessemier et al. 2021). The exact mechanism through which gut microbiome participates in skin homeostasis is not fully known and is reported to be related to the modulatory effect of gut commensal on systemic immunity (Salem et al. 2018).

The microbiome is a key regulator for the immune system, as it aims to maintain homeostasis by communicating with tissues and organs in a bidirectional manner. Hence, dysbiosis in the skin and/or gut microbiome is associated with an altered immune response, promoting the development of skin diseases, such as atopic dermatitis, psoriasis, acne vulgaris, dandruff, and even skin cancer. Here, we focus on the associations between the microbiome, diet, metabolites, and immune responses in skin pathologies. An enhanced understanding of the local skin and gut microbiome including the underlying mechanisms is necessary to shed light on the microbial involvement in human skin diseases and to develop new therapeutic approaches.

(a) *Competition for dietary ingredients (like growth substrates)*: Probiotic culture when consumed modulate the microbiome and/or change the metabolic attributes through competition for nutritive substrates. Gordon and colleagues performed transcriptional microarrays to elucidate changes in the mouse gut on consumption of probiotics (Sonnenburg et al. 2006). A shift in genetic expression of bacteroides was observed in germ-free mice on *Lactobacillus casei* administration. The probiotics compete for availability of substrate and alter carbohydrate usage pattern (O'Toole and Cooney 2008).

(b) *Bioconversion of sugars into fermentation products with inhibitory properties: L. plantarum* ferments sugars into sugar alcohol which possess an antibacterial action (Hedberg et al. 2008). The bioconversion of sugars through fermentation into sugar alcohols results in production of antibacterial products with inhibitory properties which aid resistance against pathogenic organisms.

(c) *Secretion of antimicrobial substances like bacteriocins with direct antagonistic effect on pathogens:* The host's skin health is influenced by the commensal microbiome which provides protection from an array of infectious organisms.

Bacteriocin produced by these commensal microbiota is synthesized by ribosomes and is stable to heat. They serve as antimicrobial proteins having broad as well as narrow inhibition of infectious bacteria (O'Sullivan et al. 2019). There is a close interconnection between microbiome and host's mucosal homeostasis. Probiotics introduce bacterial factors involved in killing of infectious agents which aid promotion of health. These low molecular weight agents include organic acids, namely, lactic and acetic acid, and antimicrobials like bacteriocin. Common examples of bacteriocins include lactacin B produced by *Lactobacillus acidophilus* and plantaricin produced by *Lactobacillus plantarum.* Organic acids and bacteriocin compounds have been shown to be successful in inhibiting gram-negative bacteria like *Helicobacter pylori* (Maldonado Galdeano et al. 2019). In immunocompromised mice models, a bacteriocin agent produced by *Bifidobacterium bifidum*, namely, bifidocin B, is reported to inhibit infection caused by *Salmonella typhimurium* (Matsumoto et al. 2008).

(d) *Competitive exclusion for binding sites:* Competitive exclusion against pathogenic bacteria can be achieved by directly or indirectly competing for nutrients. Surface ligands present in probiotics compete with pathogens for attachment sites. Furthermore, probiotics also enhance the host immunity, aiding in resistance against pathogenic organisms (Callaway et al. 2008). Colonisation by probiotics induces production of short-chain fatty acid molecules. Compounds situated on bacterial surface act as a ligand attaching to receptors of the host intestinal epithelium. This attachment transcends a cascade of signaling pathway (Monteagudo-Mera et al. 2019). *Lactobacillus rhamnosus* acts by competitive exclusion of *Enterococcus faecium* against vancomycin-resistant enterococci bacteria (Tytgat et al. 2016). Steric hindrance facilitating competitive exclusion of *Escherichia coli, Salmonella typhimurium*, and *Yersinia pseudotuberculosis by Lactobacillus acidophilus* (Coconnier et al. 1993). Heat-inactivated forms of *Lactobacillus reuteri* strains are successful in competing and hence displacing selected pathogens. However, this inhibition is strain dependent with the LR6 strain possessing the highest inhibiting property against *Listeria monocytogenes* and *Enterococcus faecalis* (Singh et al. 2017).

(e) *Colonize and adhere to the colon and reinforce the barrier function of the intestinal mucosa helping in the management of intestinal infection and food allergies:* The epithelial barrier is pertinent in resisting attacks by bacteria as well as preventing their dissemination into deeper tissues. The disrupted barrier leads to inflammation and loss of tolerance to microbiome. Probiotic consumption contributes to a healthy epithelial barrier by reducing the paracellular permeability. These probiotics provide a defensive recourse against infective organisms (Ohland and Macnaughton 2010). Transcription of mucin MUC3 induced by Lactobacillus enhances protection of epithelium which is a prime target for causative organisms (Mack et al. 2003). *Lactobacillus fermentum* ME-3 is a probiotic with dual function against microbes and oxidative stress. It can reduce gram-negative bacteria as well as Enterococci and *Staphylococcus aureus*. Capsules containing ME-3 were tested for their efficacy in both open

placebo-controlled and randomized double blind placebo-controlled clinical studies. Results showed benefits in maintaining the gut microbiome health (Mikelsaar and Zilmer 2009). Another study on HT-29 intestinal cell lines reported that *Lactobacillus plantarum* interact with dendritic cells of the small intestines and influence T cell response as well as translocation of NF-κB p65 in the epithelium. Administration of probiotic enhances barrier health by rearranging protein conformation of tight junctions towards toll-like receptors signalling pathways present in the intestines (Zhai et al. 2016). MUC2 mucin and cytokines influence the immune system of the gut wall. In another study *Lactobacillus acidophilus* A4 was reported to upregulate MUC2 mucin and cytokines and was successful in inhibition of attachment of *Escherichia coli* (Kim et al. 2008).

(f) *Reduction of inflammation enhances the nonspecific immunophagocytic activity of circulating blood granulocytes*: *Lactobacillus casei* alleviates inflammation of skin by influencing both protein-specific and hapten-specific $CD4^+$ T cells. A reduction in recruitment of $CD4^+$ T cells in the skin is observed during symptomatic phase of skin disorders. Probiotic treatment in hapten-sensitized mice enhances the presence of FoxP3+ T-reg and synthesis of IL-10 by $CD4^+CD25^+$ regulatory T cells in skin's lymph nodes (Hacini-Rachinel et al. 2009). *Bacteroides fragilis* polysaccharide A elicits an IL-10-mediated response in the T cells of the cut. Hereby, preventing the evolution of TH17 cells which curate derangement in the intestinal wall (Cristofori et al. 2021). *Lactobacillus reuteri* 100–23 promotes induction of anti-inflammatory cytokines, namely, IL-10. Pro-inflammatory cytokines are inhibited by soluble factors derived from *Lactobacillus reuteri.* Strain K12 of *Streptococcus salivarius* inhibits synthesis of pro-inflammatory cytokines IL-8 in the keratinocytes and epithelial cells by inhibiting the NK-kappa B pathway (Kober and Bowe 2015).

2.3.1.2 Barrier Recovery and Hydration of Compromised Skin

The mammalian epidermis is capable of renewing itself in times of disruption of homeostatis and on the infliction of any injury. The stratified epidermis does this by preserving cells that can undergo mitosis to become active. The genesis of this barrier originates in utero and continues re-establishing whenever desired (Segre 2006). In normal conditions, the infliction of wound stimulates cytokine release and growth of keratinocytes. T-Lymphocytes reachs the site of damaged skin. In contrast, when the skin is compromised, barrier recovery fails and the skin proceeds for inflammation. In absence of barrier recovery, the moisture homeostasis collapses leading to aggravation of disorder since allergens and chemicals further penetrate deeper into the skin. Furthermore, there has been an established genomic link for inflammatory disorders like psoriasis and atopic dermatitis on chromosomes which facilitate building and regulation of barrier. The genomic link necessitates maintenance of barrier function to fight against inflammatory disorders. Barrier function recovery can provide aid in various inflammatory disorders (Segre 2006). Another study by Horii et al. (2014) reported that oral administration of *Lactobacillus brevis* in rodents substantially reduces moisture content loss by enhancing serotonin

production via the enterochromaffin cell situated in the intestines. The serotonin reduces arterial sympathetic output and, on the other hand, enhances the cutaneous blood flow (Horii et al. 2014). Administering probiotics topically or orally can facilitate barrier recovery.

2.3.1.3 Regulating Skin Homeostasis

Skin homeostasis influences inflammatory response and immunogenic cell components. The skin flora enjoys a pertinent role in maintaining skin homeostasis. However, this homeostasis varies frequently and depends on a wide array of elements namely diet, exercise, drugs, surgery, mental health, climatic conditions, and physical well-being. The dominating species of every region in the skin is unique to its location. Species that are influenced by moisture include *Staphylococcus* and Corynebacterium. On the contrary, Propionibacterium grows in sebaceous conditions. Probiotics also aid in modulating the systemic immunological response which involves the stimulation of cytokine release impacting the regulation of skin homeostasis. Commensal bacteria residing on the skin protect *Staphylococcus aureus* by increased production of antimicrobial peptides. Additionally, for atopic dermatitis, these bacteria enhance resident skin ceramides, improving erythemas and scaling of the skin, and, hence, decrease the pathogenic bacteria and sustain skin homeostasis (Knackstedt et al. 2020). Our skin is constantly exposed to reactive oxygen species sourced from environmental factors mainly UV radiation and other metabolic processes. These species are potential carcinogenic substances, and hence, the availability of antioxidant formulations would curb the imbalance and restore skin homeostasis. *Cutibacterium acnes* releases a protein called RoxP which has antioxidant potential. It has also been shown to furnish human skin colonised bacteria (Andersson et al. 2019). Besides this, probiotics also improves systemic immune response by modifying the release of cytokines which maintains the skin homeostasis (Farage et al. 2010).

2.3.1.4 Reducing Skin Inflammation

Skin inflammation is manifested by substance P acting as the key mediator for the enhanced inflammatory response and excess sebum released. Gueniche et al. reported that *Lactobacillus paracasei* can modulate the immunological homeostasis of the skin. They observed a reduction in levels of substance-P along with enhanced dilation of vessels and degranulation of mast cells. All these factors contributed to the establishment of skin homeostasis (Gueniche et al. 2010). Another group evaluated the immunogenic response of *Lactobacillus casei* in dermatitis. A downregulation of skin inflammatory response characterised by a reduction in $CD8^+$ effector T cells was seen hence causing a reduction in the severity of the disease (Chapat et al. 2004). In acne, the peroxidation of lipids is extremely high, varying with location. This creates the need for antioxidants to establish homeostasis. Oral probiotics reduce oxidation-derived stress. This mechanism regulates the production of inflammation-mediating molecules, namely, cytokines and interleukin alpha (Bowe and Logan 2011). Another organism *Streptococcus salivarius* is present as the normal constituent of oral microbiome. It has been observed to release a

bacteriocin-like substance possessing an inhibitory action against *Staphylococcus epidermidis, Propionibacterium acnes,* and *Staphylococcus aureus* which are responsible for skin disorders. Bacteriocin-like substance is capable of directly inhibiting inflammatory stress and hence can prove to be an effective antimicrobial therapeutic (Oh et al. 2006).

2.3.1.5 Ameliorating Hair Growth

On feeding yogurt or purified bacteria to female mice, improvements in hair health were observed in a study where beneficial effect is attributed to consumption of naturally present bacteria in yogurt, namely, *Lactobacillus reuteri* (Levkovich et al. 2013). Another in vivo study in mice utilising *Lactobacillus reuteri* suggested that the display of healthier skin possessing constant folliculogenesis and enhanced hair growth was due to the heat-stable, non-protein components derived from the bacteria. These components were successful in inhibition of TNF-α responsible for inflammatory-mediating hindered hair growth (Lee et al. 2016).

2.3.1.6 Reduced Peripheral Tissue Response to Stress

Administration of probiotics is reported to reduce stress implicated induction of neurological skin inflammation, thus ameliorating the peripheral tissue responses. The scientific evidence suggests immunogenic modulation at the local as well as systemic levels. Microbes and their metabolites interfere in the neurological endocrine pathways which influence stress-mediated responses in the skin. Administration of *Lactobacillus helveticus* to rodents supported the proposed probiotic health benefit and resulted in lowering the levels of corticosterone and adenocorticotropic hormone which causes tissue damages under stress (Sarkar et al. 2016). In a mice model, *Lactobacillus reuteri* was seen to reduce stress-induced inflammation (Mu et al. 2018). Gut lactobacilli were shown to stimulate the synthesis of neurologically active compounds, namely, catecholamines and gamma-aminobutyric acid. Reduction in cortisol levels was observed in the urine of humans and rats on the administration of formulations consisting of *Lactobacillus helveticus* and *Bifidobacterium longum*. As a result, a reduction in the stress response was also consequently observed (Lukic et al. 2017).

2.3.2 Direct Effects on Topical Application

2.3.2.1 Pathogen Inhibition by Competitive Exclusion

Greenberg was the first to use the term "competitive exclusion" (Greenberg 1969). The basic concept is that this is the situation where two or more than two microbial species compete for the same target. The one with highest efficiency will bind to the receptor site excluding others and thus will express itself by silencing others. The exclusion mechanisms are different for different species. Some of the mechanisms are elimination of available bacterial receptor sites, creation of a hostile mircoecology, competitive depletion of essential nutrients, and secretion and production of selective metabolites or antimicrobial substances (Mead 2000).

Bifidobacteria and Lactobacilli are reported to hinder a broad range of pathogens, including *E. coli*, *Salmonella*, *Helicobacter pylori*, and *Rotavirus* (Bermudez-Brito et al. 2012). Probiotics inhibit pathogen adhesion by stimulating the intestinal epithelial cells. The intestinal bacteria show a complicated bacterium-to-bacterium interaction. This interaction mediates the competition for present nutrients and for the mucosal adhesion sites as well. For gaining additional competitive advantage, the probiotic microbes can also modify their environment for creating a hostile mircoecology for other competitive pathogens. Production and secretion of antimicrobial substances like lactic and acetic acid can be considered as an example of environmental modification (Schiffrin and Blum 2002). Few Lactobacilli and Bifidobacteria represents carbohydrate-binding specificities with certain enteropathogens. This specificity makes them competitive with specific pathogens for the receptor binding sites present on the host cell (Mukai et al. 2002). Probiotic strains are reported to develop the steric hindrance at enterocyte pathogen receptors and thus obstructing the attachment of pathogenic bacteria (Bernet et al. 1994).

2.3.2.2 Production of Bacteriocins as Antimicrobials

Bacteriocins are the antimicrobial substances produced by probiotics. These are one of the most discussed elements responsible for the various activities of the probiotics (Bermudez-Brito et al. 2012). Some of the bacteriocins produced by gram-positive bacteria are plantaricin from *L. plantarum*, lactacin B from *L. acidophilus*, and nisin from *Lactococcus lactis*. Lactic acid bacteria produce bacteriocins, a form of antibacterial peptide. These bacteriocins are generally reported to possess a narrow window of activity and that too for closely related bacteria. On the other hand, some are highly active against foodborne pathogens (Nielsen et al. 2010). Bifidocin B, a unique bacteriocin produced by *B. bifidum*. NCFB 1454, is reported to active towards gram-positive bacteria (Yildirim et al. 1999). Generally bacteriocin either inhibits the cell wall synthesis or forms the pore in the cell wall of the target cell and thus mediates the killing of the pathogen cell (Hassan et al. 2012). For example, nisin targets the lipid II, the ultimate cell wall precursor. Lipid II is inhibited by complex formation, and hence cell wall biosynthesis is inhibited. Consequently, the formed complex aggregates and integrates target cell wall peptides to form pores in the membrane (Bierbaum and Sahl 2009). Strains able to produce bacteriocins are reported to be competitively efficient with the complex microbial environment. This can be considered as a result of their concomitant antimicrobial activity (O'Shea et al. 2012).

2.3.2.3 Maintenance of Skin Hydration and Elasticity

Maintaining an adequate skin hydration is one of the prime requirement of a healthy skin. Skin hydration is directly related to the moisture value of skin, which is a basic component of skin care. Stratum corneum is the prime layer which holds water and also acts as a barrier to water loss (Verdier-Sevrain and Bonte 2007). The barrier contains a "brick and mortar" structure characterised by keratin, filaggrin, and cornified envelope coverings. Additionally, lipids are present which act as a seal for the envelope. Various protein molecules, namely, loricrin and filaggrin help in

facilitating the barrier by making multiple junction types including tight, adherens, and gap junctions (Jung et al. 2019). *Bifidobacterium longum* is a nonreplicating bacterium that was utilised for human studies. *Bifidobacterium longum reuteri* lysate active extract was seen to provide relief from sensitive skin and enhance the resistance of the skin for physical and chemical aggression (Arck et al. 2010). In a clinical study, done on 20 healthy Caucasian female subjects to study the influence of ceramide levels and skin aging, it was observed that treating the skin with the preparation of *Streptococcus thermophilus* enhanced sphingomyelinase enzyme activity leading to increased ceramide in the corneum layer. This led to an overall improvement in barrier recovery and transdermal moisture retention (Di Marzio et al. 2008). Skin elasticity is the property of the skin which maintains the appearance of the skin. The more the skin elasticity, younger the skin will look. Decreased skin elasticity is considered to be directly related with the wrinkle formation. There are several reports justifying the role of skin elasticity with aging and wrinkles (Fujimura et al. 2007). Probiotics are reported to increase the skin hydration level. Fermented milk or probiotics were reported to interfere with the normal moisture level of the stratum corneum and were found to increase the hydration level of it (Nakamura et al. 2016). There are several reasons associated with the skin elasticity. Major being change in skin microflora, altered stratum corneum lipid composition, and collagen level reduction. A study utilising the lysates of *Lactobacillus rhamnosus* on a human epidermal reconstructed model called as Keraskin™ observed that the use of probiotics improves the expression of junction protein molecules, namely, occludin and claudin and proteins aiding skin barrier recovery and hydration, namely, filaggrin and loricrin (Jung et al. 2019).

2.3.2.4 Remodelling of Epidermal and Dermal Tissues

The epidermal and dermal tissues are the most affected tissue during any topical skin ailment mainly in case of wound. Any damage to these layers may result into the formation of a wound and scar. Remodelling of these tissues is obviously the prime concern for good skin health and appearance. Probiotics are reported to be beneficial in wound healing due to their action on epidermis and dermis layers. Probiotics are reported to mediate the production and release of beta-defensins and thus enhance the immunity of skin. In this way, they act as signalling receptors against pathogens (Fijan et al. 2019). *Lactobacillus rhamnosus* GG is reported to exhibit mitogenic effects and also increase the mucosal regeneration (Caballero-Franco et al. 2007). Hyaluronic acid is the matrix forming element of the skin, responsible for maintain the normal structure and epidermal barrier functions. It influences cell proliferation, differentiation, and thus tissue repair. Various microbial probiotics are able to produce hyaluronic acid by their own and can even enhance its level in skin and helps in tissue remodelling (Lew et al. 2013).

2.3.2.5 Generation of Ceramide and Mucin

Ceramides are chief water-holding molecule present in the intercellular spaces of stratum corneum. These are the major lipid constituent and thought to maintain the barrier property of the epidermis. These play essential role in maintaining the water

permeability through the skin as well (Coderch et al. 2003). Any interference in the ceramide level can result into various skin ailments like atopic dermatitis (Jensen et al. 2005).

Mucus layer represent the first line of defence for any epithelial layer. Mucin is the main constituent produced and secreted through this layer. Mucins can be defined as large complex glycoproteins. They limit the access of environmental elements to the epithelial cells. Any alteration in the mucin concentration can cause inflammation. They are involved in both barrier and repair functions (Caballero-Franco et al. 2007; Gaudier et al. 2005). Probiotics are reported to enhance the ceramide level on stratum corneum in both the healthy subjects and in atopic dermatitis patients. Increased amount of ceramide is responsible to reduce dryness, water loss, skin tone loss, and fullness (Jensen et al. 2005). Numerous probiotics are reported for their activity to enhance mucin level and thus able to positively treat various inflammation related ailments. Probiotics are also reported to modulate the thickness of mucus layer by modulating the mucin gene (Caballero-Franco et al. 2007).

2.3.2.6 Maintenance of Healthy Skin pH

The pH of skin plays a very crucial role in maintenance of its healthy condition including structure (stratum corneum integrity and permeability) and functions (homeostasis) (Feingold 2007). The slightly acidic pH (5.5–6.5) of skin is responsible for neutralizing the alkaline-based products (harsh surfactants), inhibits growth of pathogens, and provides an optimal environment for natural skin flora. Latter aids in the prevention of pathogens colonization, maintenance of appropriate moisture levels, and regulation of normal enzyme activities (Mauro 2006). Any kind of imbalance in the skin pH, acidic or alkaline, results in compromised barrier functions. The synthesis of essential skin lipids is impaired on elevated pH leading to excessive water loss and hence dry skin.

Similarly, increase in stratum corneum pH is also associated with some cutaneous disorders, viz. atopic dermatitis, seborrheic dermatitis, and acute eczema (Roudsari et al. 2015). A compromised skin also looks less resilient, becomes sensitive/hypersensitive to environment triggers, and is more susceptible to several skin infections. Moreover, at molecular levels, the alterations in aging skin include an increase in skin pH along with reduced ability to quench reactive oxygen species (Cinque et al. 2010) and triggered protease activity (Hachem et al. 2003).

Several studies and research experts have suggested that probiotics and their components including metabolites, lysates and bioactives might alter above-listed aspects including skin aging. Apart from bacteriocins, probiotics also produce other acidic molecules like free fatty acid, lipoteichoic acid, and conjugated linoleic acid as a metabolic end product. Moreover, use of probiotics with lactose, glucose, and other sugars result in fermentative metabolism that produce lactic acid via homofermentative metabolism (Roudsari et al. 2015). Acids released by probiotics are responsible for maintaining the weakly acidic environment of the skin. Hence probiotics are reported to restore the normal skin pH and protease activity levels as in case of a younger and healthier skin (Kober and Bowe 2015).

2.3.2.7 Effect of Probiotic Metabolites, Byproducts, and Structural Components

The effects of live probiotics on oral and topical administrations are well established and documented. However, several research groups and marketing companies have also suggested the use of other probiotic components including its metabolites and extracellular components. Latter include acids (hyaluronic, acetic, and lactic), peptidoglycan, diacetyl, sphingomyelinase, and lipoteichoic acid. There are evidences suggesting that such components could enhance skin homeostasis, barrier, and immune systems (Lew et al. 2013).

An extracellular matrix protein, hyaluronic acid (HA) majorly present in skin, is well-known to provide constructive support to epidermal cell and is responsible to bind and retain water molecules in stromal cells (Papakonstantinou et al. 2012). HA is also found to be involved in angiogenesis, tissue repair, and migration of fibroblasts. Levels of HA in epidermal cells are found to decrease drastically with ageing. Hence, HA is a vital component required to maintain of skin in healthy condition. Several strains of bacteria including *Lactobacillus* and *Bifidiobacterium* have shown to induce production of HA (Ouwehand et al. 2017). *Streptococcus thermophilus* YIT2084, *Pasteurella multocida, Streptococcus zooepidemicus*, *Bacillus subtilis*168, *Streptococcus equisimilis*, *Lactococcus lactis* LL-NAB, *Bifidobacterium longum* BL 8643, *B. longum* BB 8843, *B. bifidum* BB 12, *Lactobacillus rhamnosus* FTDC 8313, and *Lactobacillus rhamnosus* FTDC 8131 are some strains that are reported to produce hyaluronic acids both extracellularly and intracellularly (Lew et al., 2013). Commercially HA is produced from attenuated group C *Streptococcus* known to be comparatively more pure than obtained from animal sources (Lew and Liong 2013). Similarly, sphingomyelinase, an enzyme present in epidermal lamellar bodies and intrices of stratum corneum, is responsible for production of ceramides and phosphorylcholine and hence maintains skin barrier functions. Besides maintaining skin hydration levels, ceramide sphingolipids also demonstrate antimicrobial effects against *Propionibacterium acnes* (Kober and Bowe 2015). Few strains of lactobacillus, i.e. *L. casei* BT 1268, *L. rhamnosus* FTDC 8313, and *L. gasseri* FTDC 8131, and bifidiobacterium, i.e. *B. animalis* subsp. lactis BB 12, *B. longum* BL 8643 (Lew et al. 2013), are known to alleviate skin barrier functions by production of shingomylinase. Increased levels of ceramide are also reported in human keratinocytes (Di Marzio et al. 1999) and in healthy Caucasian volunteers on treatment with *Streptococcus thermophilus*.

Peptidoglycan (PG), a vital component abundantly found in cell wall of gram-positive bacteria, is responsible for shielding skin against pathogens by stimulation of innate immunity via activation of Toll-like receptor-2 (TLR2) (Niebuhr et al. 2010), nuclear factor kB (Sorensen et al. 2005), hBD, antimicrobial peptide (cathelicidin LL37) (Ruiz-Gonzalez et al. 2009) and IL-8 production (Matsubara et al. 2004). PG is also known to initiate the recruitment of killer cells at the site of infection mediated by PG recognition molecules, viz. CD14, PG recognition proteins (PGRPs), lysozyme, and amidase (Lew and Liong 2013).

Similarly, lipoteichoic acid (LTA), a major immunostimulatory principle of pathogenic and non-pathogenic gram-positive bacteria, can be easily located in

their cell walls. Aulock et al. found this cellular component are responsible for release of the chemoattractants, viz. LTB_4, IL-8, complement factor 5a (C5a), macrophage chemoattractant protein-1 (MCP-1), and the colony-stimulating factor G-CSF in human system. LTA was established to be a strong stimulant for relocation of phagocytes to the infected site (Aulock et al. 2003). *Lactobacillus plantarum* K8 was found to exert anti-inflammatory, wound healing, and anti-pathogenic effects on keratinocytes via inhibition of interferon-gamma (IFN-γ) or tumor necrosis factor-alpha (TNF-α) through LTA (Brandi et al. 2020).

Lactic (LA) and acetic acid (AA) are primary products of bacterial fermentation. Lactic acid-producing bacteria generate lactic acid by either homo- or heterofermentation of carbohydrates (Lew and Liong 2013). Way back in 1996, Walter established that treatment with 12% lactic acid can increase skin (epidermal and dermal) firmness and thickness. They also confirmed minimisation of fine lines and wrinkles, giving a smooth appearance to skin (Smith 1996). This α-hydroxy acids (AHA) is popularly used in cosmetics and other topical preparations for ameliorating photoageing, hyperpigmentation, acne, dry skin, and ichthyosis. It is widely used as an exfoliator and peeling agent due to its skin desquamation effects which results in a younger looking skin (Lew and Liong 2013). Both LA and AA are also known to have wound-healing effects. The latter acts as a potential antibacterial agents having effects against various pathogenic species including *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Hansson and Faergemann 1995).

2.3.2.8 Enhancement in Immune Function and Regulation of Production of Inflammatory Cytokines

Probiotics are reported to enhance the immune function. Several mechanisms can be thought to be related with this action, but the most prominent mechanism came out to be the regulation of the inflammatory cytokines (Medina et al. 2007). A significant reduction in the level of the proinflammatory cytokines in plasma and lymphocyte of the patients with ulcerative colitis was reported after the administration of *Lactobacillus paracasei* (Federico et al. 2009). Reduction in the levels of proinflammatory cytokines of mucosa and restoration of barrier integrity and colonic physiology was reported when the experimental animals were fed with probiotics (Madsen et al. 2001). *Lactobacillus casei* was reported to augment total and pathogen-specific secretory IgA levels in infected mice (Maldonado Galdeano and Perdigón 2006). *Lactobacillus casei* was found responsible for downregulating the transcription of genes encoding for inflammatory cytokines resulting in an anti-inflammatory effect (Tien et al. 2006). Probiotics can modulate immune system depending upon their species or even on the strain of same species. Probiotics have the potential to regulate the immune responses in different directions, which makes them an ideal product for different formulations (Medina et al. 2007).

2.3.2.9 Cross-Talk Between Microbiota and the Skin Immune System

Skin microbiome is successful in induction of protection through immune system. Gut bacteria are effective in controlling the homeostasis between T effector and regulatory T-cells. The characteristic mechanisms of innate and adaptive immunity

regulation by the skin microbiota are strain-dependent. *Staphylococcus epidermidis* induces $CD8^+$ T cells homing primed by $CD103^+$dendritic cells into epidermal cells which promotes skin's antimicrobial responses through IL-17. Tissue repair is promoted by $CD8^+$ T cell response specific to *Staphylococcus epidermidis.* This response is restricted to non-classical major histocompatibility complex class I molecules. Additionally, the cell wall components of S. epidermidis supress skin inflammation by inhibiting release of inflammatory cytokines which also aids in healing of wounds. Colonization with skin microbiota is especially crucial in the neonatal period for establishment of immune tolerance by accumulating active T regulatory cells (Zheng et al. 2020). Skin disorders like psoriasis and atopic dermatitis involve Th17 induced inflammation. Continuous exposure of microbiome strengthens inflammation's Th17/IL-23 axis and regulatory pathways (Sherwani et al. 2018). Nakatsuji et al. showed the implications of commensal skin bacteria in reduction of inflammation during wound healing by regulation of TLR3-dependent inflammation, displaying how microflora can modulate particular cutaneous inflammation response (Lai et al. 2009).

2.4 Conclusion and Future Perspective

According to a study, the prevalence of skin and subcutaneous disease increased by 46.8% between the periods 1990–2017 and is the fourth leading cause of disability, excluding mortality (Giesey et al. 2021). The cost and psychological burden associated with skin diseases is high and has significant personal and social implications. Currently existing treatment options include antimicrobials, retinoids, steroids, antifungals, and herbals. Continued treatment with several of these agents can induce resistance making the treatment ineffective and costly. Some of these, viz. antimicrobials including antifungals and steroids, lack specificity and hence show poor treatment outcomes. Further, they tend to alter the commensal microflora leading to adverse effects. Therefore, the expansion of probiotic-microbiome-targeted interventions, and an evolving landscape for implementation across consumer spheres, portends significant scope.

Use of oral probiotics for gut and dermal health is well established. Upon ingestion, probiotics exert beneficial dermal effects via gut-skin cross talk involving mechanisms, viz. competitive inhibition, secretion of antimicrobial substances, bioconversion of sugars, stimulation of immune response, and generation of biohealers. Existence of a gut-skin axis (Bowe and Logan 2011) indicates that altering the gut flora, which is quintessential for maintaining the normal and healthy skin state, may help in the management of various skin ailments including infections. The inflammatory skin diseases like acne can be suitably manipulated by oral administration of probiotics (Kober and Bowe 2015); however this pathway is majorly immune system mediated. Thus it may have a limited scope for the control of infections, while a direct manipulation/modification of skin flora to restore its robustness may be a more favourable option. The transiently administered oral probiotic may not survive adverse conditions of the gut, and also fail to establish

themselves in the gut for long-term effects. Topical route is the most suitable and easily acceptable route of application for any medication (Witting et al. 2015). However, evaluation of effects of probiotics on skin following direct application to local area has been undertaken only recently. Topical probiotics containing preparations are now gaining popularity due to a multitude of physiological effects on skin without any adverse effects. Consumer awareness towards the natural, organic, and environment friendly alternatives is the reason for their popularity.

However delivery of these beneficial microorganisms to skin is challenged by formulation aspects including (1) maintenance of viability during manufacture and storage; (2) retention on skin for sufficiently long periods to elicit effect; and (3) their germination on skin surface after application. As an alternative to these formulation challenges, several research groups and manufacturing companies are incorporating probiotic ferments, lysates, byproducts, and structural components instead which may not harness all the effects of live probiotic cells. Looking at the potential of these microorganisms and dearth of organized research and resulting marketed products, there is a lot desired to be done and achieved.

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3 Skin Microbiome and Host Immunity: Applications in Regenerative Cosmetics and Transdermal Drug Delivery

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Abstract

Recent advances in our understanding of the function of the skin and its microbiome have shown that there is a strong symbiotic relationship between the microbiota of the skin and its host immune functions. The dysbiosis or imbalance of the microbiome and other factors that have an influence on the surface microbiota can influence keratinocyte regulation and homeostasis as well as the skin barrier function. In this perspective paper, we review the evidence that connects the skin's microbiome and the barrier function of the epidermis and explore the future potential for applying this unique dialogue in developing innovative cosmetics and transdermal drugs for well-being and beauty. Lay abstract: The microbiome on the skin has a unique dialogue with the host through the host immune system. This dialogue makes the basis of several host immune responses and helps shape the host immunity. In this article, we explore this microbiome and host interaction and see how this can influence our understanding of skin barrier function and future applications toward transdermal delivery of topicals.

Keywords

Barrier function · Cosmetics · Skin · Surface microbiome · Transdermal drugs

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3.1 Introduction

The primary role of the skin is to serve as a physical barrier, protecting our bodies from potential assault by foreign organisms or toxic substances. The skin is also an interface with the outside environment and, as such, is colonized by a diverse collection of microorganisms—including bacteria, fungi, and viruses (Grice and Segre 2011). Metagenomic sequencing of the microbiome on many human body sites gives insight into how the microbial biodiversity of the skin is influenced by several factors including environment, ecology, host immunity, genetic predisposition, and host lifestyle (Cogen et al. 2008; Zeeuwen et al. 2012). Skin injury disrupts the homeostasis of the host tissue and its commensal microbiota. We look at the literature-based evidence that shows a connection in how the dynamic state of the microbiome can influence keratinocyte function and vice versa (Cogen et al. 2008; Zeeuwen et al. 2012). This perspective aims to explore the influence of the host–microbiome relationship on skin protective functions and barrier stability.

3.2 Invasion of Cutaneous Pathogen and Response of Skin Ecosystem

Skin microorganisms extend from the surface of the skin to the deeper layers. Almost 25% grow in the deeper layer of the dermis and sebaceous glands (Lange-Asschenfeldt et al. 2011). These microorganisms are classified as resident and transient (Grice et al. 2009). The resident bacteria transmitted during birth from the mother or acquired from contact with daily life surroundings (animals, plants, persons, chemicals and climates) are long-lasting. On the other hand, the exposition to new settings (e.g., changes in the environment due to traveling) leads to the development of new transient microorganisms groups. However, these transient groups are eradicated once back to usual conditions and surroundings. Therefore, each individual has a unique and specific signature of skin microbiota encountered during infancy and stabilized during adulthood (Cho and Blaser 2012). Several papers have documented connections on the microbiome and the host systemic immune system (Belkaid and Tamoutounour 2016).

A tight relationship within this symbiosis regulates pathogen recognition, barrier function, host immune response, and evolution of skin diseases like atopic dermatitis, acne, and psoriasis (Belkaid and Tamoutounour 2016; Belkaid and Segre 2014). Species of Staphylococci are common bacterial colonizers of human skin and, hence, have been studied extensively to see the correlation and influence of their dysbiosis and the impact of this dysbiosis on skin functions. *Staphylococcus epidermidis*, in particular, is the most frequent microorganism isolated from human epithelia and is an essential member of skin resident microflora (Otto 2009). Staphylococcus epidermidis has an adaptable relationship with its host, and it has the ability to form biofilms that are extremely hard to clear, due in part to the difficulty in bypassing the extracellular matrix and also to the development of antibiotic resistance and immune resistance (Thien-Fah and Toole 2001). This matrix acts as a

physical barrier restricting many antibiotics and chemical diffusion and as a mechanical barrier restraining immune cell passage. *Staphylococcus aureus* is another important and prevalent member of the Staph family of resident microbes and is studied extensively due to its correlation to multiple cutaneous conditions. It is an excellent model of bacteria, being part of a semi-resident flora but able to switch to a pathogen as soon as it is left uncontrolled by other members of resident flora (Spaan et al. 2013).

The specific interaction of this particular microorganism with the host's systemic immune system gives it the ability to attain specific virulence genes easily. *Staphylococcus aureus* produces δ toxins triggering local allergic cutaneous responses which may also prevent wound healing and cause epithelial barrier deterioration (Nakamura et al. 2013; Feuerstein et al. 2016). More recently, there has been increasing awareness of the importance of fungi and their interactions with the immune system influencing the immune homeostasis and inducing disease. When the chemical composition (pH, pathological sweat secretion) of host epidermis is disrupted, *Malassezia* spp. gain in pathogenicity and release lipases, phospholipases, and an array of bioactive indoles. These molecules alter the function of the epithelial barrier resulting in immune deregulation and diseases (Xu et al. 2007). The ability of the innate immune cells, macrophages, dendritic cells, and natural killer cells to communicate with epithelial cells, leading to an effective immune response, is a key feature of the cutaneous immune system (Bangert et al. 2011). It is of great importance to understand how the cellular and structural composition of the skin dictates the hierarchy of the skin immune response. The epidermis is separated from the dermis by the dermo-epidermal junction and from the external environment by the stratum corneum. The latter represents a true barrier of protection. It is composed of cells made up mainly of proteins called corneocytes, whose intercellular space is highly constituted of lipids. The dynamic interaction between all these cells coordinates the immune response.

The advances in metagenomic data analysis with the 16s ribosome compared with regular cultural techniques have helped understand the dysbiosis on the surface of the skin and specifically have been helpful in understanding this dynamic in certain skin conditions such as atopic dermatitis (Abdallah et al. 2017). The skin is a primary immunological barrier to the external environment and has the following interactions. The uppermost "corneal layer" is composed of dead keratinocytes that provide a physical barrier. However, the pathogens can directly access the interior of the host through skin wounds and by outcompeting the normal flora. Toll-like receptor-bearing cells, keratinocytes, and Langerhan's cells recognize pathogens and establish a highly coordinated immune response, which includes antimicrobial production to neutralize the pathogen; inflammatory mediator secretion to alert the immune cells; activation of innate immune cells such as natural killer cells to induce cell lysis; and/or phagocytosis such as macrophages to engulf pathogens.

In the adaptive immune pathway, the immature dendritic cells play a role. The mature dermal dendritic cells migrate into draining lymph nodes to prime T-cell responses to create antigen-specific antibodies through the clonal proliferation of T-

and B-cell lymphocytes in the lymph nodes (Feuerstein et al. 2016). When the innate immunity and signaling are insufficient to clear a pathogen and to resolve pathogen invasion, the adaptive immune system becomes involved. The coordination between innate and adaptive immunity is assured by dermal dendritic cells, which are professional antigen-presenting cells known as immune system gatekeepers (Haniffa et al. 2015). Exploring the skin–gut and skin–brain axis through the microbiome's interactions with the host immune system and defining the deep and intricate connection which the microbiome has with its host lead one to explore the effects of the microbiome on the host's cutaneous and systemic functions—in particular, on those functions influenced by the immune system. A correlation within the skin–gut axis is evident, as shown by connections between psoriasis and Crohn's disease, for example. The crucial interface organs gut and skin have much in common with regard to commensal bacteria. The communication and symbiotic balance of these with microbe-heavy sites is intricate (O'Neill et al. 2016).

Gut–skin dysbiosis in many related skin and gut conditions can therefore theoretically be put forward as an explanation for pathophysiology that leads to disruption of barrier functions in the respective organs, so leading to permeability and inflamed states (Craig 2016). Much exploration is needed in the field to understand the immunological crosstalk between the skin and gut microbiomes in healthy and diseased states. The skin–brain axis is also an interesting hypothesis that has been recently examined by connecting post-traumatic stress disorder to skin conditions such as atopy, and exploration is underway to understand how emotional states can affect the skin microbiome and vice versa (Gupta et al. 2017). Skin cells manufacture and metabolize steroid hormones, peptide neurohormones, and neurotransmitters. Some of these are disseminated further by sweat and sebum (Paus 2016). On making contact with cutaneous microbes, they can influence virulence, growth, and adhesion. For example, experimental studies have suggested a relationship between psychological stress-induced increases in local substance P (linked to eczema, acne, and barrier dysfunction (Zhan et al. 2017; Lee et al. 2008)) production (Pavlovic et al. 2008) and changes in skin microbiota (Mijouin et al. 2013; N'Diaye et al. 2016). However, some paradigm-shifting studies have provided a different perspective. Here, pathology is not entirely mediated in a unidirectional manner from brain to skin.

Recent research has placed epidermal keratinocytes at the forefront of sensory systems, showing that they influence whole-body states and even emotions by generating a variety of hormones and neurotransmitters (Denda et al. 2013). This includes the capacity for glucocorticoid production via elements of the local hypothalamic–pituitary–adrenal axis—acting as an independent steroidogenic organ, in addition to the sensors of mechanical stress, temperature, and chemical stimuli (Negi et al. 2012). Cutaneous cortisol production is stimulated by skin stressors (e.g., dryness and barrier disruption); it is possible that this action occurs through activation of inflammatory cytokines such as IL-1β and has systemic implications (Prescott et al. 2017). Conclusion and future perspective: The cosmetic microbiome and regenerative therapies for the skin: We have tapped into a wealth of information on the surface of our skin that can help identify and channel a deeper

understanding of skin structure and regulatory functions. This science now serves as a platform for various diverse applications in understanding the host immune system in different diseased states (Nakatsuji et al. 2013). The future of skin microbiome research will be heavily influenced by a more clear understanding of the resident commensal microbes as a facilitating tool to connect external factors to the host. A paper published by Nakatsuji et al. shows the presence of active bacterial components through 16s ribosomal and pyrosequencing and in the deeper layers of the skin like the dermis and subcutaneous areas. This raises the possibility of surface microbes interacting with the deeper microbial components, deeper immune cells, and pathways by a complex dialogue.

A better understanding of this dialogue will then help us to design cosmetics that can be applied topically but without needing penetration and that can have deeper effects in the dermal tissue and immune functions of the host skin and body. This study also confirms human skin actively regulates bacterial entry or penetration into dermis through the skin barrier function and antimicrobial peptide (AMP) secreted by keratinocytes, and these are perhaps key regulators to maintain dermal microbiome homeostasis (Nakatsuji et al. 2013). AMPs are gene-encoded peptides that comprise a highly conserved component of the innate immune system and contribute to direct microbial destruction and tissue repair pathways. A study by Plichta et al. examined the impact of burn injury on the epidermal barrier and AMP production at the donor graft site. They concluded that graft rejection can be a result of the impairment of the AMP regulation and barrier permeability. More studies are needed to clarify the influence of microbes on AMP regulation and their impact on improving graft uptake (Plichta et al. 2017).

Certain bacteria in the *Propionibacterium* species are capable of producing their own AMP, and this might be of future interest in studying and designing topicals that can influence barrier function and perhaps enhance regenerative pathways through AMP. Another possibility of exploration is looking at the influence of cosmetic products (Negi et al. 2012; Prescott et al. 2017), such as epidermal exfoliating treatments for anti-aging, and the possibility of designing novel cosmetics that kick-start a regeneration process within the epidermal layer by influencing local immune signals that in turn govern host regenerative pathways. With the evidence of how dysbiosis, or a change in the homeostasis between microbiome and host, can influence host immunity, we can begin the quest to understand the different ways by which this aspect can be further explored so as to fully grasp the regenerative pathways of the skin. Several applications can be cited, including wound healing, and innovative cosmetics that can influence the slowing of cellular aging, as well as influence deeper penetration of actives. Another avenue in innovation to explore is using drug delivery devices like ultrasound and radiofrequency for delivery of cosmetics (Issa et al. 2013), and the change in surface microbiome in certain skin conditions like acne. Based on the paper by Nakatsuji (Nakatsuji et al. 2013), we are left with several hypotheses of how altering surface bacteria, with its very complex host relationship, can significantly change host regenerative processes that could also be used for aesthetics and enhancing dermal collagen synthetic activity.

The very interesting relationship explored between the skin and gut opens avenues of understanding of the diet and its influence on the skin. For centuries, ancient practices like Ayurveda have connected the importance of the gut to overall health, and now, through medical science, we are beginning to notice an interaction between the skin and the gut, and their respective microorganisms, and the connection to a healthy, balanced, immune system. This leads us to speculate that much of ancient eastern science has always seen this unique relationship. Ayurveda also emphasizes the importance of emotional health and its connectivity to the health of the rest of the organs by a dosha evaluation. In the pathways that are modulated in a neuroendocrine-based system as cited in the previous section of the article, we can appreciate how this connection is possible. Vibration science (Beri 2018) interconnects the ancient philosophy of interconnectivity of all living organisms through energy, and it would be fascinating to explore the possibilities of altering the microbiome to influence emotions, especially through cosmetics that can trigger local neuroendocrine pathways or perhaps vibrational healing methods like reiki or sound vibration (Denda et al. 2013). Another field of study for understanding the skin–brain axis could be in assessing any impact of meditations and mindfulness in the change of the surface microbiome and looking for changes or improvement in skin conditions or skin aging. Various studies that correlate breathing and yoga to improvement in overall well-being can also guide the design of testing this axis more deeply through changes in the microbiome and correlating it to blood antioxidant levels [34, 35]. More work is needed in using devices with penetrating active ingredients that affect regenerative and immune pathways that can then stimulate alterations in the deeper layers of the skin microbial components and cause more significant host response. Finally, we should point to the application of the microbiome and host immune interaction as a possible basis for regulating claims by cosmetic products and perhaps creating guidelines that can make for a safe and clear demarcation of the topical action on the body. Regulatory bodies like the FDA could focus on using the unique host–microbiome interaction post-topical application as a fundamental guideline to regulate cosmetics. The various possibilities expand our horizons to consider a concept of the "cosmetic microbiome," which can influence the skin–gut–brain relationship [36, 37], and therefore result in design of innovative cosmetics and transdermal drugs that change the perception of beauty, health, and well-being. Cosmetics in the future could have the potential to claim that they can not only make you look good but also that make you "feel" good.

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Probiotics and Their Various Forms Supporting Skin Health

4

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Abstract

There is increasing evidence for a gut-skin axis and a potential influence of the skin microbiota on skin health. With this in mind, there is an opportunity for probiotics, even though they have traditionally been investigated for their intestinal and immune benefits. The skin has an important function as a barrier. Here, oral and topical probiotics, parabiotics, and postbiotics have been observed to have positive benefit, also in supporting the skin against UV-induced damage. The manufacturing of probiotics for skin health, especially for topical applications, may have very specific requirements, different from current oral applications, because the intended use and regulation are different. For the future, the field would benefit from rigorous human intervention studies as are becoming more common in the nutritional field. While currently mainly typical probiotic organisms such as lactobacilli and bifidobacteria are used, it can be foreseen that in the future also organisms representative for the skin microbiota will find application.

Keywords

Probiotic · Parabiotic · Postbiotic · Oral · Topical · *Lactobacillus* · *Bifidobacterium* · Manufacturing · Regulation

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4.1 Introduction

Probiotics research has focused primarily on the effects on the health of the gut, and the rising interest in skin and scalp health has evolved only over the past decade. Many studies, also in the clinical setting, have shown that probiotics have benefits on the skin, as topical application that acts locally, or when ingested orally with more systemic effects through the gut-skin axis. The definition of a probiotic is unclear in terms of skin health, and there are questions over whether bacterial components, such as the metabolites that are produced by the probiotics, are to be considered probiotics. According to the official definition, this is not the case.

This chapter will discuss the skin and its microbiota, focusing on the bacterial component, considering the various definitions of probiotics for skin health and describing the benefits of topical and oral use of probiotics. Although fermented dairy products and other fermented ingredients have been used for skin health purposes, we will not address them in this chapter but will direct the reader to other recent publications on this topic (e.g., Vaughn and Sivamani 2015; Chen et al. 2014; Chan et al. 2014). In this chapter, we will not consider cosmeceuticals, which refer to the combination of cosmetics and pharmaceuticals, but will review the cosmetic effects of probiotics on non-diseased skin conditions, such as chronological aging and photoaging and skin barrier and hydration defects, in alleviating the symptoms that are associated with sensitive, dry, and aged skin. The demand for anti-aging, moisturizing, skin-whitening, and UV- and pollution-protective products has risen with the aging population and the desire for more functional ingredients.

The effects of probiotics on dermatological diseases in adults are not included in this chapter but have been reviewed for instance by Notay et al. (2017) and Yu et al. (2020). A meta-analysis on the oral probiotic treatment of atopic dermatitis in children was recently reported by Huang et al. (2017). The function of probiotics and other beneficial microbes in wound-healing has been examined by Lukic et al. (2017).

4.1.1 Definitions of Probiotics, Postbiotics, Parabiotics, and Prebiotics

Microbes, their components, and metabolites can be used to alter the composition and/or activity of the host microbiota or directly influence the host, through immune modulation or changes in barrier function, for example.

The administration of live microbes refers to probiotics that are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al. 2014). Although this definition is most commonly applied to consumed microbes, the term “administered” specifically allows for other routes of exposure. Because the definition requires a “health benefit,” it is uncertain whether cosmetic benefits are covered by this definition. However, for simplicity, we will assume in this chapter that cosmetic benefits are also included and refer to topically applied live microbes as probiotics.

Metabolites that are produced by probiotics have been referred to as "postbiotics" and were defined by Johannsen and Prescott (2009) as "the biologically active by-products of probiotic cultures (e.g. short-chain fatty acids produced by bacterial fermentation such as butyrate), which can be added to health supplements." Tsilingiri and Rescigno (2013) expanded this definition to include secreted non-metabolites: "any factor resulting from the metabolic activity of a probiotic or any released molecule capable of conferring beneficial effects to the host in a direct or indirect way." There is, however, no consensus on the definition of postbiotics. Further, it is unclear whether the producing strain must be a probiotic as defined above, i.e., whether it must have a health benefit *per se* or whether it can be any strain that produces postbiotics with health benefits.

Killed microorganisms with a health benefit have been referred to as "parabiotics" and are loosely defined as "non-viable microbial cells (intact or broken) or crude cell extracts which when administered (orally or topically) in adequate amounts, confer a benefit to the human or animal consumer" (Martin and Langella 2019). Also, the term "parabiotic" has no generally agreed-on definition. To add to this confusion, the term "parabiosis," referring to "living beside," has various meanings in other scientific disciplines.

The microbiota can also be influenced by providing selective energy and carbon sources, allowing specific groups to be promoted or have their metabolism altered. This approach is referred to as "prebiotic," defined as: "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al. 2017). Prebiotics will not be the focus of this chapter but are mentioned here for exhaustiveness.

4.1.2 Definition of Probiotic Skin Care Products and Their Associated Challenges

Skin care products that contain probiotics have been suggested to alleviate skin problems, such as redness, irritation, and swelling; counteract skin diseases, for instance, by providing antimicrobial effects in acne; and be suited for certain skin types, such as sensitive, oily, or dry skin. Probiotic-containing skin care products may also have general positive health effects on the skin, such as eliciting better skin balance, moisturizing, softening, strengthening the skin's defense mechanisms, stimulating and calming, providing anti-aging effects, and altering the composition of the skin's microbiota (Warming et al. 2018; Sharma et al. 2016). Research on the skin microbiota in the past decade has raised the interest of consumers to commercially available probiotics that improve skin health, and several topical formulations have been proposed to correct dysbiosis of skin microbiota and enhance the function of the skin immune system (Lee et al. 2019a; Knackstedt et al. 2020). This development has brought opportunities for various commercial entities to incorporate live probiotics, parabiotics, or postbiotics into skin care products to rebalance the skin microbiota and promote healthier and more radiant-looking skin (Tkachenko et al. 2017). Currently, probiotics, parabiotics, and postbiotics are included in various

types of cosmetic products, including creams, lotions, facial scrubs, hand soaps, hair care products, and toothpaste, but historically, those that were intended for skin were based initially on yoghurts with probiotics (Lee et al. 2019a; Tkachenko et al. 2017; Sharma et al. 2016). Often, they are marketed as luxurious products (Warming et al. 2018). As an ingredient, probiotics are considered to be natural, which resonates with the modern social trend toward healthy lifestyles (Siegel et al. 2019).

The definition of probiotics by the food industry as living organisms might pose challenges for cosmetics and personal care products (PCP). By definition, probiotics are live microorganisms. For food and dietary supplement applications, there is good understanding in the industry how an adequate dose of live microbes should be delivered until the end of a given product shelf-life, which might require adaptation of the food recipe and production methods. Similarly, storage conditions might need to be adjusted. It might also require excess of the probiotic to have sufficient counts at the end of the product shelf-life. For long storage times (up to 24 months), probiotics are commonly freeze-dried and incorporated in a low-water-activity ($Aw < 0.20$) delivery format, such as capsules and sachets (Jackson et al. 2019). For topical applications, there are other challenges that might not have been encountered regularly by the probiotic industry. Cosmetics and PCP commonly are typically lotions and creams with shelf-life of 12–24 months at ambient temperature, which can be harmful for live microbes. In general, probiotics are stable in oil-based matrices, such as in chocolate or oil drops, even at ambient temperatures and humidity (Possemiers et al. 2010). Most skin creams and lotions, however, are water-oil emulsions, stabilized by emulsifiers. Probiotics that are incorporated into such products will fail to remain in the oil phase and thus migrate to the water phase or collect at the water-oil interphase. This situation would expose the probiotics to a high-Aw environment, which is detrimental to their survival. Consequently, substantial product development might be required to successfully incorporate live microbes into skin products. Instead of the typical probiotic lactobacilli and bifidobacteria, spore-forming organisms, such as *Bacillus*, could be considered; the spores provide substantial stability (Elshaghabee et al. 2017). However, it is unknown whether there is sufficient moisture on the skin to allow the spores to germinate.

Lactobacilli, bifidobacteria, and spore-formers are also uncommon on the skin (Table 4.1), perhaps necessitating the development of characteristic skin microbes as probiotics, as part of the so-called next generation probiotics. These organisms will have specific growth requirements, processing challenges, and delivery issues but will be adapted to life on the skin (Paetzold et al. 2019).

Preservatives are used in cosmetic products for microbial control, rendering them unable to contain probiotics as such (Warming et al. 2018). Cosmetic products also often contain amino acids, vitamins, minerals, and free fatty acids, which are potential sources of energy for microorganisms; unwanted growth of living probiotics could cause spoilage of the product itself. Culture supernatants and dead bacteria—intact or as lysed crude cell extracts—have beneficial effects on the skin when applied topically, indicating that strictly living probiotics are not necessary in topical skin care products (Foligne et al. 2013). Further, the technical

Table 4.1 Non-exhaustive list of common microbes (Zhernakova et al. 2016) in the intestine and on the skin (Byrd et al. 2018)

Intestine	Skin
Bifidobacterium adolescentis	*Corynebacterium tuberculostearicum*
Eubacterium rectale	*Corynebacterium fastidiosum*
Ruminococcus bromii	*Corynebacterium afermentans*
Faecalibacterium prausnitzii	*Micrococcus luteus*
Subdoligranulum unclassified species	*Cutibacterium acnes*
Ruminococcus unclassified species	*Staphylococcus aureus*
Bifidobacterium longum	*Staphylococcus epidermidis*
Collinsella aerofaciens	*Staphylococcus hominis*
Dorea longicatena	*Staphylococcus captis*
Akkermansia muciniphila	*Streptococcus mitis*
Eubacterium hallii	*Streptococcus oralis*
Ruminococcus torques	*Pseudomonas fluorescens*
Dialister invisus	*Veillonella parvula*
Ruminococcus obeum	*Malassezia globosa*
Coprococcus unclassified species	*Malassezia restricta*
Streptococcus thermophilus	*Malassezia sympodialis*
Prevotella copri	
Dorea formicigenerans	
Bacteroides uniformis	
Eubacterium biforme	

challenges in the colonization efficiency, stability, and viability of living probiotics could be circumvented by postbiotics by simplifying the issues with shelf-life, packaging, transportation, and storage (Wegh et al. 2019). Most so-called probiotic skin care products do not contain living bacteria (Warming et al. 2018). However, solutions, such as microencapsulation technologies, are tested with living and stable probiotic bacteria in PCP (da Silva et al. 2014).

For these reasons, in personal care, probiotics have been proposed to be divided into living and nonliving entities: "living cosmetics" would indicate the presence of living probiotics, without preservatives in the product, whereas "probiotic cosmetic" would describe products with lysates of bacteria and preservatives (Tkachenko et al. 2017). However, these terms, the effective dose, and the categories into which the postbiotics and fermentates would fall under this definition must be validated. The distinction between parabiotics, postbiotics, and probiotics is not straightforward, either. A "probiotic" preparation, especially for research purposes, can contain two, or even all, of the "biotics." For instance, a postbiotic preparation, indicated to include only metabolites, could harbor components of the bacterial cell wall or other elements that originate from the bacterial cells themselves (Wegh et al. 2019). Further, the preparation method—for instance, denaturation by heat and sterilization by irradiation—can pose challenges in the compositions due to microbial protein denaturation or mutations in the microbial nucleic acids, respectively

(Wegh et al. 2019). The type, form, and effective and safe dose for probiotics in PCP must thus be determined—a process that will rely on manufacturers (Siegel et al. 2019).

4.1.3 Briefly on Regulatory Environment for Probiotics in Skin Care Products

Various jurisdictions differ substantially with regard to regulatory and legislative environments for the general definition of cosmetics and the evidence that is needed for their efficacy, for which there is a lack of international harmonization (Foligne et al. 2013; Singh et al. 2018; Nohynek et al. 2010). Japanese regulations are among the most stringent, wherein cosmetic products must be registered with evidence of their safety and efficacy (Nohynek et al. 2010). This strict process has been followed by other Asian countries, such as China, South Korea, and Taiwan (Nohynek et al. 2010).

In the EU, directive EC 1223/2009 states that the cosmetic product must be safe *a priori* and that the responsibility for the safety of cosmetic products is placed entirely on the manufacturer; thus, there is no premarketing clearance (Pauwels and Rogiers 2010). Several annexes of the EU directive describe the ingredients that are allowed and banned in cosmetic products (Pauwels and Rogiers 2010). The EU Scientific Committee on Consumer Safety (SCCS) provides guidance on the safety assessment of cosmetic ingredients.

In the USA, the Food and Drug Administration (FDA) is the health authority that controls cosmetic products. The US Food, Drug, and Cosmetic Act of 1938 designated the FDA as the agency that is responsible for the safety of PCP and cosmetics (Nohynek et al. 2010). As in the EU, no preapproval of cosmetic products for marketing or distribution is needed, but the cosmetics industry should ensure that every ingredient and composition is substantiated as safe (Nohynek et al. 2010; Singh et al. 2018). There are, however, differences between these regions: certain products that are regarded as cosmetics in the EU are considered over-the-counter (OTC) drugs in the USA (Nohynek et al. 2010). Also, although the FDA classifies probiotics into various categories, such as foods, food additives, dietary supplements, medical devices, drugs, and even cosmetics on a case-by-case basis, no regulatory definition for topical probiotic products exists, nor does an agency that specifically addresses probiotics in cosmetic products, rendering them the least regulated area among foods in this respect in the USA (Singh et al. 2018; Hoffmann et al. 2012). In the EU, the term "probiotic" is considered to be an unauthorized health claim in terms of nutrition, but in PCP, it is widely used (European Parliament 2006; Warming et al. 2018).

Probiotics for human oral consumption have been selected based on strict safety and clinical evidence and meet certain requirements with regard to their passage through the gastrointestinal tract (acid and bile tolerance) and their absence of toxins and transferable antibiotic resistance genes (Martin and Langella 2019; Pereira et al. 2018). Probiotics for human consumption are regarded as safe through GRAS

(generally regarded as safe) designation in the USA and QPS (qualified presumption of safety) in the EU (Martin and Langella 2019; Sanders et al. 2010). Often, probiotic strains fall into a limited set of food-grade bacterial genera, such as lactobacilli, *Bifidobacterium* spp., minor *Bacillus* and *Escherichia coli* strains, the yeast *Saccharomyces*, but *Enterococcus* spp., *Lactococcus* spp., and *Propionibacterium* spp. are also used (Lew and Liong 2013; Martin and Langella 2019; Foligne et al. 2013). The lack of a definition for the term "probiotic" in the personal care and cosmetics industry is causing confusion among consumers over the functions of the products, especially because the evidence that supports their use is largely believed to be less vigorous and lower in quality (Siegel et al. 2019). The issue is also complicated by the fact that consumers are only beginning to understand the probiotic concept in foods, beverages, and dietary supplements and do not have this awareness in cosmetics (Martin and Langella 2019). Further, there are restrictions in how cosmetic and personal care ingredients and their safety and efficacy is determined—for instance, in the EU, animal experimentation in the safety assessment and marketing of cosmetic products has been banned since 2009 (Pauwels and Rogiers 2010). This constraint applies in other countries as well, such as India, and many other nations are interested in pursuing a ban for animal testing of cosmetic products (Singh et al. 2018).

PCP and cosmetics are marketed for their performance characteristics, not their chemical content or origin (Morganti and Coltelli 2019). Because the personal care and beauty sector relies on the wellness category, it will need an official definition to aid in labeling and claiming (Martin and Langella 2019). There are no official guidelines on probiotics in PCP, and structure-function claims that are substantiated with proper scientific evidence are allowed in dietary supplements in the USA but not similarly in cosmetics (Siegel et al. 2019; Foligne et al. 2013).

In the USA, the label can feature indications for function and instructions on use, if necessary, and precautions on safety (Singh et al. 2018). In the EU, product claims for cosmetics can be used, but there must be no implication that the product that bears the claim has characteristics or functions that they do not have, and the claims on cosmetic products are considered informational tools for the end-consumer (Singh et al. 2018). However, the claim in the EU must be proven and substantiated and based on six distinct principles: legal compliance, truthfulness, evidential support, honesty, fairness, and informed decision-making (Singh et al. 2018; Warming et al. 2018). The structure and function of the product must be documented, and such methodological evidence must be provided that is precise and standardized with regard to its efficacy, safety, and tolerability and the mechanisms through which the probiotics function in the skin (Siegel et al. 2019; Lee et al. 2019a). To this end, properly designed preclinical or clinical placebo-controlled randomized studies are recommended and needed (Siegel et al. 2019; Foligne et al. 2013).

4.1.4 Comparison of Skin and Gut Microbiota

Much of the research on the human microbiota has traditionally focused on the intestinal microbiota, which in practice has been the fecal microbiota and primarily its bacterial component. Recently, however, more attention has been paid to other microbial inhabitants of the gut, such as fungi, archaea, and viruses. The fecal microbiota is characterized by the abundance of obligate and facultative microbes.

An expert group at the North American branch of the International Life Sciences Institute (ILSI) recently attempted to define a healthy human gut microbiome (McBurney et al. 2019). Instead of listing common or key organisms, they concluded that microbiota communities are highly individualized, show a high degree of interindividual variation to perturbations, and tend to be stable over years. However, the dietary, ethnic, physical, and economic diversity of neighborhoods (urban or rural) complicates the determination of factors that affect the diversity of the gut microbiome and its associations with human health. Due to challenges that are associated with identifying and characterizing major gut microbiome patterns, the enormous ecological gut microbial diversity among healthy adults, and their ethnic origins and geographic locations, it will likely be extremely difficult to define a single, healthy microbiota (McBurney et al. 2019), regardless whether one examines the skin microbiota or any other human microbiota.

Notwithstanding these issues, what are the commonly observed organisms in the intestinal microbiota? Most species belong to the phylum *Firmicutes*, followed by *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Euryarchaeota*, and *Proteobacteria*. In samples from 1135 healthy Dutch adults, 632 unique species were identified, 170 of which were present at levels of at least 0.01% of the total microbiota in over ten individuals (Zhernakova et al. 2016). When the threshold was set to 0.1%, 74 species were present in at least 70 individuals, whereas a 1% threshold yielded 22 species in at least 381 individuals. These 22 species are listed in Table 4.1, in which several unclassified species were detected as major components of the intestinal microbiota. With regard to the mycobiota, Huseyin et al. (2017) reported more than 200 species, belonging primarily to the phyla *Ascomycota* and *Basidiomycota*. The most prevalent organisms belonged to the genera *Saccharomyces* and *Candida*.

Similar to the intestinal microbiota, it is impossible to define a healthy human skin microbiota for similar ethnic, cultural, geographic, and lifestyle reasons. Further, the skin has many ecological sites, which, in contrast to the intestine, are easily accessible. These sites can be sebaceous, moist, or dry and have disparate temperatures (Grice 2014). Skin bacteria belong mainly to the phylum *Actinobacteria* (51.8%), followed by *Firmicutes* (24.4%), *Proteobacteria* (16.5%), and a minority belonging to *Bacteroidetes* (6.3%) (Grice et al. 2009). Species of the genera *Corynebacterium*, *Cutibacterium* (formerly *Propionibacterium*), and *Staphylococcus* are the most common (Table 4.1) (Zhai et al. 2018; Byrd et al. 2018). *Malassezia* is the most predominant fungus; it is lipophilic and most often associated with sebum-rich areas of the skin (Balato et al. 2019).

The various skin micro-organisms can be permanently colonizing or inhabit the skin transiently (Dréno et al. 2016). Many of them, however, are necessary for the health of the skin, producing, for instance, antibacterial substances and preventing pathogenic bacterial colonization and influence the skin's immune function. Despite external factors, the human skin microbiota can be stable for long periods, despite the high variation between locations and individuals (Oh et al. 2016). For example, *Corynebacterium* species can be found in moist sites with *Staphylococcus*, whereas sebum-rich areas are inhabited by *Cutibacterium* and, to a lower extent, staphylococci. Dry skin areas, in turn, are colonized by β-*Proteobacteria* and *Flavobacteriales* (Grice et al. 2009; Findley et al. 2013). It has been also suggested that ethnicity and, particularly, soap and shampoo practices are secondary factors compared with the ecological site in determining the composition of cutaneous microbiota, whereas in each location, the host origin is a determinant for cutaneous microbiota composition (Perez Perez et al. 2016).

Many skin diseases have been proposed to be linked to disturbances in the composition of skin microbiota; there is a well-balanced interaction between the resident and short-term microbiota of the skin (Table 4.1). Changes in this balance cause dysbiosis, but it is often unknown whether they are the cause or the effect of the disease (Egert and Simmering 2016). In addition to intrinsic factors, such as sex-related differences in the microbiota composition (Egert and Simmering 2016; Giacomoni et al. 2009), extrinsic factors, such as the use of personal hygienic products and cosmetics and exposure to UV irradiation, can alter the skin microbiome, but they might be secondary (Egert and Simmering 2016; Burns et al. 2019).

4.1.5 Skin Barrier Function

The epidermis continuously replaces itself every 30–40 days. During normal differentiation, keratinocytes that originate in the basal layer of the epidermis move toward the skin surface and change their function and composition (Fig. 4.1). There, the terminally differentiated keratinocytes are filled with keratin and shed. Skin hydration and skin barrier regulate the balanced maturation of keratinocytes. Epidermal turnover is governed by various external and host-dependent factors, such as genetic background, sex hormones, and chronological age. For example, psoriasis is considered a skin disease with a markedly increased rate of epidermal turnover and defects in formation of the skin barrier. Relocalization of the tight junction components zonula occludens-1 (ZO-1) and occludin to the spinous layer instead of the granular layer is associated with the pathology of psoriasis (Pummi et al. 2001).

Typically, aging skin experiences decreases in lipid content, gland secretion, and keratinocyte proliferation and differentiation, which impairs its barrier function and hydration. The dryness of aging skin is partly caused by lower blood circulation and reduced synthesis of matrix components in the dermis. Ceramides are the major lipids in the *stratum corneum* (SC), maintaining the skin barrier and functioning as

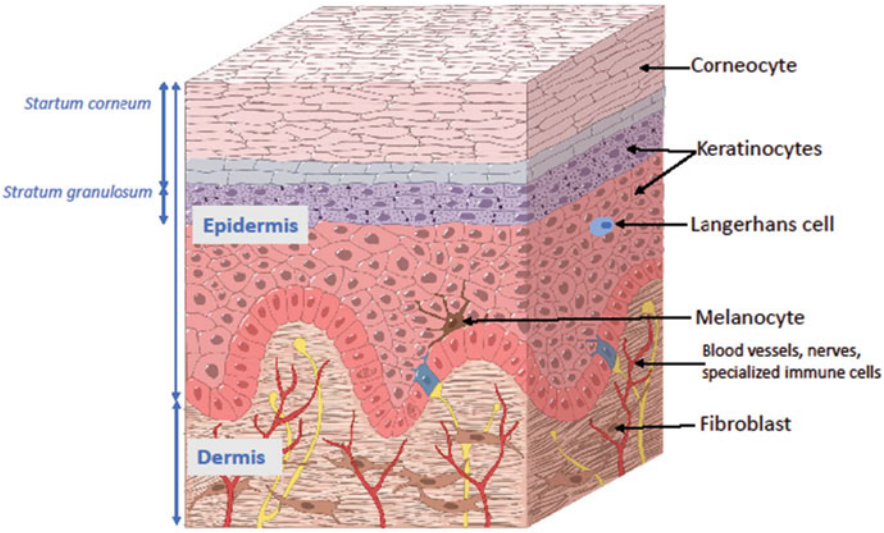

Fig. 4.1 The main structures and the cell types of the hairless skin. (© Pinja Kettunen/SciArt and International Flavors & Fragrances, with permission)

signaling lipids to regulate cellular functions (Uchida 2014; Uchida et al. 2015; Cinque et al. 2011). Ceramides can be synthesized *de novo* and through hydrolytic enzymatic activity of sphingomyelin and glucosylceramide (Uchida et al. 2015). In aged skin, ceramide synthase activity decreases (Jensen et al. 2005), weakening the skin barrier function.

In addition to chronological aging, the skin and its microbiota are continuously exposed to various external factors, such as ultraviolet (UV) radiation, pollution, PCP, food, lifestyle, and even clothing. Cigarette smoke, active or passive, is a common pollutant that is linked to reduced skin barrier function and various skin disorders (for a review, see Prieux et al. 2020). Exposure to these and other environmental toxic chemicals induces proinflammatory and oxidative markers in various organs, including the skin.

Low temperature and humidity negatively affect the skin barrier in the winter months, especially in persons who live far from the equator (Engebretsen et al. 2016). Dry skin is more fragile and reactive to skin irritants. However, the upper layer of the epidermis can adapt to a dry environment and restore barrier function through metabolic changes and by thickening. Transepidermal water loss (TEWL) is a parameter that is used to measure water diffusion from deeper layers to the skin surface and is linked to characteristics of the skin barrier. Disturbances in the barrier often result in higher TEWL values, whereas a healthy skin barrier has lower values; TEWL decreases during aging (Akdeniz et al. 2018). Clinical studies often enroll participants with increased TEWL values to monitor the potential beneficial effects of an investigational oral supplementation or topical application product on skin

hydration and barrier function. In experimental settings, stress conditions, such as tape stripping and capsaicin, are used to enhance water loss and induce irritation.

Research on skin hydration and moisturization often involves the in vitro evaluation of hyaluronic acid (HA) production. Both epidermal keratinocytes and dermal fibroblasts produce HA which is involved in various physiological functions (Papakonstantinou et al. 2012; Hynes and Walton 2000). HA maintains the structure of the *stratum corneum*, the epidermal barrier, and the extracellular dermal matrix; regulates the immobilization of water; and functions in immune modulation (Papakonstantinou et al. 2012; Lew and Liong 2013). It is synthesized by HA synthase and is degraded by hyaluronidase (HYAL), with a half-life of less than 1 day (Papakonstantinou et al. 2012). Aging causes changes in hyaluronan function, which might underlie some of the alterations in human skin with aging (Lee et al. 2016a).

The absence of UV exposure in winter also affects the physiology of the skin. Low-dose ultraviolet B (UVB) radiation reinforces skin barrier function, activates cutaneous vitamin D synthesis, and upregulates antimicrobial peptides (Hong et al. 2008). Nonetheless, prolonged exposure to UV radiation is a major risk factor for skin cancer and increases the expression of matrix metalloproteases (MMPs), which disrupt the skin's structure. The potential function of probiotic products in UV-induced photodamage is discussed later in this chapter (see Sects. 4.2 and 4.3 on Oral and Topical Probiotics).

4.1.6 Microbial Interactions in the Skin

The skin immune system and the impact of skin microbiota on its function were reviewed recently by Abdallah et al. (2017), who described the communication between the external environment and the complex network of immune and epithelial cells. Skin microbiota-host interactions in the outermost cornified layer and its associated structures have been reviewed by Chen et al. (2018) and Schommer and Gallo (2013), and the interplay between the dominant eukaryote, *Malassezia* fungi, and the host has been discussed by Grice and Dawson (2017). Intestinal microbes also influence skin functions systemically (see Sect. 4.2 on Oral Probiotics).

Compared with the intestinal epithelia, the skin surface is a completely different environment: dry, acidic, aerobic, and lacking an external nutrient supply. However, hair follicles and glands that secrete sweat and sebum provide an anaerobic and lipid-rich environment for microbes (Matard et al. 2013). The metabolism of skin microbes depends almost entirely on the unique chemical structures of the lipids and proteins that are produced by keratinocytes. These complex and unusual epidermal lipid structures have antibacterial effects, (for review Drake et al. 2008; Kwiecien et al. 2019) and have been suggested to contribute to the selective survival of commensal microbes (Kobayashi et al. 2019). Due to the poor nutrient supply, and constant shedding of the keratinocytes, the microbial biomass on the skin surface is small, rendering the profiling of skin microbes challenging.

In addition to the outermost skin surface, bacteria are present in the deeper epidermis of normal human skin and even the dermis and skin adipose tissue (Nakatsuji et al. 2013). Thus, commensal skin microbes might influence the functions of dermal tissue. However, the function of the skin barrier in regulating the entry of skin microbes into the deeper skin layers is not understood.

4.2 Effects of Oral Probiotics and Parabiotics on Skin Health

The influence of diet, the gut microbiota, and their metabolites on skin through various routes has been reviewed by many groups and will be detailed in other chapters of this book. We will briefly introduce the mechanisms that are involved in the gut-skin axis and describe examples of the use of oral probiotics in various skin ailments, which are nonserious inconveniences or cosmetic defects, such as photo-aging, chronological aging, weakened skin barrier function, sensitive skin, and dry skin, rather than concentrate on diseases or medical conditions (atopic dermatitis, psoriasis, acne, allergy, cancer, wounds).

Studies conducted in vitro with different keratinocyte and tissue models may be used to model both oral and topical applications of probiotics, parabiotics, and postbiotics for skin health. We will attempt to simplify the topic and focus on research on specific single-form products. Research on mixtures, such as fermented products that generally contain live bacteria, parabiotics, and postbiotics (Baba et al. 2010; Kano et al. 2013; Kimoto-Nira et al. 2014; Kwon et al. 2019; Mori et al. 2016; Nagino et al. 2018; Puch et al. 2008), and studies that combined bacteria with other ingredients, such as carotenoids (Bouilly-Gauthier et al. 2010), are excluded, because the effects of each component on the skin might be difficult to separate.

Tables 4.2–4.4 list examples of various research approaches for oral probiotics and parabiotics on skin health, divided into several categories: Table 4.2 includes in vitro and ex vivo research, Table 4.3 lists animal models, and Table 4.4 presents human clinical interventions.

4.2.1 Mechanisms Involved in the Gut-Skin Axis: Brief Overview

Several reviews have implicated the immunomodulatory mechanisms of gut microbiota as being important for cutaneous health (Friedrich et al. 2017; Kumar et al. 2014; Lee et al. 2018; O'Neill et al. 2016; Yu et al. 2020). The connection between the gut and skin has been observed in, for example, inflammatory bowel disease (IBD), in which individuals can have various cutaneous manifestations of the disease (Huang et al. 2012). Salem et al. (2018) covered this subject broadly, discussing how gut microbiota affect skin homeostasis through systemic immunity and their metabolites—citing acne, atopic dermatitis, and psoriasis as examples—and how ingested probiotics might exert their beneficial effects through these mechanisms.

Table 4.2 Examples of in vitro and ex vivo studies on the effects of probiotics and parabiotics for oral administration

Health area	In vitro/ex vivo subjects	Bacteria for experiments	Stress model	Endpoints	References
Skin hydration	Hs68 human dermal fibroblasts	Heat-treated *L. plantarum* HY7714; *L. plantarum* 27; *L. gasseri* 51; *L. gasseri* 82, bacteria 5×10^7 CFU/mL and 2×10^9 CFU/well	NA	Hyaluronic acid, serine palmitoyltransferase (SPT) and ceramidase	Ra et al. (2014)
Neurogenic skin inflammation	Caco-2/PBMC-co-culture followed by ex vivo skin explants	Fresh overnight culture of live *L. paracasei* NCC 2461 (ST11), 1×10^7 CFU/mL	Substance-P (SP) for ex vivo skin explants	Histamine, tumor necrosis factor-α (TNF-α), histological evaluation of the inflammatory reaction	Gueniche et al. (2010b)
Skin barrier function	Caco-2/PBMC-co-culture followed by ex vivo skin explants	Fresh overnight culture of live *L. paracasei* NCC 2461 (ST11), 1×10^7 CFU/mL	Sodium lauryl sulfate (SLS) for ex vivo skin explants	TEWL	Gueniche et al. (2010b)
Photoaging	Hs68 human dermal fibroblasts	Heat-treated and frozen *L. plantarum* HY7714; *L. rhamnosus* 24; *L. rhamnosus* 25; *L. plantarum* 26, 5×10^8 CFU/mL bacteria	UVB irradiation	Type I procollagen, MMP-1 and -13, total and phosphorylated extracellular regulated kinases (ERKs), total and phosphorylated p38, total and phosphorylated c-Jun N-terminal kinases (JNKs), total, and phosphorylated c-Jun	Kim et al. (2014)
Skin moisturization	HaCaT epidermal keratinocyte cells	*L. plantarum* K8 bacteria lysates (sonicated), 10^8 to 10^{11} CFU/mL	NA	Hyaluronic acid	Kim et al. (2015a)
Skin moisturization	BALB/c mice cultured primary epidermal cells, BALB/c mice spleen cells followed by BALB/3T3 fibroblast	Heat-treated *L. plantarum* L-137 bacteria, 20%	Ammonium pyrrolidinedithiocarbamate (PDTC) with BALB/3T3 fibroblasts	Hyaluronic acid, IL-12, hyaluronan synthase (HAS), cytokines (IL-12, IFN-γ, TNF-α,), NFκBp65	Nakai et al. (2019)

Table 4.3 Examples of in vivo animal studies on the effects of probiotics and parabiotics for oral administration

Health area	In vivo subjects	Bacteria	Stress model	Endpoints	Intervention length	References
Skin hydration	Hairless mice	Heat-treated *L. plantarum* HY7714 bacteria, 1×10^9 CFU/day	UVB irradiation	Epidermal hyperplasia, epidermal thickness, skin hydration, TEWL, serine palmitoyltransferase (SPT) and ceramidase, ceramide, hyaluronic acid, and filaggrin	8 weeks	Ra et al. (2014)
Photoaging	Hairless mice	Heat-treated *L. plantarum* HY7714 bacteria, 1×10^9 CFU/day	UVB irradiation	Wrinkle formation, epidermal thickening, MMP-2, MMP-9, and MMP-13	12 weeks	Kim et al. (2014)
Cutaneous immune system	Skh:hr1hairless mice	Frozen *L. johnsonii* La1 bacteria, 10^8 CFU/day	UV irradiation, sensitization with dinitrofluorobenzene (DNFB)	Assessing erythema and edema, interleukin 10 (IL-10), Langerhans cells	23 days	Gueniche et al. (2006)
UV-induced skin damage	Hos:HR-1 hairless mice	*B. breve* strain Yakult (BBY) powder mixture, 1×10^5 to 10^9 CFU/day	UV irradiation	TEWL, skin hydration, H_2O_2, carbonylated proteins, lipid peroxidation, antioxidant capacity, xanthine oxidase (XO) activity	9 days	Ishii et al. (2014)
Anti-aging	C57BL/6 wild type and IL10-deficient mice and outbred CD-1	*L. reuteri* ATCC 6475 culture, 3.5×10^5 CFU/day	NA	Fur luster and growth, tissue pH, skin thickness, hair cycle, sebocytes	20–24 weeks	Levkovich et al. (2013)
Skin barrier function	Skh:hr1 hairless mice	*L. paracasei* CNCM I-2116 (ST11), 10^5 to 10^9 CFU	Repeated tape stripping	TEWL	24 days	Philippe et al. (2011)

Skin inflammation	Skh:hr1 hairless mice	*L. paracasei* CNCM I-2116 (ST11), 10^5 to 10^9 CFU	Sensitization with dinitrochlorobenzene (DNCB)	Ear thickness	23 days	Philippe et al. (2011)
Photoaging	HOS:HR-1 hairless mice	Lyophilized *B. breve* B-3, 2×10^9 CFU/day	UVB irradiation	Epidermal thickness, TEWL, skin hydration, epidermal thickening, IL-1β, claudin-1, laminin, and collagen IV	7 weeks	Satoh et al. (2015)
UV-induced skin damage	HOS:HR-1 hairless mice	*B. breve* strain Yakult (BBY) in saline, 1×10^3 to 10^9 CFU/day	UV irradiation	Epidermal thickness, skin elasticity, skin surface properties, elastase, IL-1β	9 days	Sugimoto et al. (2012)
Skin moisturization	HOS:HR-1 hairless mice and BALB/c mice	0.002% heat-treated *L. plantarum* L-137	NA	*Stratum corneum* water content, cytokine mRNA (IL-12, IFN-γ)	6 weeks	Nakai et al. (2019)
Skin moisturizing	SKH-1 hairless mice	*L. plantarum* K8 lysate (sonicated), 1×10^9 CFU/day	Dinitrochlorobenzene (DNCB)-treatment	Horny layer thickness, epidermal thickening, TEWL	8 weeks	Kim et al. (2015a)
UV-induced skin damage	HR-1 hairless mice	Tyndallized *L. acidophilus* ID-ACT3302, 100 mg/kg body weight/day	UVB irradiation	Skin hydration, TEWL, SAPK/JNK, mitogen-activated protein kinases (MAPKs), MMP-1, and MMP-9, epidermal thickness, collagen fibers	12 weeks	Im et al. (2016)

Table 4.4 Examples of clinical studies on the effects of orally administered probiotics and parabiotics

Health area	Clinical subjects	Investigational product	Endpoints	Intervention length	Reference
Skin moisturization	Healthy volunteers aged 25–60 years with dry and dark skin	2.1% *L. plantarum* K8 lysate (sonicated) in a candy	*Stratum corneum* hydration, horny layer thickness, TEWL	8 weeks	Kim et al. (2015a)
Skin hydration	Healthy volunteers aged 21–59 years with slightly elevated TEWL	Capsule of heat-killed *L. brevis* SBC8803, 25 mg/day and 50 mg/day	*Stratum corneum* hydration, TEWL, skin surface evaluation, questionnaire on skin conditions	12 weeks	Ogawa et al. (2016)
Reactive skin	Healthy female volunteers aged 18–40 years with sensitive skin	A sachet of *L. paracasei* NCC 2461 (ST11) in powder, 1×10^{10} CFU/day	Skin sensitivity, TEWL, clinical score, self-assessment, natural moisturizing factor (NMF) components urea and sodium lactate, fecal microbiota, serum cytokines (IL-10, IL-12, and TGF-β)	2 months	Gueniche et al. (2014)
Skin aging	Healthy female volunteers aged 41–59 years with dry skin and wrinkles	*L. plantarum* HY7714 in powder, 1×10^{10} CFU/day	Skin hydration, TEWL, facial wrinkles, skin gloss, and elasticity	12 weeks	Lee et al. (2015)
Cutaneous immune system	Healthy male volunteers aged 20–40 years receiving solar-simulated UV irradiation	*L. johnsonii* La1 supplementation, 1×10^{10} CFU/day	Immunohistochemical analysis (CD1a, HLA-DR, CD36, CD86, CR7, DC-Lamp), mixed epidermal cell-lymphocyte reactions (MECLRs)	66 days	Peguet-Navarro et al. (2008)
Scalp	Healthy male volunteers aged 18–60 years with moderate to severe dandruff	A sachet of *L. paracasei* NCC 2461 ST11, 1×10^{9} CFU/day	Dandruff, erythema, scalp seborrhea, global clinical score, self-assessments, scalp microbiota	56 days	Reygagne et al. (2017)
Skin condition	Healthy women aged 20–64 years with relatively high TEWL	A tablet of heat killed *L. casei* 327 (L. K-1), 50 mg (approximately 1×10^{11} CFU/day)	TEWL, *stratum corneum* hydration, self-assessment	8 weeks	Saito et al. (2017)

The favorable effects of probiotics in skin disorders through modulation of the immune system have been examined in another recent review (Szanto et al. 2019). The gut-skin axis and the function of probiotics in acne severity have been reviewed by Bowe et al. (2014). Maarouf et al. (2019) evaluated how nutrition exerts its effects on skin directly through metabolites and indirectly through alterations in the gut microbiota, also focusing on such disorders as acne. The influence of diet on cutaneous health through secreted metabolites has been well described by O'Neill et al. (2016), as well as via neurotransmitters that are produced by intestinal microbiota.

Many studies have concentrated on the effects of the gut-skin axis, but there are indications that the skin influences the gut (O'Neill et al. 2016). An Australian group found that exposing shaved skin to UV radiation altered the fecal microbiota in mice (Ghaly et al. 2018). Recently, a Canadian group reported that exposing skin to narrow-band UVB light (NB-UVB) increased serum vitamin D levels, which in turn improved the intestinal microbiota, especially in subjects who were vitamin D-deficient (Bosman et al. 2019).

4.2.2 In Vitro and In Vivo Research on Oral Probiotics and Parabiotics in Barrier Recovery and Hydration Related to Compromised Skin Barrier

The effects of orally administered probiotics and parabiotics on skin hydration and barrier recovery have been determined in in vitro (Table 4.2), animal (Table 4.3), and clinical intervention studies (Table 4.4), often applying stress to enhance water loss and irritation. There are various challenges in studying the effects of oral probiotic supplementation in vitro, because they are ingested and have their effects systematically through the gut. One should model the effects of a probiotic, which first enters the gut and then disseminates its effects through the gut epithelium systemically to the deeper layers of the skin and epidermis.

An interesting approach has been to study the effects of oral probiotics in vitro on skin barrier function and reactive skin using the Caco-2/peripheral blood mononuclear cell (PBMC) coculture model (Table 4.2) (Gueniche et al. 2010b). After topical *Lacticaseibacillus paracasei* CNCM-I 2116 (ST11) treatment, the basolateral conditioned cell culture medium from Caco-2/PBMC cocultures prevented substance P-induced inflammation and protected and enhanced barrier recovery after sodium lauryl sulfate (SLS)-induced irritation stress in an ex vivo human abdominal plastic skin explant model (Gueniche et al. 2010b).

The effects of probiotics on skin barrier and skin inflammation were further studied in vivo in hairless mice (Table 4.3) (Philippe et al. 2011). Oral *L. paracasei* CNCM I-2116 (ST11) decreased skin thickness on topical dinitrochlorobenzene (DNCB)-induced stress, dose-dependently. The beneficial effect of oral bacteria supplementation on TEWL after tape-stripping improved significantly, but only on long-term application (22 days) with a higher dose of bacteria.

The effects of oral supplementation with *L. paracasei* NCC 2461 (ST11) were studied in a randomized, double-blind, placebo-controlled clinical study in subjects with sensitive skin (Table 4.4) (Gueniche et al. 2014). Subjects who received the probiotic experienced lower sensitivity of the skin, as measured by capsaicin test, and improved barrier recovery after tape-stripping, based on TEWL, after 2 months compared with the control group. The probiotic also prevented a decrease in the natural moisturizing factor (NMF) components urea and sodium lactate during the winter and spring (Gueniche et al. 2014).

In addition to studying *Limosilactobacillus reuteri* ATCC 6475 probiotic yoghurt on skin and hair anti-ageing effects Levkovich et al. (2013) measured the effects of orally fed *L. reuteri* ATCC 6475 without yoghurt matrix to differentiate the functions of probiotics from that of probiotic yoghurt (Table 4.3). Purified bacteria had significant effects only on female mice fur luster and the pH of the vagina, skin, and mouth. Moreover, probiotic-fed mice experienced faster regrowth of fur compared with controls. The effects in probiotic-fed aged mice were similar to those in mice that ate the probiotic yogurt with regard to skin thickness, sebocytes, subcutaneous hair follicles, and follicle phase. These results were reproducible in genetically outbred adult Swiss mice that were fed purified *L. reuteri* ATCC 6475. Studies in interleukin 10 (IL-10)-deficient C57BL/6 mice also indicated that these anti-aging effects are mediated by an anti-inflammatory route—no parameter changed when these mice received probiotic supplementation (Levkovich et al. 2013).

Also, orally administered parabiotics show effects on skin hydration. In a 12-week double-blinded, placebo-controlled study, oral administration of a capsule of heat-killed *Levilactobacillus brevis* SBC8803 to healthy volunteers with slightly elevated TEWL values in the forearm experienced a marginal decrease in TEWL in neck and low eye region and greater hydration of the stratum corneum in the neck (Table 4.4) (Ogawa et al. 2016). In an analysis of a population than consumed lactic fermentation products less than once per week, corneal hydration increased significantly (in the neck at a lower dose and in the eye at a higher dose) (Ogawa et al. 2016). In another study, 8 weeks of orally supplemented heat-killed *Lacticaseibacillus. casei* 327 in healthy women with relatively high TEWL values had significantly lower TEWL in the arm at week 4 compared with the placebo control, but no other significant differences were noted in TEWL or hydration parameters (Table 4.4) (Saito et al. 2017).

The effects of heat-killed *Lactiplantibacillus plantarum* L-137 (HK L-137) parabiotics on skin moisturization have been examined in vitro and in vivo (Tables 4.2 and 4.3) (Nakai et al. 2019). In mice, oral HK L-137, administered for 6 weeks, reduced the decrease in skin water content, and in cultured mouse primary epidermal cells, treatment with this parabiotic upregulated HA and IL-12. In mouse spleen cells, stimulation with HK L-137 increased the production of IL-12, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), and in mouse fibroblast cultures, HK L-137-conditioned spleen cell culture medium upregulated HAS mRNA and HA production. Based on the neutralization of TNF-α and IFN-γ, the authors discussed that these cytokines are involved in enhancing HA production (Nakai et al. 2019).

The moisturizing effect of an orally administered parabiotic in lysed form, *L. plantarum* K8, was determined in healthy volunteers with dry skin (Table 4.4) (Kim et al. 2015a), first by assessing HA expression in HaCaT keratinocytes, the thickness of the horny layer and epidermis, and TEWL in DNCB-treated SKH-1 hairless mice. The parabiotic decreased TEWL in mice and human volunteers after 8 weeks of oral administration (Kim et al. 2015a).

An oral *L. paracasei* NCC 2461 (ST11) supplement has shown antidandruff effects in healthy male volunteers who suffer from dandruff (Table 4.4) (Reygagne et al. 2017). The probiotic improved the restoration of the scalp microbiota and decreased the signs and symptoms of dandruff, suggesting that the effects are attributed to indirect actions on the skin barrier and skin immune system.

4.2.3 In Vitro and In Vivo Research on Oral Probiotics and Parabiotics in Effects Associated with Exposure to UV Radiation

UV irradiation stimulates inflammatory cytokines which in turn have various effects on the skin (Nasti and Timares 2012). UV radiation is used widely to model the photoaging effects on skin with regard to protein degradation, dehydration, and inflammation (Table 4.3). Oral probiotics and parabiotics have been studied in association with short-term and chronic UV exposure, wherein such parameters as hydration, extracellular matrix (ECM) and tight junction barrier-related proteins, erythema, edema, and inflammatory markers have often been analyzed.

Bifidobacterium breve strain Yakult (BBY) was studied in vivo in HOS:HR-1 hairless mice on short-term exposure to UV irradiation. The mice received the probiotic strain for 9 days and a daily dose of UV irradiation on days 6 to 9 (Table 4.3) (Sugimoto et al. 2012). BBY preserved the skin that was exposed to short-term UV irradiation and mitigated the UV-induced increase in elastase activity and IL-1β (Sugimoto et al. 2012). When fed to mice, BBY also inhibited the UV-induced rises in TEWL, hydrogen peroxide, and oxidized protein and lipid levels and the decrease in hydration (Ishii et al. 2014). However, there were no changes in the levels of antioxidative defense molecules between the active and control groups, but BBY suppressed the activity of XO, an enzyme that generates superoxide (Rinnerthaler et al. 2015; Ishii et al. 2014).

Chronic UV-exposed (3 times per week) mice that were fed the *B. breve* B-3 strain daily for 7 weeks experienced a reversal of the increase in TEWL, less UV-induced epidermal thickening, and the suppression of abnormal claudin-1 expression in the epidermis and disruption of the basement membrane components laminin and collagen IV (Table 4.3) (Satoh et al. 2015).

Also, the UVB irradiation induced effects in hairless mice; such as decrease in skin hydration, increases in TEWL and epidermal thickness, were reduced when tyndallized *Lactobacillus acidophilus*, a parabiotic, was administered (Table 4.3) (Im et al. 2016). By histology, the amount of collagen fiber was lower in the UV-treated group without bacteria compared with those that received the parabiotic.

The parabiotic also downregulated ECM-degrading proteases and mitogen-activated protein kinases.

In a separate study with hairless mice and probiotics, the administration of *Lactobacillus johnsonii* La1 did not affect the post-irradiation erythema and edema (Table 4.3) (Gueniche et al. 2006). In turn, the UV irradiation inhibited the response to DNFB, which La1 largely rescued. La1 protected against the UV-induced decrease in Langerhans cell (LC) density and rise in serum IL-10. A clinical study showed that when subjects were given La1 for 66 days and exposed to UV radiation twice within 10 h on day 56, the LC phenotypic activation and maturation were similar between the control and probiotic groups. In the La1-treated group, the expression of CD1a in the epidermis was normalized faster, on day 4 after UV, as well as CD36 at day 10, compared with the control group (Table 4.4) (Peguet-Navarro et al. 2008). The allostimulatory function of epidermal cells recovered more quickly, on day 4, in the UV-sensitive participants in La1-supplemented group.

The effect of *L. plantarum* HY7714 on photoaging has been studied extensively in vitro, in vivo, and in clinical settings. The administration of *L. plantarum* HY7714 to Hs68 fibroblast cells in vitro and UVB-stressed hairless mice for 8 weeks in vivo increased the messenger ribonucleic acid (mRNA) levels of serine palmitoyltransferase (SPT) and downregulated ceramidase mRNA, both of which participate in sphingolipid metabolism in the skin (Table 4.3) (Ra et al. 2014; Coant et al. 2017). Further, *L. plantarum* HY7714 reduced the epidermal thickness and prevented the UV-induced increase in TEWL and decline in skin hydration in mice. These studies indicate that *L. plantarum* HY7714 supports the maintenance of skin hydration levels during UV stress (Ra et al. 2014).

Furthermore, *L. plantarum* HY7714 induces type I procollagen production and inhibits the collagen-degrading MMP-1 in human dermal fibroblasts in vitro, possibly by impeding the c-Jun N-terminal kinases (JNK) phosphorylation pathway (Table 4.2) (Kim et al. 2014). In hairless mice, this probiotic reduced UVB-induced wrinkle formation and inhibited epidermal thickening, MMP-2 and -9 activity, and MMP-13 expression (Table 4.3) (Kim et al. 2014). In a clinical trial, the anti-aging effects of *L. plantarum* HY7714 were studied in healthy female volunteers with dry and wrinkled skin (Table 4.4) (Lee et al. 2015). During the 12-week intervention, in the control and probiotic groups, skin hydration, gloss, elasticity, and wrinkle parameters improved, and TEWL decreased. Although the groups had similar responses, the enhancements in skin aging parameters were greater in the probiotic group after the 12-week supplementation.

4.2.4 Conclusion of Oral Probiotic and Parabiotics Studies on Skin Health

Most studies on the effects of postbiotics examined probiotic fermentates, in which all bacterial cell forms are present, in addition to the metabolites. The in vitro modeling of the oral application of probiotics, parabiotics, and postbiotics is challenging, and the results that are obtained with these methods should always be

verified by in vivo studies. However, in vitro models can provide useful mechanistic information for specified contexts.

The evidence from in vivo studies with orally administered probiotics and parabiotics indicates that the former has effects on skin barrier function, hydration, and UV-induced cellular damage. In chronic intervention studies, probiotics, lysates, and heat-killed probiotics have shown to improve the skin barrier in subjects with sensitive skin. On UV challenge, probiotics have shown to improve anti-aging parameters in humans and cell and animal models. However, the clinical relevance of the probiotic effects in UV challenge remains to be determined.

4.3 Topical Probiotics, Parabiotics, and Postbiotics on Skin Health

An emerging approach is to treat various skin conditions, including the external signs of acne, rosacea, psoriasis, allergies, and atopic eczema, using topical probiotics. We will not focus on these areas in this chapter but direct the reader to other reviews, such as Yu et al. 2020 and Knackstedt et al. 2020. Instead, we will concentrate on how topical probiotics can be used in cosmetics and PCP for skin-rejuvenating purposes. In this part of the chapter, the key components that arise from probiotics, parabiotics, and postbiotics, and some of the mechanisms of their topical application will be described.

4.3.1 Mechanisms of Topical Probiotics, Parabiotics, and Postbiotics in Skin Health

There is increasing evidence that inactivated bacteria, metabolites, bacterial DNA, cell wall components, and fragments—including extracellular polysaccharides (EPSs), lipoteichoic acid, peptidoglycan, and their peptide derivates—and bacterially derived nucleic acid may have health benefits in the skin, rendering them an interesting option when the delivery of live microbes is not feasible due to stability issues (Lew and Liong 2013; Sarkar 2018).

Many of these compounds are believed to function as immunomodulatory factors through innate immunity, based on their recognition by same pattern recognition receptors (PRRs) that sense pathogenic microbial structures. These receptors are important for recognizing pathogens, such as bacteria, fungi, and viruses—for instance, *Staphylococcus aureus*, *Candida albicans*, herpes simplex virus, and varicella zoster virus—but have also been implicated in several skin diseases (Miller and Modlin 2007). For example, toll-like receptor 2 (TLR2), a PRR, can bind to diacylated membrane anchors of lipoproteins, lipoteichoic acids, and peptidoglycan, whereas TLR9 binds bacterial CpG DNA. Subsequently, depending on the receptor and its adaptors, the response can be proinflammatory or anti-inflammatory (Bermudez-Brito et al. 2012).

Table 4.5 Various components and metabolites associated with postbiotics and parabiotics

Metabolite/bioactive cosmeceutical	Suggested benefit associated with skin	Examples of references
Small organic acids—e.g., lactic acid, butyric acid, acetic acid, formic acid, succinic acid	• Lowering of the skin pH to preserve the skin barrier	Halstead et al. (2015), Keshari et al. (2019), Krejner et al. (2018), Lastauskiene et al. (2014), Rawlings et al. (1996), Ryssel et al. (2009), Wang et al. (2014)
	• Inhibition of the growth of pathogens	
	• Moisturization	
	• Anti-inflammatory function	
Diacetyl	• Antimicrobial effect	Lau and Liong (2014), Hor and Liong (2014)
Hydrogen peroxide	• Antimicrobial effect	Zhu et al. (2017), Lau and Liong (2014), Hor and Liong (2014)
Antimicrobial peptides/ bacteriocin	• Antimicrobial effect	Deidda et al. (2018), Rosignoli et al. (2018), Oh et al. (2006), Bowe et al. (2006), Kang et al. (2009), Cebrián et al. (2018)
Hyaluronic acid	• Maintenance of the skin's normal structure and skin barrier	Lew and Liong (2013), Lew et al. (2013b), Hor et al. (2014)
	• Immobilization of water and moisturization	
	• Influencing cell proliferation, differentiation, and tissue repair	
Sphingomyelinase (SMase)	• Normal skin permeability barrier maintenance through production of ceramides	Di Marzio et al. (2003), Di Marzio et al. (2008), Di Marzio et al. (1999), Lew et al. (2013a)
Lipoteichoic acid	• Immunomodulatory function	Lew and Liong (2013), You et al. (2013), Hong et al. (2015), Kim et al. (2015b)
	• Photoaging prevention by reducing MMP-1 and increasing procollagen type I	
	• Photoaging prevention by reducing the amount of reactive oxygen species (ROS)	
	• Antihyperpigmentation through inhibition of tyrosinase	
Peptidoglycan	• Immunomodulatory function	Sorensen et al. (2005), Yuki et al. (2011), Kuo et al. (2013)
	• Tight junction barrier enhancement	

(continued)

Table 4.5 (continued)

Metabolite/bioactive cosmeceutical	Suggested benefit associated with skin	Examples of references
Exopolysaccharides (EPSs)	• Anti-inflammatory function	Noda et al. (2019), Gorska et al. (2016), Gotoh et al. (2017), Shirzad et al. (2018), Morifuji et al. (2017)
	• Reduction of MMP and elastase activity and antioxidant function	
CpG-rich DNA	• Immunomodulatory function	Ghadimi et al. (2008)

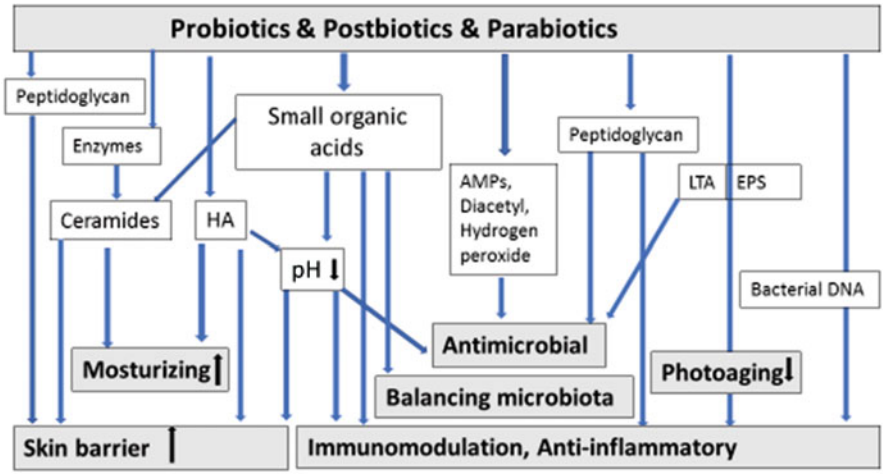

Fig. 4.2 Mechanisms of topically applied probiotics, postbiotics or parabiotics on skin health. *HA* hyaluronic acid, *AMPs* antimicrobial peptides, *EPS* exopolysaccharides, *LTA* lipoteichoic acid, *DNA* deoxyribonucleid acid

The key difference between responses that are elicited by pathogens and probiotics is that whereas the immune system functions to eliminate pathogens, probiotics stimulate the host's nonspecific, innate immune response towards pathogens and aid in their eradication (Ashraf and Shah 2014). In the skin, several toll-like receptors have been suggested to participate in the innate immune response and are expressed in various cell types in the skin, including keratinocytes, Langerhans cells, trafficking immune cells, and various dermal cells (Miller and Modlin 2007). Like intestinal epithelial cells, keratinocytes have a prominent function in immunity as first responders to the external environment (Juranova et al. 2017; Jeong et al. 2016). Table 4.5 summarizes the various components and metabolites that are associated with postbiotics and parabiotics, and Fig. 4.2 illustrates the impact of these components in skin.

4.3.1.1 Small Organic Acids Produced by Probiotics and Maintenance of pH in the Skin

The slightly acidic pH of the surface of the skin and the acid mantle are important for its maintenance, barrier function, maturation, and antibacterial activity (Panther and Jacob 2015; Proksch 2018). Several intrinsic and external factors affect the pH of the skin, including age, body part, skin type, sweat, and also the usage of PCP (Ali and Yosipovitch 2013). Aging, for instance, is associated with increases in skin pH (Trojahn et al. 2015), and small organic acids, such as free fatty acids and HA that are produced by probiotics, could be used to restore the normal acidic milieu of the skin (Sharma et al. 2016). In aged skin, the recovery from acetone-induced barrier disruption is enhanced after treatment with an emulsion at pH 4 compared with pH 5.8 (Angelova-Fischer et al. 2018). Also, the ceramide content and number of lipid lamellae in the skin increase when it is treated with an acidic skin care product (Blaak et al. 2017). In an atopic dermatitis animal model, the application of acids decreased the TEWL and improved SC hydration, indicating improved epidermal barrier function (Lee et al. 2016b).

Short chain fatty acids (SCFAs) and lactic acid are fermentation products that are generated by probiotics and have been implicated in skin health (Collins and Reid 2016). L-lactic acid, produced by lactic acid bacteria as a metabolic fermentation product, has been shown to increase ceramide production; improve barrier function, as measured by TEWL (Rawlings et al. 1996); and function as an antimicrobial agent in the skin and for skin-brightening purposes (Algiert-Zielinska et al. 2019). Butyrate, another microbial small organic acid, has been shown to have anti-inflammatory activities (Krejner et al. 2018; Keshari et al. 2019).

It remains to be determined whether these short-chain organic acids have benefits apart than their acidifying effects; many effects have been described for SCFAs in the gastrointestinal tract and systemically, from improving mineral and fluid absorption to providing a versatile energy source for the gastrointestinal epithelium and regulating various satiety-linked signaling and metabolic functions (Tungland and Tungland 2018). SCFAs can be used in cross-feeding by other microbes (Tungland and Tungland 2018), which could be used to balance the skin microbiota during microbial insults that disrupt the microbiota composition (Reid et al. 2011).

4.3.1.2 Pathogen Inhibition by Competitive Exclusion, as Well as by Production of Small Organic Acids and Bacteriocins

In gastrointestinal applications, a key function of probiotics is their anti-pathogenic function. This activity is elicited by competitive exclusion through competition for nutrients or cell surface adhesion sites or through the production of metabolites that inhibit the growth of pathogens. The production of acids is one such key mechanism, reducing the luminal pH to acidic levels in the gastrointestinal tract, rendering the environment unfavorable to many pathogens (Collado et al. 2009). Bacterial adhesion is the first step in the colonization of beneficial and pathogenic bacteria and in biofilm formation (Hori and Matsumoto 2010). In skin, pathogenic bacteria can adhere, for instance, to ECM proteins, such as fibronectin, collagen, and laminin (Singh et al. 2012; Hammerschmidt et al. 2019). The adherence of probiotics to the

same ECM proteins and competition for adhesion sites can be used to inhibit pathogenic adherence, as seen for *L. plantarum* CRA21, which competed with *S. aureus* for the same adhesion sites in collagen and reduced levels of the former (Mukherjee and Ramesh 2015).

Many pathogenic skin bacteria, such as staphylococci and cutibacteria, thrive at neutral pH (Ali and Yosipovitch 2013), for this reason a topical probiotic strategy, similar to the gastrointestinal tract, would be to create an acidic milieu to prevent pathogenic growth. Acetic acid, for instance, is an antimicrobial agent that impedes biofilm formation (Ryssel et al. 2009; Halstead et al. 2015) and has antifungal activity, similarly as formic acid (Lastauskiene et al. 2014). Certain lactobacilli and bifidobacteria can produce hydrogen peroxide and diacetyl, which can also function as antipathogenic agents (Lew et al. 2013b). Hydrogen peroxide is used as an antipathogenic mechanism by vaginal lactobacilli and has been implicated in bacterial skin infections and wound management (Kovachev 2018; Zhu et al. 2017; Hor and Liong 2014; Lau and Liong 2014). In a recent study, *Staphylococcus epidermidis* that fermented succinic acid from glycerol inhibited the growth of *Cutibacterium acnes* (Wang et al. 2014). Further, postbiotics that are synthesized during fermentation by several lactic acid bacteria have been shown to prevent the growth of *S. aureus* and *S. epidermidis* (Hor and Liong 2014; Lau and Liong 2014).

Bacteriocins are an abundant and diverse group of antimicrobial peptides that are produced by bacteria and archaea (Dobson et al. 2012) and are effective in fighting pathogenic microbes and balancing the skin microbiota (Cruz et al. 2014). Many naturally inhabiting skin microbes, such as isolates of staphylococci, can produce bacteriocins (O'Sullivan et al. 2019). It has been suggested that bacteriocin production by probiotics could be used in the management of acne and atopic dermatitis, but many such reports have been in vitro studies, and clinical evidence is largely absent (Mottin and Suyenaga 2018). For instance, strains of *Ligilactobacillus salivarius* LS03 (Deidda et al. 2018), *L. johnsonii* NCC 533 (Rosignoli et al. 2018), *Lactococcus lactis* HY 449 (Oh et al. 2006), *Streptococcus salivarius* (Bowe et al. 2006), and *Enterococcus faecalis* SL-5 (Kang et al. 2009; Cebrián et al. 2018) produce bacteriocins that act against skin pathogenic bacteria.

Bacteriocins can function as signaling molecules for other microbes and the immune system and facilitate the colonization of microbes in the gastrointestinal tract (Dobson et al. 2012). Bacteriocins might have similar signaling roles in the skin, which remains to be examined.

4.3.1.3 Enhancement in Skin Barrier by Hyaluronic Acid and Lipid Production

Hyaluronic acid (HA), a component that is widely used in cosmetics industry, is produced by several prokaryotes, including lactobacilli (Lew et al. 2013b; Choi et al. 2014; Hor et al. 2014), and *Bifidobacterium*-fermented soy milk have been reported to enhance HA production by keratinocytes (Miyazaki et al. 2003). Several gram-positive and gram-negative bacteria synthesize HYALs, enzymes that degrade HA, which are used as part of the pathogenic mechanisms of pathogenic bacteria, such as *C. acnes* and *S. aureus* (Hynes and Walton 2000; Nazipi et al. 2017; Ibberson et al.

2014). It would be interesting to examine the function of HA-producing versus HA-degrading bacteria and determine whether the HA that is produced by bacteria can be used as a therapeutic against HA-degrading bacteria, especially because HA has antimicrobial properties (Ardizzoni et al. 2011; Pirnazar et al. 1999).

Another example of role of probiotics in skin permeability barrier relates to effect on ceramides. The probiotics *Streptococcus thermophilus* and *Lacticaseibacillus rhamnosus* FTDC 8313 express SMase and thus contribute to ceramide synthesis when applied topically, as indicated by in vitro studies (Di Marzio et al. 1999; Lew and Liong 2013) and in a clinical intervention study (Di Marzio et al. 2003, 2008). Certain ceramide sphingolipids, such as phytosphingosine, also have antimicrobial effects against *C. acnes*, and this is another reason, why probiotics have been suggested in the management of acne (Lee et al. 2019b). In an atopic dermatitis mouse model, oral lactic acid bacterial postbiotics increased ceramide production in the skin (Tokudome 2018), indicating that even oral administration of probiotics and postbiotics has a benefit on skin permeability barrier.

4.3.1.4 Enhancement in Immune Function by Bacterial Cell Wall Components and Bacterial DNA

Parabiotics contain either intact but inactivated probiotic cells or lysed bacterial cells and thus might harbor various metabolites, bacterial DNA or cell wall components, and fragments that are released into the surrounding matrix. Lipoteichoic acid (LTA) is a major structural component of the cell wall of gram-positive bacteria (Lew and Liong 2013). It has been implicated in bacterial cell adhesion, and through its binding to TLR2 and TLR6, LTA has immunomodulatory functions, rendering it an important virulence factor of gram-positive bacteria (Bermudez-Brito et al. 2012; Kang et al. 2016). There are structural differences in the composition of LTA between bacteria, affecting the immunological response (Lew and Liong 2013). For example, LTA from staphylococci in normal flora has been shown to have anti-inflammatory functions (Lai et al. 2009). In skin, LTA has been implicated in the stimulation of the host defense against microbial threats (Lebeer et al. 2012).

There indications for interesting anti-aging functions for LTA. In studies on dermal fibroblasts, LTA from *Latilactobacillus sakei* KCCM 11175P and *L. plantarum* have been shown to reduce the amount of UV-irradiation induced MMP-1 expression suggesting benefits against photoaging (You et al. 2013; Hong et al. 2015). Further, *L. plantarum* LTA promotes type 1 procollagen synthesis and has antioxidative effects, reducing UV-induced ROS generation (Hong et al. 2015). In mouse melanoma cells, LTA from *L. plantarum* was able to reduce tyrosinase activity and melanogenesis (Kim et al. 2015b).

Peptidoglycan is another major structural component abundant in the cell wall of gram-positive bacteria, differing in composition between various bacteria (Navarre and Schneewind 1999). It activates TLR2 and stimulates innate immunity (Bermudez-Brito et al. 2012). Mammals have several peptidoglycan recognition proteins that are expressed in skin that might function in antibacterial defense and under inflammatory conditions (Park et al. 2011). Peptidoglycan stimulates human keratinocyte β-defensin production, which has been implicated in the resistance of

epithelial surfaces to microbial colonization (Sorensen et al. 2005). Stimulation of TLR2 on epidermal keratinocytes with peptidoglycan has been shown to enhance the tight junction barrier and upregulate tight junction proteins, suggesting contribution to the maintenance of the cutaneous permeability barrier and preventing the invasion of pathogens (Yuki et al. 2011; Kuo et al. 2013).

Extracellular exopolysaccharides (EPSs) that are produced by lactic acid bacteria have many proposed health benefits, such as cardioprotective, antiulcer, antioxidant, and cholesterol-lowering effects and the management of allergies and dermatitis (Korcz et al. 2018; Xu et al. 2019). These polysaccharides have been shown to enhance wound healing by improving re-epithelialization (Trabelsi et al. 2017). EPSs have anti-inflammatory functions, for instance, in a contact dermatitis mouse model (Noda et al. 2019; Gotoh et al. 2017) and in in vitro studies (Gorska et al. 2016). In an in vitro assay, EPS that was produced by lactobacilli showed anti-collagenase, anti-elastase, and antioxidant activities in fibroblast cultures, indicating that EPS could have role as anti-aging and regenerative functions (Shirzad et al. 2018). The benefit of EPS has been indicated by a study in which mice were administered oral EPS and exposed to UV radiation, experiencing attenuation of the erythema and modulation of the immune response (Morifuji et al. 2017).

Bacterial DNA, especially CpG DNA and unmethylated CpG oligonucleotide mimetics, is recognized by toll-like receptors—specifically, by TLR9—and certain CpG structures that are released by lactobacilli have been shown to mediate anti-inflammatory functions (Bermudez-Brito et al. 2012). CpG oligonucleotides have been suggested as a potential treatment for allergies, because they can direct the production of Th2 cytokines, which are prominent in allergies, toward the Th1 type (Inoue et al. 2005; Kim et al. 2007). DNA from various lactobacilli and bifidobacteria was shown dose-dependently to inhibit Th2 cytokines and modulate the Th1/Th2 response to allergens (Ghadimi et al. 2008). CpG oligonucleotides also enhance wound healing by increasing basic fibroblast growth factor levels and keratinocyte migration (Sato et al. 2010; Yamamoto et al. 2011).

4.3.2 Effects of Topical Probiotics, Parabiotics, and Postbiotics on Skin Health

There is increasing interest in using topical probiotics, parabiotics, and postbiotics in dermatology (Knackstedt et al. 2020; Lolou and Panayiotidis 2019). Many in vitro studies have concentrated on the antimicrobial effects of topical probiotics (Lopes et al. 2017) and parabiotics and postbiotics against, for instance, *C. acnes* (Al-Ghazzewi and Tester 2010; Kang et al. 2012; Deidda et al. 2018) and *S. aureus* (Mohammedsaeed et al. 2014; Rosignoli et al. 2018). Clinical trials have examined the effects on atopic dermatitis (AD) patients (Blanchet-Rethore et al. 2017; Gueniche et al. 2008), and there are several studies on effects against skin pathogens infecting skin wounds or causing skin microbiota dysbiosis (Cabrera et al. 2016; Hafez et al. 2013; Jebur 2010; Prince et al. 2012; Ramos et al. 2015; Valdez et al. 2005; Lau and Liong 2014).

In this section, we will not delve into these antibacterial effects, diseases, or clinical conditions, such as atopic dermatitis (Kawahara et al. 2018; Park et al. 2014) and acne (Muizzuddin et al. 2012). The studies that tested combinations of bacteria and postbiotics (Im et al. 2018, 2019) and fermentation products (Baba et al. 2006; Kaur and Rath 2019; Kim et al. 2017; Lee 2018; Rong et al. 2017) are not reviewed here, either.

Although we only present research on individual strains and metabolism products from individual strains, cocultures of single bacterial strains might yield beneficial molecules. For instance, a quorum-sensing molecule, produced by a coculture of *L. plantarum* DC400 with *Fructilactobacillus sanfranciscensis* DPPMA174, enhanced the proliferation and migration of human keratinocytes in vitro (Pinto et al. 2011), decreased ROS levels, and upregulated human β-defensin-2 (Marzani et al. 2012).

In addition to the studies that are presented here, some of the in vitro research in the previous section (oral probiotics and parabiotics) might be equally relevant to topical probiotic and parabiotic prescreening with skin cells. Tables 4.6 and 4.7 list examples of various research approaches for topical probiotics, parabiotics, and postbiotics in skin health, comprising in vitro and ex vivo research and clinical trials, respectively.

4.3.2.1 Strain-Specificity and Dependence of the Application Form of Topical Probiotics

Sultana et al. (2013) (Table 4.6) and Putaala et al. (2012) (Table 4.6) have been studying the effects of parabiotics from various strains of lactobacilli on human primary keratinocyte tight junction function. In addition to studying lysed probiotic cells, Putaala et al. (2012) compared the effects with intact cells and postbiotics, from the same strains. Strain-, species-, and application form-dependent effects on transepithelial electrical resistance (TEER) values were observed when differentiated normal human epidermal keratinocytes (NHEKs) were treated with *L. acidophilus* NCFM, *Bifidobacterium animalis* subsp. *lactis* 420 (B420), *L. acidophilus* La-14, *Ligilactobacillus salivarius* Ls-33, and *Propionibacterium jensenii* P63. Sultana et al. (2013) made similar observations of strain-dependent behavior with lysed parabiotics from *Bifidobacterium longum* and *L. rhamnosus* GG strains, measuring the expression of the tight junction proteins claudin-1 and -4, ZO-1, and occludin from keratinocytes with and without TLR2 inhibition (Sultana et al. 2013). Like those from *B. longum* and *L. rhamnosus* GG, lysates from *L. plantarum* and *L. reuteri* enhanced the TEER of keratinocytes, whereas those from *Limosilactobacillus fermentum* decreased such values over time compared with control.

Strain-dependent effects on the expression of NHEK differentiation markers have been observed. Heat-treated *B. longum* NCC2705 (BL/81) upregulated all markers (keratin 1 (KRT1) and 10, transglutaminase 1 (TGM1), filaggrin, loricrin, and involucrin); extract from sonicated *B. longum* Reuter (BL/84) upregulated only some of them (KRT1 and 10, TGM1), whereas heat-treated *B. longum* NCC3001 (BL/64) had no effects (Table 4.6) (Szollosi et al. 2017). The effect of the BL/81 was

Table 4.6 Studies on the effects of topical probiotics, parabiotics, and postbiotics in vitro and ex vivo

Health area	In vitro/ex vivo subjects	Bacteria for experiments	Stress model	Endpoints	References
Reactive skin	Ex vivo skin explants	*B. longum reuter* lysate, 10% (40 µL/cm^2)	Substance P (SP)	Histological evaluation of inflammatory reaction, TNF-α	Gueniche et al. (2010a)
Reactive skin	Dorsal root ganglia (DRG) nerve cell cultures	*B. longum reuter* lysate, 3%, 1%, and 0.3%	Capsaicin	Calcitonin gene-related peptide (CGRP)	Gueniche et al. (2010a)
Skin microbiota homeostasis, anti-inflammatory	Bacteria cells, normal human epidermal keratinocytes (NHEKs)	*L. brevis* DSM17250 cell-free extract, 0.2 mg/g to 4 mg/g	LPS exposure	*Staphylococcus epidermidis* growth, IL-1α	Holz et al. (2017)
UV-induced skin damage	Human dermal fibroblasts (HDF)	*L. plantarum* lipoteichoic acid (pLTA), 0.1–100 µg/mL	UV irradiation	MMP-1, extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNKs), activator protein-1 (AP-1), (NF-kappa B), type 1 procollagen, ROS	Hong et al. (2015)
Melanogenesis	Acellular	Crude culture filtrates of *Enterococcus* sp. EA3 and EB2, *Pediococcus* sp. PC2 and PD3	NA	Tyrosinase inhibitory activity, DPPH radical scavenging activity, ABTS radical scavenging activity, and superoxide dismutase (SOD)-like activity	Ji et al. (2018)
Antimicrobial, anti-inflammatory, anti-aging	Bacteria, fungi, HaCat keratinocytes, and fibroblasts ATCC CRL—2072	Live and inactivated (freeze-thaw) *B. longum* 5^{1A}, *B. pseudolongum* 119^{1A}, *B. bifidum* 162^{2A}, and *B. breve* 110^{1A}, 10^8 to 1 CFU/well	Inactivated *S. aureus* ATCC 29213 for cytokine assay	Antagonism, cytotoxicity, adhesion and internalization to keratinocytes, cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-18, and IL-19), collagen, lumican, and periostin	Silva et al. (2018)

(continued)

Table 4.6 (continued)

Health area	In vitro/ex vivo subjects	Bacteria for experiments	Stress model	Endpoints	References
Skin barrier	Normal human epidermal keratinocytes (NHEKs)	Lysates from *B. longum* ATCC 51870, *L. plantarum* ATCC 10241, L. *reuteri* ATCC 55730, *L. rhamnosus* GG ATCC 53103, and *L. fermentum* ATCC 14932, 10^8 to 10^2 CFU/mL	TLR 2 inhibition	Viability, transepithelial electrical resistance (TEER), claudin-1 and-4, occludin, zonula occludens-1 (ZO-1)	Sultana et al. (2013)
Skin barrier	Normal human epidermal keratinocytes (NHEKs)	Live and lysed bacteria, bacterial metabolites of *L. acidophilus* NCFM, *B. lactis* B420, *L. acidophilus* La-14, *L. salivarius* Ls-33, and *P. jensenii* P63, 1.1×10^7 CFU/well and 10% bacterial culture media	NA	Transepithelial electrical resistance (TEER), claudin-4, occludin, zonula occludens-1 (ZO-1)	Putaala et al. (2012)
Differentiation and cellular functions	Normal human epidermal keratinocytes (NHEKs)	Heat-treated *B. longum* NCC3001 (BL/64) and *B. longum* NCC2705 (BL/81), sonicated *B. longum* Reuter (BL/84), 0.3–3%	NA	Viability, differentiation markers (KRT1 and 10, TGM1, filaggrin, loricrin, and involucrin), β-defensin 1, cathepsin S, B, D, and H	Szollosi et al. (2017)
Antioxidant, whitening, and moisture	Acellular	*L. rhamnosus* LRH113-produced metabolites, 10–100 v/v %	NA	DPPH and ABTS+ radical scavenging, tyrosinase inhibition	Tsai et al. (2013)
UV-induced skin damage	Normal human dermal fibroblasts (NHDFs) and human monocyte-like cell line THP-1	*L. sakei* KCCM 11175P lipoteichoic acid (sLTA), 0.01–10 μg/mL	UVA irradiation, TNF-α, LPS	Viability, TNF-α, MMP-1, MAPK signal (p38, p-SAPK/JNK, phospho-NF-kappa B P65)	You et al. (2013)

Skin barrier	Reconstructed human epidermis (RHE)	*L. rhamnosus* lysate, exact concentrations not disclosed	Sodium lauryl sulfate (SLS)	Claudin 1, occludin, loricrin, filaggrin, desmosomes, rhodamine B permeability	Jung et al. (2019)
Anti-inflammatory and skin barrier	Human native epidermal explants and reconstructed human epidermis (RHE)	Live and lysed *L. reuteri* DSM 17938, live: 6×10^9 to 3×10^5 CFU/mL, lysates: 1.69×10^5 and 1.69×10^2 CFU/skin piece, and 6×10^5 and 3×10^2 CFU/mL	UVB irradiation	IL-6, IL-8, laminin A/B, aquaporin 3 (AQP3), kallikrein 5 (KLK5), occludin, claudin-1	Khmaladze et al. (2019)
Skin moisture	HaCat keratinocytes	Sonicated *S. thermophilus* S244, 50 mg/5×10^6 keratinocytes/10 mL	NA	Sphingomyelinase (Smase), ceramides, ceramide synthase, choline, cholesterol	Di Marzio et al. (1999)

Table 4.7 Clinical studies on the effects of topical probiotics, parabiotics, and postbiotics

Health area	Clinical subjects	Bacteria	Endpoints	Intervention length	References
Reactive skin	Female volunteers with reactive skin	Topical cream with *B. longum* Reuter lysate, 10%	Skin sensitivity, TEWL, natural moisturizing factor (NMF) components (pyrrolidone carboxylic acid, sodium lactate, serine, and urea)	2 months	Gueniche et al. (2010a)
Skin microbiota homeostasis	Volunteers aged 19–47 years, with dry skin	Topical cream with *L. brevis* DSM17250 cell-free extract, 0.88 mg/g	TEWL, xerosis cutis symptoms, stinging, skin microbiota	4 weeks	Holz et al. (2017)
Anti-aging	Volunteers aged 19–61 years with clear skin	Live *N. eutropha* in buffer, 1×10^9 and 8×10^9 cells/mL	Facial wrinkles, pigmentation, and radiance	7 days	Notay et al. (2020)
Skin moisture	Healthy volunteers aged 24–47 years	Sonicated *S. thermophilus* S244 (1.7 g/5 mL) mixed with 20 mL of a base cream	*Stratum corneum* ceramides	1 week	Di Marzio et al. (1999)
Skin hydration	Healthy females aged 65–71 years	Sonicated *S. thermophilus* S244 (1.7 g/5 mL) mixed with 20 mL of a base cream	Skin hydration, TEWL, *stratum corneum* ceramides	15 days	Di Marzio et al. (2008)

the strongest among the three strains also on analysis of antimicrobial peptide production (β-defensin 1 and cathepsin S) and the expression of molecules that are involved in wound healing (cathepsins B, D, and H).

The effects of live and lysed products of *L. reuteri* DSM 17938 on inflammation and skin barrier were studied and compared using ex vivo human epidermal explants and a reconstructed human epidermis (RHE) model with UVB irradiation stress. *L. reuteri* DSM 17938 decreased UV-induced inflammation and the cytokines IL-6 and IL-8, similar to lysed and live cells on RHE. With regard to skin barrier function, only live *L. reuteri* upregulated aquaporin 3 (AQP3) and downregulated kallikrein 5 (KLK5), whereas laminin A/B expression rose only with lysate after 24-h topical

incubation on RHE. Live and lysed cells had no effects on the expression of occludin and claudin-1 (Table 4.6) (Khmaladze et al. 2019).

These effects vary within a genus. Silva et al. found that four *Bifidobacterium* spp.—*B. longum* 5^{1A}, *Bifidobacterium pseudolongum* 119^{1A}, *Bifidobacterium bifidum* 162^{2A}, and *Bifidobacterium breve* 110^{1A}—after 6-h stress with inactivated *S. aureus*, had strain- and application form (live or inactivated)-dependent effects on cytokine production in keratinocytes and fibroblasts (Table 4.6) (Silva et al. 2018). Further, live *B. longum* 5^{1A} had the highest antimicrobial activity against pathogenic indicator strains, and all four strains underwent adhesion to and internalization by keratinocytes. Differences in extracellular matrix protein production were also noted (Silva et al. 2018).

The beneficial effects on skin health might depend on the culture time of the probiotic cells. This pattern was observed in a study on four lactic acid bacterial strain isolates—*Enterococcus* sp. EA3 and EB2 and *Pediococcus* sp. PC2 and PD3—which showed varying tyrosinase inhibitory activity, depending on the culture conditions (time, temperature, pH, and NaCl concentration) (Table 4.6) (Ji et al. 2018). The antioxidant potential and radical scavenging activity of these strains varied between culture condition parameters (Ji et al. 2018).

4.3.2.2 Topical Probiotics, Parabiotics, and Postbiotics: Emerging Evidence of Health Benefits

The modeling of topical probiotics, parabiotics, and postbiotics with in vitro studies that use ex vivo skin explants, RHE, and cultured keratinocytes (Table 4.6) is more understandable from the perspective of end-consumers, compared with modeling the effects of orally ingested products in in vitro models. However, as reviewed below, the field is complicated, due to differences in probiotic strains and strain specificity, in vitro models, biomarkers, modes of application, and dosages. These characteristics render this area challenging and definite conclusions on the effects difficult to make. Further, the effects are often observed without proper controls and might be marginal, complicating even further. Thus, to obtain reliable evidence on the effects, clinical intervention trials should be conducted. This pursuit will be further complicated by the individual differences in the skin microbiota, when more evidence on its function accumulates.

The effects of *B. longum* Reuter, that has been inactivated by ultrasonography, have been studied in vitro for reactive skin benefits using ex vivo skin explants. The explants were treated systematically with substance P and topically with bacterial lysate. Further, dorsal root ganglia (DRG) neuronal cells were treated with lysed bacteria for 6 h, followed by capsaicin stress. The bacterial lysate attenuated substance P-induced inflammatory reactions in ex vivo skin explants and calcitonin gene-related peptide (CGRP) neuropeptide release from nerve cells after capsaicin stress (Table 4.6) (Gueniche et al. 2010a)—substance P and CGRP are important neuropeptides in the skin (N'Diaye et al. 2017). In a clinical trial of females with sensitive skin, skin sensitivity, as analyzed by lactic acid stinging test, decreased after 2-month daily application of cream that contained the inactivated *B. longum* lysate, providing evidence of its effects in a clinical setting. It also inhibited the

decrease in the levels of natural moisturizing factor (NMF) component urea, but no effects on other NFM components were noted (Gueniche et al. 2010a).

Lysates of *L. rhamnosus* improved skin barrier function after 16 days of every-other-day application in an RHE model in vitro by immunohistochemical and immunofluorescence analysis of claudin-1 and occludin and the skin barrier proteins loricrin and filaggrin (Table 4.6) (Jung et al. 2019). The upregulation of loricrin and filaggrin on RHE by the bacteria lysate was confirmed by gene expression assay after 2-day topical application. *L. rhamnosus* lysate protected against sodium lauryl sulfate (SLS) irritant-induced damages in skin barrier, based on tissue cytotoxicity, rhodamine B penetration, and desmosome degradation (Jung et al. 2019).

Treatment of keratinocytes in vitro with sonicated *S. thermophilus* strain S244 extract resulted in time-dependent increases in ceramide levels but no significant effects on cholesterol levels or ceramide synthase activity. The rise in ceramide production was reproduced in human volunteers who received topical sonicated bacterial extract-containing cream for 7 days (Table 4.6) (Di Marzio et al. 1999). Ceramide levels were also higher with base cream that contained purified neutral SMase (nSMase) from *Bacillus cereus*. When bacterial cell extract with high nSMase activity was incubated with pure sphingomyelin (SM), a dose-dependent generation of ceramides was observed (Di Marzio et al. 1999).

Di Marzio et al. (2008) (Table 4.7) examined the effects of 2-week topical treatment with a cream that contained sonicated *S. thermophilus* cells on the skin barrier, hydration, and *stratum corneum* ceramide levels in healthy female volunteers aged 65–71 years. The bacterial lysate was suggested to benefit aged skin that was prone to dryness, based on the increase in skin hydration and *stratum corneum* ceramides, despite no changes in TEWL values, compared with the vehicle control after 2-week application (Di Marzio et al. 2008). They concluded that the effects were likely attributed to an increase in ceramides by bacterial SMase activity through sphingomyelin hydrolyzation in the skin (Di Marzio et al. 2008).

The effects of topical LTA from *Latilactobacillus sakei* (KCCM 11175P) on UVA-induced skin damage and TNF-α were examined in normal human dermal fibroblasts (NHDFs) (Table 4.6) (You et al. 2013). Topical LTA inhibited the MMP-1 production that was induced by UVA irradiation or TNF-α stress. In addition, LTA suppressed the phosphorylation of mitogen-activated protein kinase (MAPK) signaling in LPS-stressed THP-1 cells and UVA-stressed NHDFs, indicative of its anti-photoaging and immunomodulatory benefits (You et al. 2013). Further, LTA from *L. plantarum* inhibited UV irradiation-induced MMP-1 expression dose-dependently through extracellular signal-regulated kinase (ERK), JNK, and NF-kappa B activation in human dermal fibroblasts, decreased ROS generation, and upregulated type I procollagen production (Hong et al. 2015).

L. rhamnosus-derived metabolites were studied acellularly for their antioxidative, whitening, and moisture functions (Table 4.6) (Tsai et al. 2013). Preliminary data suggest that *L. rhamnosus* metabolites have beneficial dose-dependent effects in skin care, based on DPPH and ABTS+ radical scavenging activities, tyrosine inhibition, and moisture retention, despite undergoing heat treatment, which can affect their composition due to denaturation of proteins, for example.

Notay et al. (2020) (Table 4.7) studied the effects of topical spray of live ammonia-oxidizing bacteria *Nitrosomonas eutropha*, after 7-day use on facial wrinkles, pigmentation, and radiance. This open-label study did not contain a control group but compared higher and lower doses, observing reduction in wrinkle depth, as assessed by high-resolution facial photography, and radiance improved in both groups, although likely to be not clinically significant effect. This study was a subproject of a larger study on the effects on the face microbiome, which will be published separately.

There are indications that topical skin care products have effects on microbial and molecular diversity in the skin (Bouslimani et al. 2019). With regard to homeostasis of the skin microbiota, in a screen of hundreds of lactobacilli, a cell-free extract of *L. brevis* DSM17250 was one of the four candidates that promoted the growth of *S. epidermidis* DSM20044 by agar diffusion test dose-dependently, based on measurements of growth kinetics by optical density (Table 4.6) (Holz et al. 2017). A chemically synthesized peptide, based on the active component of the extract, had anti-inflammatory effects, decreasing the secretion of IL-1α from NHEKs after exposure to LPS.

To examine these effects further, healthy volunteers with dry skin used *L. brevis* extract cream or placebo cream for 2 months (Table 4.7). The cream with *L. brevis* extract increased commensal bacterial amounts and *S. epidermis* counts compared with placebo, whereas *S. aureus* numbers were unchanged in both groups. There were also decreases in TEWL values and xerosis symptoms among those had applied the cream with *L. brevis* extract for 4 weeks (Holz et al. 2017). In addition to the health effects presented above, and the observations of their effects on the homeostasis of skin microbiota, live or lysed probiotics and their metabolites could be used to manage body odor—especially axillary malodor (Callewaert et al. 2017).

4.3.3 Conclusions of Topical Probiotic, Postbiotic, and Parabiotic Studies on Skin Health

Significant research has been performed on the antimicrobial effects of topical probiotics and their derivatives. They can also modify the homeostasis of skin microbiota by promoting the growth of commensal microbes, such as *S. epidermis*. The effects of probiotics depend significantly on the strain, genus, and form of the probiotic. In vitro studies have reported strain-specific differences in epidermal barrier and differentiation and the production of cytokines and matrix proteins. Certain probiotic lysates have indicated also protection against UV irritation and inflammatory reactions in skin models. Few human studies have shown that topically applied probiotic lysates and cell extracts may improve skin hydration and attenuate sensitive skin.

4.4 Manufacturing and Quality-Related Challenges of Postbiotics and Parabiotics

The history and experience in producing live microbes as starter cultures in various food fermentation processes and for the purpose of producing probiotics are extensive. In general, these processes are optimized to yield a high cell count of stable microbes. The production is also subject to certain limitations, e.g., ingredients in the fermentation broth must be food-grade. Further, microbes might require production in the absence of certain common allergens, such as soy, milk, and gluten, etc. (Fenster et al. 2019).

Postbiotics and parabiotics have similar, yet slightly different, requirements.

Postbiotics are primarily metabolites. The focus should thus be on the production of metabolites, not cells. It is especially helpful if the metabolites that are responsible for the desired health effect are known, to which the production process should be adapted accordingly. Certain culture conditions and fermentation ingredients might stimulate the production of the desired metabolites. There might also be opportunities to concentrate or isolate the metabolite from culture liquid. Postbiotics could be standardized toward a minimum level of the desired metabolite to ensure a specific quality of the product. If the culture liquid is the main carrier of the postbiotic, the absence of potential contact allergens is important, as discussed for oral probiotics.

Parabiotics are essentially non-viable microbes or fragments of microbial cells. It might be tempting to consider that obtaining a high cell density should be the main goal in the production of parabiotics. However, it is possible that specific structures must be present and expressed, as in the production of probiotics, necessitating examination at an early stage: does the culturing of the microbes influence their efficacy, even if they are subsequently killed? If this is solved, an appropriate way for inactivating the microbes needs to be found. Although the method of inactivating microbes might seem mundane, it is actually a crucial step to consider in the production of parabiotics (Ouwehand et al. 2000).

In industrial settings, heating is probably the most common way to kill microbes. However, heat-treatment of complex substances, such as microbes and their culture media, can lead to browning reactions, such as caramelization and Maillard-reactions. The influence of heating must thus be taken into account when the process is developed. In particular, the differences between laboratory and industrial scales, with their disparate process durations and hold times, need to be considered. Techniques that minimally influence the physicochemical properties should be contemplated, such as UV irradiation. Thus, products from industrial-scale manufacturing might need to be tested for their efficacy.

Although postbiotics and parabiotics do not contain viable components, their stability must be determined. Enzymatic reactions and oxidation of sensitive components can occur and should be controlled, or it must be ensured that such reactions do not affect the efficacy of the product.

4.5 Future Outlook: Need for Harmonization, Validated Methods, and Well-Designed Clinical Trials

As reviewed by Shane et al. (2010), several relevant points must be considered in designing clinical trials for probiotic applications, such as study design, subjects, investigational products (active and placebo), follow-up time, endpoint measurements, safety evaluations, and regulatory aspects. The firmest evidence of treatment effects is obtained with well-performed, double-blind, randomized, and placebo-controlled trials.

Subjects in the trial should represent the target population of the investigative product, and an appropriate participant number is needed to reach sufficient statistical power in the study and achieve clinical relevance of the results (Bhardwaj et al. 2004). Depending of the research question, exclusion and inclusion criteria should be carefully prepared. The age distribution of subjects can be wider in studies on, for example, skin hydration versus skin aging, wherein the target population would be subjects who are already experiencing the features of aging. If the skin is already in good condition and no inconveniences are encountered, the effects of the investigational product are likely to be invisible.

With regard to probiotic research, subgroup analyses of the subjects can demonstrate additional effects of probiotics, as seen in a trial by Ogawa et al., in which heat-killed probiotic had beneficial effects on dry skin in subjects who consumed low amounts of fermented dairy products (Ogawa et al. 2016). In some studies, only subjects who were generally consuming low amounts of fermented dairy products were included (Gueniche et al. 2014) or excluded (Saito et al. 2017).

A placebo group is important to include when studying the effects of probiotics, as with mixed products, such as probiotic yoghurts, because yoghurt as such contains bacteria and other ingredients (minerals, such as calcium, fats) has been noticed to have an effect on skin health (Vaughn and Sivamani 2015). The appropriate type of placebo group should be established with the study objectives in mind. The placebo group can reveal the effects of, for example, seasonal changes and changes in washing procedures in both study groups, as in a dandruff study (Reygagne et al. 2017). The participants might change their habits, although they are instructed not to do so in many study protocols.

The placebo effects in dermatological itch (van Laarhoven et al. 2015) and clinical painkiller trials (Vachon-Presseau et al. 2018), although performed with patients, might provide good observations when planning, for example, clinical trial protocols for sensitive skin, because differences in brain structure and function (Vachon-Presseau et al. 2018) can result in certain subjects responding effectively to placebo treatment.

In planning studies on skin hydration, one should consider the climate and geographical location. Several clinical studies have reported that seasonal changes affect the skin—during winter in the northern hemisphere, the low temperatures outside increase the TEWL in the skin and decrease its hydration, especially when the humidity is low (Engebretsen et al. 2016), a common situation indoors during winter. When planning a skin hydration study during the moist summer season, the

investigational product might not have effects, because the skin might already be moisturized by the warm and moist season. Drier and colder seasons might therefore be preferred to examine the benefits on hydration, when the skin encounters a stress.

The study length should be considered with the objective and investigational product type in mind. Orally consumed probiotics can take longer to elicit systemic effects, whereas topical products can have faster local effects. Regarding the various health targets in in vivo animal and clinical trials (Tables 4.3, 4.4, and 4.7), the study intervention times vary from 1 week (skin hydration) to 12 weeks (anti-aging). If clinical studies are too long, are participants willing to continue with the study protocol, and are they even willing and patient enough to consume the product to obtain the benefits on their skin?

In Europe, a nonprofit group, the European Group on Efficacy Measurement and Evaluation of Cosmetics and Other Products (EEMCO), provides science-based guidance on study methods for efficacy and safety assessments on several skin-related topics (EEMCO-Group 2019). Several of their guidelines, such as the TEWL assessment (Rogiers 2001) and in vivo assessment of skin surface pH (Parra and Paye 2003), have been used as the basis for study methods in many clinical trials. The uniform use of validated research methods would help standardize and compare the results from trials with inherently many variables. Although the guidelines might be intended for the testing of cosmetics products, they can be valid for efficacy assessments of oral therapeutics for skin health. If there is interest, the necessary research methods that assist in proving the possible claims should be included.

Adding subjective and objective methods to clinical trials on the benefits of therapeutics for skin health is advantageous. Subjective analysis of the investigative product, such as self-assessment questionnaires, discloses important information about the use of the product by consumers. When the subjective analysis results correspond to the objective measurements, there is greater weight on product use, but the subjective evaluation might not always match the objective results.

Dermatological conditions have various symptoms, and their evaluation can be subjective. Certain dermatological conditions have standardized methods for generating visual descriptions, such as the SCORAD evaluation for atopic dermatitis, but it is prone to investigator bias.

The microbiome has myriad potential applications. Every year brings more knowledge on how important our microbiota is and how disruptions in its homeostasis might result in illnesses. The environment and our modern lifestyle have spawned several stress factors that the skin must counter—microbes and microbe-derived molecules might be important aids in normalize such stressful situations.

Advances in technologies, such as sequencing, imaging, -omic technologies, machine learning, and portable machines, might enable the use of new biomarkers and endpoints for future dermatological research.

4.6 Concluding Remarks

There is no definition for the healthy skin microbiota, because it is highly variable, depending on the individual and anatomical site. However, species- and strain-specific effects of probiotics on skin health have been identified. Oral probiotics have been shown to influence skin barrier function, hydration, and UV-induced immune suppression and cellular damage. Topical application of probiotics would represent an advance in skin health by improving the skin barrier and inhibiting pathogenic skin microbes. However, more research is needed to understand the interaction of probiotics, parabiotics, and postbiotics with the skin microbiota and skin physiology. To follow with the growing interest in the use of probiotics, parabiotics, and postbiotics for skin health, official guidelines on their definitions, manufacturing, and quality are needed to create common frameworks in the personal care and cosmetics industries and improve consumer confidence in these products.

There are also technical questions that are related to viable topical probiotics and whether it is possible to incorporate viable probiotics in topical products, given the issues with emulsifiers, water activity, and preservatives. At the moment, probiotics for skin care are the same species that have been regarded as being safe for human consumption in oral applications. Next-generation probiotics from healthy skin and true inhabitants of the skin might provide solutions in the future for this complicated issue, at least partially.

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Topical Probiotics: Scope and Challenges

5

Aakriti Sethi and Jinyan Tao

Abstract

A significant upsurge has been discerned on the utilization of probiotics in the amelioration of skin associated disorders since the commencement of the twenty-first century. An extended therapeutic profile where topical probiotic therapies can be exercised has been discovered, such as inflammation, fungal infections, microbial infection, and treatment of atopic dermatitis. Topical therapy with *Lactobacillus*, *Nitrosomonas*, *Streptococcus*, and *Bifidobacterium* has shown to ameliorate skin inflammation by prompting decolonization of pathogens that reside on the skin such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Cutibacterium acnes*, *Acne vulgaris*, etc. However, none of the probiotics have been approved to be labelled as "drugs" by the US Food and Drug Administration. Even though the emerging therapeutic effects of probiotics potentiate a wider therapeutic usage, it still necessitates a thorough assessment for its safety and efficacy profile. A review on the present-day topical probiotic therapy was formulated, and the scope and challenges associated with the therapy are discussed in the chapter.

Keywords

Probiotics · Topical therapy · Dermatitis · Acne · Aging

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5.1 Introduction

With advances of next-generation sequencing and rapid development of bioinformatics analyzing tools, researchers have achieved breakthroughs investigating the microbes inhabiting humans, soils, oceans, environment, etc. The microbial colonies which are usually found to inhabit humans in different passages, in GIT, nasal, urogenital, oral cavity, and skin, have been highlighted to be crucial for the health of humans. The varying microbial colonies present in humans including bacteria, skin mites, viruses, and eukaryotes exhibit a considerable interaction with the host, and any interference with such association might disrupt the homeostasis of the human body (Grice and Segre 2011). The human microbiota profoundly safeguards against the progression of serious pathological diseases by shielding against exogenous pathogens and initiating immune response. Therefore, alteration in the gut microbiome might impede with the autoimmune activity of the system, leading to the development of inflammatory disorders, not only in the gut but also on the skin as shown in Fig. 5.1. Even though the concentration and colonies of bacteria present on the skin are comparatively less than what is found in the gut, the imbalance in the former might also obstruct the regulation of immune system (Sanford and Gallo 2013).

A wide range of studies have revealed that the administration of probiotic supplements could ameliorate several GIT disorders, such as lactose intolerance, diarrhea, enterocolitis, colorectal cancer, irritable bowel syndrome, and ulcerative colitis (Pace et al. 2015). Hence, probiotics can be defined as "living microorganisms, which upon administration in the sufficient amounts, extend health benefits to the host organism" (Fuller 1991). Although a number of strains of microorganisms are present, the ones that manifested significant importance in

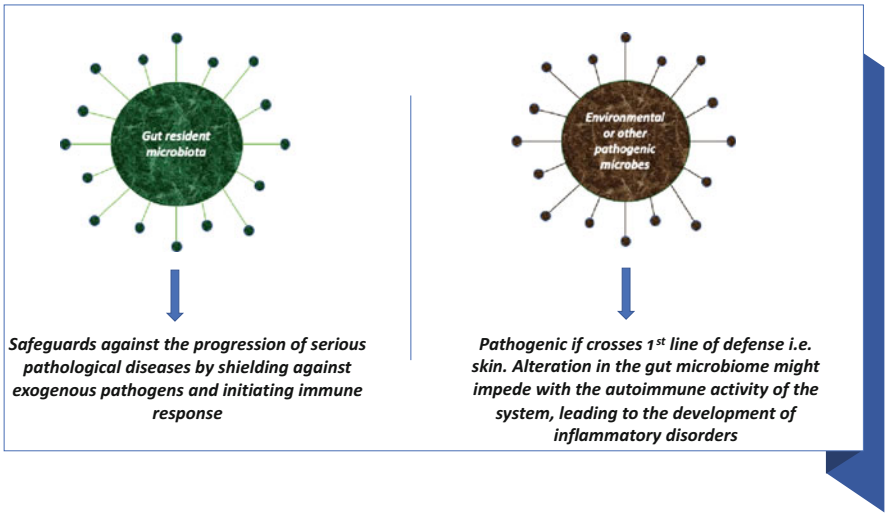

Fig. 5.1 Comparison between the gut resident and pathogenic microbes

improving the state of the skin, when administered orally, include *Lactobacillus* and *Bifidobacterium*. The mechanism by which both these strains of bacteria exert propitious effects, providing health benefits, are summarized as follows:

- Modulating the immune activity by initiating antioxidant and anti-inflammatory effects.
- Producing substances which act against the other deleterious microbes.
- Compete with other detrimental pathogens, leading to their exclusion.
- Induction of apoptosis (Ohashi and Ushida 2009).

In addition, probiotics have exhibited beneficial effects such as healing of wounds, safeguarding against photoaging, and relieving symptoms associated with skin diseases such as psoriasis and dermatitis. However, due to limited clinical evidence supporting the mechanism of action of probiotics, there lack guidelines and recommendations for proper administration and use (Lukic et al. 2017). Herein, this chapter reviews the topical use of probiotics (in vitro and in vivo studies) in certain skin disorders such as acute dermatitis, seborrheic dermatitis, wound, acne, and psoriasis (Fig. 5.2). Also, the chapter focuses on the future challenges and the present research direction in the area of probiotic therapy.

5.2 In Vitro Assays to Determine the Efficacy of Probiotics Used in Topical Application

5.2.1 Determining In Vitro Adhesion Characteristics of Probiotics

To test the topical therapeutic effect of probiotics, an in vitro study can be performed which evaluates the adhesion percentage of different probiotic strains on keratin obtained from human epidermal skin. A comprehensive adhesion study showed that different probiotic strains exhibited different adhesion percentages to the human keratin. *L. plantarum* 226v, *Lactobacillus salivarius* 20,555, *Lactobacillus casei* 01, *Bifidobacterium longum, Lactobacillus brevis* D-24, *Lactobacillus rhamnosus* 20,021, and *L. casei* 431 showed the lowest adhesion percentages (below 5%), whereas *Propioniferax innocua, Lactobacillus delbrueckii, Lactobacillus paracasei* LA-26, *Bifidobacterium* Bb12, *Lactobacillus acidophilus* LA-5, *L. acidophilus* LA-10, and *Bifidobacterium lactis* B-94 showed high adhesion percentages. *Bifidobacterium* Bb12 and *Bifidobacterium lactis* B-94 showed best adhesion to the human keratin with a percentage of 35 and 31.1% (Ouwehand et al. 2003; Moisés Laparra et al. 2011). Apart from this, the study also revealed that probiotics could change the affinity of certain pathogenic strains to keratin as well as inhibit the pathogenic activity as shown in Table 5.1.

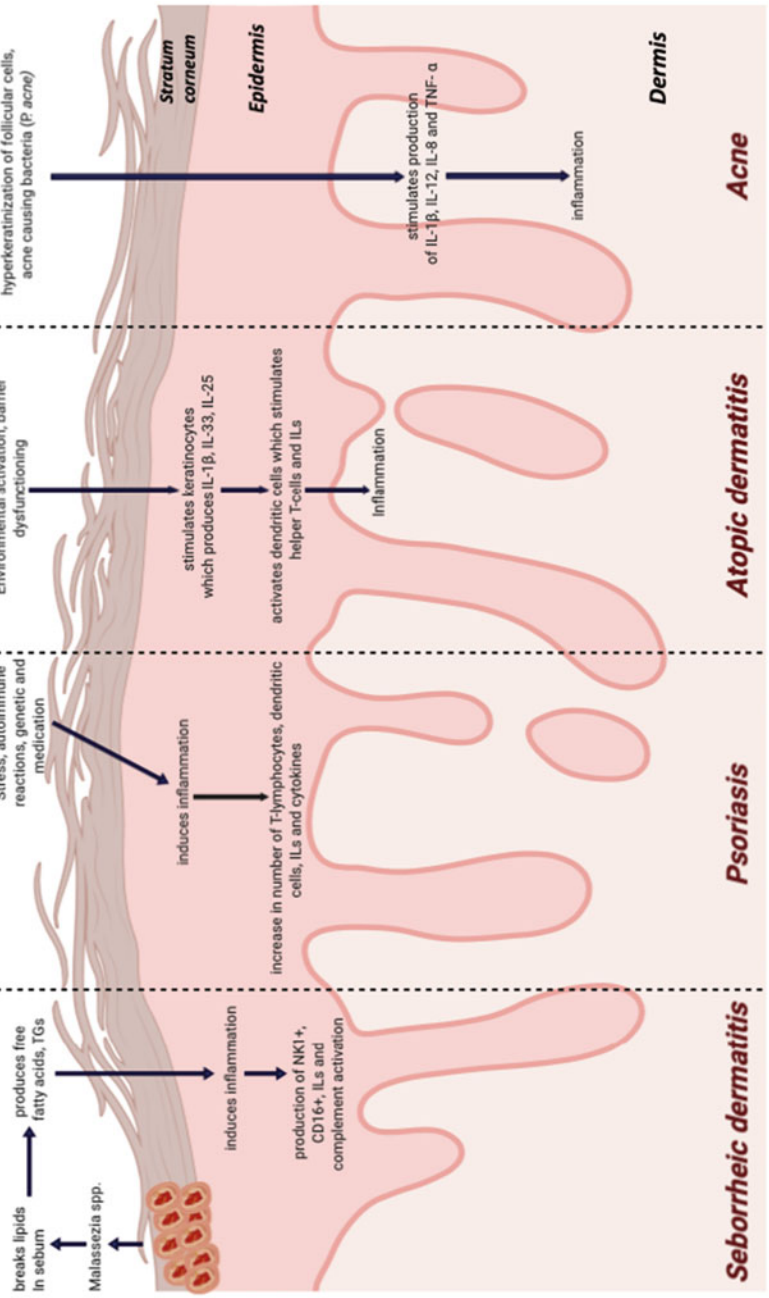

Fig. 5.2 Mechanism underlying the pathophysiology of skin disorders such as seborrheic dermatitis, psoriasis, atopic dermatitis, and acne

Table 5.1 Effect of probiotics on affinity of certain pathogenic strains towards keratin (Lopes et al. 2017b)

Pathogenic strain	Selected probiotic strain	Effects
Propionibacterium acnes	*P. innocua*	Significantly reduced the adherence of bacterial strain
Escherichia coli	*B. animalis* Bb12	Significantly increased bacterial strain adherence
Pseudomonas aeruginosa	*B. animalis* Bb12, *B. lactis* B-94	Both probiotic strains significantly increased bacterial strain adherence to keratin
Staphylococcus aureus	*P. innocua, B. animalis* Bb12, *B. lactis* B-94 and *L. acidophilus* LA-10	All the probiotic strains significantly increased bacterial strain adherence to keratin

5.2.2 Determining In Vitro Antimicrobial Characteristics of Probiotics

Two antimicrobial assays can be performed to determine the antimicrobial activity of probiotics: well diffusion assay and quorum sensing assay. To prepare for the assays, cell-free culture supernatants (CFCS) are extracted from specific probiotic strains having high adhesion percentage. After sterilization, these CFCS will be added into the sterile tubes and were preserved at −20 °C unless required.

In the well-diffusion assay, both neutralized and unneutralized CFCS were employed, and inhibition zones were recorded outside the wells. In quorum sensing assay, only unneutralized CFCS of some specific probiotic strains were used. The method detects the presence of quorum sensing (QS) antagonists in the CFCS using *Chromobacterium violaceum,* which acts as a biosensor and produces a violet colored pigment violacein. The amount of depleted violacein is measured as a potential inhibition of QS which is calculated by recording the inhibition zones outside the wells.

For well diffusion assay, among all the lactobacilli strain tested, such as *L. acidophilus* LA-10, *L. paracasei* L-26, *L. acidophilus* LA-5, and *L. delbrueckii,* all but *L. delbrueckii* showed significant inhibition zones (range 0.8–2.3 mm) against *P. aeruginosa, P. acnes*, and *E. coli. L. delbrueckii*, however, exhibited significant inhibition zone against *P. aeruginosa* (0·6 mm ± 0·1) and *P. acnes* (2·0 mm). Out of *B. animalis* Bb12, *B. lactis* B-94, and *P. innocua,* the former exhibited zone of inhibition (range 0.5–1.3 mm) against *P. acnes*, *P. aeruginosa*, and *E. coli*, while the latter two did not show inhibition against any of the pathogens.

However, when evaluated with neutralized CFCS, no antimicrobial activity was observed. The unneutralized bacteria produce organic acids as well as bacteriocins (antimicrobial substance) which exhibit the inhibition effect for the bacterial growth. The organic acids responsible for antimicrobial properties are neutralized, leading to altered antimicrobial activity (Tejero-Sariñena et al. 2012).

For quorum sensing assay, QS antagonistic activity of *L. acidophilus* LA-10, *L. paracasei* L-26, *L. acidophilus* LA-5, *L. delbrueckii*, *B. animalis* Bb-12, *B. lactis*

B-94, and *P. innocua* was tested. QS inhibition was recognized by the absence of pigmentation of *C. violaceum.* The study unveiled that selected probiotics *B. lactis* B-94 and *P. innocua* exhibited a minimized production of violet pigmentation, where their zone of inhibition values varied from 0.47 to 2.83 mm. *L. acidophilus* LA-10 showed the highest zone of inhibition, while *L. delbrueckii* showed the lowest. Therefore, the results suggested that probiotics have a tendency to inhibit AHL production (***N*-Acyl homoserine lactones**), which are involved in quorum sensing in bacteria to facilitate gene expression and group coordination. Thus, the production of QS inhibitors by probiotics creates hindrance in the production of AHL (McLean et al. 2004).

5.2.3 Determining In Vitro Antibiofilm Activity of Probiotics

Biofilms allow pathogenic microbes to confine nutrients and stand against the harsh environmental conditions. The study evaluated the effect of selected probiotic strains in inhibiting the formation of biofilms and destroying the ones which are already formed by the pathogenic bacterial strain. All five probiotic strains, *L. acidophilus* LA-10, *L. paracasei* L-26, *L. acidophilus* LA-5, *L. delbrueckii*, and *B. animalis* Bb-12 reportedly reduced the biofilm formation capability of *E. coli* (variation in the range of inhibition from 22.9 to 13.1%). Among all five, *L. delbrueckii* and *B. animalis* Bb-12 exhibited maximum inhibition. Similarly, *S. aureus* and *P. aeruginosa* also exhibited decreased cell attachment against all the five probiotic strains, where their inhibition range varied from 37.7 to 30.4% and 20.3 to 8.1%, respectively. In case of *P. acnes*, all probiotics mentioned above, including *P. innocua*, exhibited reduced biofilm formation capability with an inhibition range varying from 20.5 to 9.8% (Sandasi et al. 2010; Djordjevic et al. 2002).

5.3 In Vivo Studies to Determine the Efficacy of Probiotics Used in Topical Application

5.3.1 Seborrheic Dermatitis

Seborrheic dermatitis is a chronic and inflammatory skin disease; however, the pathophysiology of the disease is still indefinite (Berk and Scheinfeld 2010). It has been observed that an aggravation in the fungi *Malassezia* count, belonging to the yeast family, is found to be closely correlated with seborrheic dermatitis. Thus, treatment with certain antifungal agents tends to minimize the associated symptoms (Gaitanis et al. 2012). However, the precise pathophysiology behind *Malassezia* being the predominant disposing factor for causing seborrheic dermatitis is still ambiguous. Studies have shown that an alleviation in the yeast count and not the absolute values of the fungi *Malassezia* are correlated with the severity of disease. Thus, a decrease in the burden of yeast count is a desirable measure of severity of disease (Pierard et al. 1997). Due to this reason, researchers have assessed the

utilization of probiotics in treating seborrheic dermatitis. A double-blind, randomized trial was carried out in 60 patients having moderate seborrheic dermatitis of the scalp. Two groups, one treated with the vehicle while the other treated with the topical lotion of *Vitreoscilla filiformis*, were evaluated for the decrease in symptoms associated with seborrheic dermatitis. It was observed that the group of patients treated with the *V. filiformis* lotion exhibited significant amelioration (62.7% reduction in the scaling, pruritus and erythema) compared to the vehicle treated group (26.1% reduction in the symptoms mentioned above) when treated for 4 weeks, once a day. Thus, a highly significant contrast could be observed between the two groups (Gueniche et al. 2008). *V. filiformis* lysate enhances the production of IL-10 by stimulating dendritic cells and also inflates the activity of regulatory T-cells (Biedermann et al. 2015). Another study was performed on NC/Nga mice, where the cutaneous treatment with *V. filiformis* remarkably reduced the skin inflammation, thus, acting as an immunosuppressant (Volz et al. 2014).

5.3.2 Wound

As soon as the pathogen infects the skin, commencement of the skin infections begins. Any accidental cause or a disease effect might disrupt the skin adherence, leading to the wound formation (Guo and Dipietro 2010). Studies have shown that the development of wound infections take place when exogenic bacteria shows a dominant effect in comparison to the resistance offered by the first layer of defense of the host. Hence, it is necessary to maintain a balance between the two in order to allow the normal functioning of the wound healing process (Negut et al. 2018). Over the past few years, researchers have shown that the topical administration of certain probiotics has not only shown to improve wound healing but also prevent inflammation (Azad et al. 2018). Studies have shown that the use of topical probiotics, such as *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Lactobacillus brevis*, subsided the inflammation and expedited the healing process of wound (Tsiouris et al. 2017). *Lactobacillus plantarum* containing topical probiotics have shown to alleviate bacterial count and ameliorate healing mechanism in diabetic ulcers by recruiting fibroblasts and phagocytes and modulating the levels of IL-8. Likewise, in non-diabetic patients, more than 90% of shrinkage was observed in the area having chronic leg ulcers when *Lactobacillus plantarum* was applied on the affected area for a period of 30 days (Peral et al. 2010). In another study carried out in mice having burn wounds, topical application of *Lactobacillus plantarum* minimized the skin infection caused due to *Pseudomonas aeruginosa*. The effectiveness of this probiotic was compared to the use of silver sulfadiazine in humans, and the results have shown comparable effects in moderate and severe degree of burns by alleviating the bacterial count and improving wound healing (Valdez et al. 2005).

Lactobacillus fermentum 7230 used as a patch to produce nitric oxide has been found to show potential wound healing characteristics when examined on wounded rabbits. The applied patch encourages the production of cytokines, TGF-β, and IL-1, thus, stimulating immune response (Jones et al. 2010).

Lactobacillus casei and *Lactobacillus acidophilus* have exhibited antibacterial properties and are labeled as one of the most commonly used topical probiotics against the wound infecting MRSA (*methicillin-resistant Staphylococcus aureus*). Upon incubation for 24 h at a temperature of 37 °C, approximately 99% of the wound infecting MRSA showed restricted growth and eradication (Sikorska and Smoragiewicz 2013).

Lactobacillus rhamnosus and *Lactobacillus reuteri*, as topical probiotics, have shown to diminish *Staphylococcus aureus* caused cell death of keratinocytes present in the epidermal layer. The study examined the wound soothing characteristics of *L. reuteri*-based topical probiotic ointment using rat model. Increased collagen deposition, accelerated production of epidermis, and a considerable reduction in the inflammation were observed in the group treated with ointment vs control group. Further, a substantial decrease was observed in the pathophysiological functioning of the peroxidase enzyme, myeloperoxidase (MPO), in the treatment group compared to the control group. The study concluded that the use of afore mentioned topical probiotic therapy led to an amelioration in the healing mechanism. Also, the effects observed with *L. reuteri* were more pronounced in comparison to *L. rhamnosus* (Prince et al. 2012; Mohammedsaeed et al. 2014; He et al. 2019).

Surface application of kefir, a fermented milk product, has been studied for its healing and antibacterial properties in burn wounds. A variety of yeast and bacterial cultures found in kefir, such as, *Lactobacillus kefiri*, *Lactococcus*, *Leuconostoc*, *Acetobacter*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Saccharmoyces unisporus*, and *Saccharomyces exiguous*, have shown to subside pathogen expansion (Rodrigues et al. 2005). In addition, kefir has also been studied for its defensive effects in the process of wound healing. Researchers have investigated and compared the outcome of using kefir vs kefir along with silver sulfadiazine against *Pseudomonas aeruginosa* (antibiotic resistant) caused contamination of burn wounds. While the use of former exhibited a significant decrease in the wound diameter and also decreased the time of healing, the use of latter did not show any such effects (Huseini et al. 2012). Further, the effect of *Saccharomyces cerevisiae* on the wound healing mechanism was evaluated, and the study showed an improvement in the process of healing as its use led to an upsurge in the release of TGF-β1 and type-1 collagen (Medeiros et al. 2012).

In addition to the abovementioned probiotics, skin microbiota also serves as one of the potential candidates for topical probiotic therapy, wherein their types along with the functions are summarized in Table 5.2.

Recently, a probiotic—VITSAMJ1—was extracted from the goat milk and was evaluated for its wound-healing mechanism and its antibacterial properties against *Staphylococcus aureus* in the Wistar rat model. Upon topical application of gel form of the extracted probiotic on wounds, accelerated healing process was observed in the treatment group in contrast to the control group. Also, the leukocyte count observed in the treatment group was quite high (11,000 u/L) in comparison to the control group (7000 u/L). Thus, the study concluded that VITSAMJ1 could be used as a potential wound healing topical probiotic ointment (Sinha et al. 2019).

Table 5.2 The table summarizes the types and function of skin commensals which can be used as one of the potential candidates for topical probiotic therapy

S.R. No.	Skin commensals	Function
1.	*Propioniferax innocua*	Damages biofilm (Lopes et al. 2017a)
2.	*Staphylococcus caprae*	Antibacterial properties against MRSA and *Staphylococcus aureus* (mouse model) (Paharik et al. 2017)
3.	*Staphylococcus epidermidis*	Antibacterial properties against *Streptococcus pyogens* and *Staphylococcus aureus* (Christensen and Bruggemann 2014)
4.	*Cutibacterium acnes*	Bacteriostatic effects in *Staphylococcus aureus* (wounded mouse model) (Wang et al. 2014; Shu et al. 2013)

5.3.3 Psoriasis

Psoriasis, a skin inflammatory disorder, is marked by the formation of lesions due to over proliferation of keratinocytic cells present against the pathogen in the outermost skin layer (Kapp 1993). While the activity and production of TH17 cells is possibly regulated by short chain fatty acids, a decline in the number of *Faecalibacterium prausnitzii* (provides SCFAs to the gut) might lead to development of psoriasis (Eppinga et al. 2016). The different subcategories of psoriasis are either classified as pruritus or erythematous plaques (Sarac et al. 2016).

Upon comparison of skin microbiota between psoriatic and non-psoriatic patients, the former carries less varied and reduced number of bacterial colonies in contrast to the latter. The concentration of *Actinobacteria* and *Propionibacterium* species is lower while those of *Proteobacteria*, *Schlegelella*, *Rhodobacteraceae*, *Moraxellaceae*, *Firmicutes*, *Acidobacteria*, *Streptococcaceae*, and *Camphylobacteraceae* species (Chang et al. 2018). Further, a diminished colony of *Propionibacterium acne* turns on the TH2 mediated helper cells immune response in spite of TH1, leading to autoimmune disorder (Agak et al. 2014). The pathological mechanism reveals the overproduction of IL-23, IL-17, IL-6, IL-8, TNF-α, IL-β, and certain other chemoattractants (released from the keratinocytic cells) from the T-cells which inhabit the psoriatic skin surface (Baliwag et al. 2015). A study carried out in mouse model of IMQ-induced psoriasis revealed a suppression in inflammation upon topical application of a probiotic component SEL001, extracted from ethanolic concentrate of *Lactobacillus sakei* in comparison to highly potent topically applied drug clobetasol. Thus, the topical application of SEL001 inhibited the inflammation caused by IL-23, IL-19, and IL-17A by significantly reducing the production of these cytokines and also a significant decrease in the thickness of skin along with improvement in the PASI (Psoriasis Area Severity Index) (Rather et al. 2018).

5.3.4 Atopic Dermatitis

Atopic dermatitis (AD), also known as eczema, is one of the most common skin inflammatory diseases that is chronic and recurrent and predominantly occurs in infants and children. In cases where atopic dermatitis occurs in adults, symptoms usually appear in puberty and later on continue into adulthood (Spergel and Paller 2003). Onset of AD often brings dehydration, pruritus, inflammation to skin, and increases the risk of infection induced by *Staphylococcus aureus (S. aureus)* colonization in lesion areas. With known risk of progression to asthma in some patients, the exact pathogenesis of AD still remains elusive, one of the most evidenced theories indicates that a genetic defect in filaggrin protein can cause AD by disrupting the epidermis (Berke et al. 2012). Classic therapies for AD include emollients, topical glucocorticosteroids, topical calcineurin inhibitors and adjuvant therapy, for example, UV phototherapy (Rather et al. 2016).

Probiotics, on the other hand, used mostly as dietary supplement as well as topical treatment in few cases, has been tested by cohort and randomized controlled studies to manifest its alleviative and preventative effects on AD in prenatal and postnatal stages (Fuchs-Tarlovsky et al. 2016; Foolad and Armstrong 2014).

The most frequently studied probiotics strains are *Lactobacillus rhamnosus strain GG*, *L. salivarius and Bifidobacterium,* which were exercised in previous clinical studies to evaluate the effect on prevention and treatment of AD (Foolad and Armstrong 2014).

The preventive and alleviative effect on AD has been assessed by several clinical studies by measuring SCORAD (Scoring Atopic Dermatitis), an index indicating severity of AD (Spergel and Paller 2003).

In individual studies, probiotics are administered mostly as extracts with lotion, ointment, or cream.

In a human-based study where coagulase-negative *Staphylococcus* (CoNS)-containing cream were applied on AD patients, the CoNS manifested prohibitive effect on colonization of *S. aureus*. The dosage of *bacteria strains administered* was estimated by the density of bacteria on normal human skin (1×10^5 CFU/cm^2). After 24-h administration, subjects applied with antimicrobial CoNS strain(s) showed significant decrease of *S. aureus* abundance compared with vehicle ($P = 0.0402$), therefore demonstrating the antimicrobial effect of the pathologic strain in AD human subjects (Nakatsuji et al. 2017).

In 2016, Audrey et al. tested the therapeutic effect of 5% *Vitreoscilla filiformis (V.f.) extract*-containing ointment on 13 female or male patients with mild to moderate AD. The therapeutic effect was evaluated using a similar scheme as SCORAD: modified eczema area and severity index (mEASI). The results of the randomized double-blind study showed the 5% V.f. significantly alleviated the severity of AD on visit of Day 28 with twice administrations daily. At Day 28, the group treated with the V.f. extract exhibited 42.9% decrease in mEASI in comparison to a 24.8% decrease for the vehicle group ($p = 0.008$) (Guéniche et al. 2006).

In 2017, a comprehensive meta-analysis conducted to evaluate the effect of probiotics as potential therapy for AD identified and analyzed data from 13 studies

(Huang et al. 2017), result of which depicted an interesting picture. Different probiotics strains were analyzed respectively for their varied alleviative profile as well as the heterogeneous response from regional population to probiotic treatment. It is stated that the probiotic treatment in both overall population and children (1–18 years old) was favored. Among the analyzed bacteria strains, *Lactobacillus rhamnosus*($P = 0.07$) and *Lactobacillus plantarum*($P = 0.39$) showed no significant effect in terms of reducing SCORAD values in children with AD indicated by positive value of mean difference of SCORAD between treated and control group, whereas *Lactobacillus fermentum*, *Lactobacillus salivarius*, and a mixture of different probiotics strains showed promising alleviative effect. Meanwhile, the European population are reported to be less responsive to probiotic treatment than Asia group.

While reading the analysis and conclusion from previous studies, it is explicit that there is inconsistency in opinions whether probiotics has a significant therapeutic effect on atopic dermatitis or not. One of the strongest evidences supporting the implicit therapeutic effect of probiotics is the US FDA that so far has not approved any probiotics as a treatment on AD. In the meantime, the selection of studied bacteria strains can vary the therapeutic effect thus the study outcome. Yet there are studies that favor the treatment of probiotics over placebo; further studies with strong evidence should be conducted to manifest its therapeutic effect.

Possible explanation for the treatment effect of probiotics on AD is the reduction of inflammation severity via regulating immune response. Probiotics can decrease the activity of dendritic cells in vitro, thus reduce the activation of inflammation-triggering Th2 cells (Weiss et al. 2011; Kwon et al. 2010). Furthermore, It is believed that probiotics can attenuate the allergic hypersensitivity reaction via suppressing Th2-mediated response to balance Th1/Th2 immune response that is related to pathogenesis of allergic disease (Rather et al. 2016). Similarly, the picture of possible mechanism of preventive effect is yet unclear.

5.3.5 Acne

Acne is a common chronic skin disease, mostly prevailing in young adults and adolescents, which, to a large extent, negatively affect both physical and mental health of patients (Fox et al. 2016). The most notable pathophysiologic development of acne follows several processes (Gollnick et al. 2003):

- Sebaceous gland hyperplasia with seborrhea.
- Altered follicular growth and differentiation.
- *Cutibacterium acne* (formerly known as *Propionibacterium acnes*) colonization of the follicle.
- Inflammation and immune response.

These processes can induce microcomedo, which can later grow to non-inflammatory or inflamed comedo, to become what is known as acne.

The pathogenesis of acne is influenced by several factors such as emotional state (e.g., stress and depression) and dietary style that are reported to be highly associated with the fundamental changes of gut microbiota, which can lead to systemic inflammation due to increased intestine permeability. It could cause occurrence of metabolism- and inflammation-related skin diseases (Bowe et al. 2014).

Probiotics are investigated for its beneficial effect as therapy for acne due to its anti-inflammatory activity, anti-microbial activity, and function to regulate the gut microflora. As a promising therapeutic candidate, probiotics are mostly administered either orally or applied topically as lotion or cream however as an adjuvant therapy in addition to mainstream therapy against acne. There is fair amount of in vitro and in vivo studies that successfully manifested that probiotics are capable to alleviate the inflammation on skin. As applied as topical treatment, particular strains of probiotics (e.g., *L. acidophilus*) are shown to exert anti *C. acne* activity in vitro (Al-Ghazzewi and Tester 2010).

Moreover, compared with quite substantial amount of clinical studies conducted on atopic dermatitis, the studies on probiotics against acne are of less interest, however it's therapeutic potential remained to be further explored.

In one of the studies, Kang and group tested the efficacy of an *Enterococcus faecalis* SL-5 lotion against the *P. acnes*. The bacterial extraction was performed from the feces of an adult (healthy Korean male) and was allowed to grow in optimum conditions. The preparation was then ultrafiltered and lyophilized, formulating CBT SL-5, a concentrated powder formulation. This powder formulation was then used to prepare an aqueous base lotion. All other components but CBT SL-5 was present in the placebo. A double-blind, randomized Phase 3 study was then performed for 8 weeks on 70 patients (divided into two groups—CBT SL-5 and Placebo), 12 years or old, diagnosed with acne vulgaris (light or moderate). The lotion was applied topically twice daily on the areas affected by acne vulgaris. The patients were then examined first in the beginning and then followed up after 2, 4, as well as 8 weeks. After 2 weeks only, a considerable decrease in the formation of inflammatory mediators was observed in the group treated with CBT SL-5 compared with placebo. Thus, the CBT SL-5 lotion exhibited high efficacy against the growth of bacterium *P. acnes*.

In another randomized clinical study conducted during 2006–2007, the effect and safety profile of *E. faecalis* SL-5 for moderate acne were evaluated. Patients were treated with probiotics lotion containing cell-free culture supernatant of *Enterococcus faecalis*, inflammatory lesions of whom were significantly reduced compared with placebo group (Kang et al. 2009). The study result, therefore, revealed the potential use of probiotics as alternative to standard topical therapy.

Besides the bright side of its potential treatment effect, there are certain concerns in applying probiotics topically. In particular when strains, that are not commensal, we're applied on the skin, safety concern might arise due to the alternation of delicate skin microbiota environment (Yu et al. 2020).

5.3.6 Aging and Photoaging

As introduced aforehand, the therapeutic effect of probiotics on skin diseases have been extensively studied over the decades; due to its excellent safety and anti-inflammatory profile, it is natural for scientist to explore further the undiscovered potential probiotics could exert in other pertinent skin diseases, such as skin aging.

Skin aging is a rather universal process that occurs either intrinsically with natural aging or is extrinsically induced by external factors such as ultraviolet; the latter process is commonly known as photoaging (Vierkotter and Krutmann 2012). Manifestation of photoaging is mainly characterized by coarse wrinkles, solar elastosis, and pigment irregularities, whereas natural skin-aging is marked with dehydration, fine wrinkles, and a certain degree of laxity. It is believed that the UV-induced skin-aging can be triggered by several pathways such as DNA damage mediated through generation of ROS (reactive oxygen species) (Cinque et al. 2017).

Probiotics has manifested its anti-oxidative activity in previous studies. In an aged mice model, the increased level of antioxidant enzymes (such as superoxide dismutase, catalase, glutathione peroxidase) in liver was demonstrated to be associated with *Lactobacillus rhamnosus oral* supplement (Sharma et al. 2014). Topically, it's assumed that the acidic substances produced by probiotics such as free fatty acid (Nakatsuji et al. 2017, 2018) and conjugated linoleic acid (Peguet-Navarro et al. 2008) could alter the skin's pH environment and might be able to attenuate the increasing protease activity accompanied by natural aging. The slightly acidic skin pH among healthy subjects usually contributes to regularization of enzyme activity and prevention of microbial colonization and dehydration, however, will rise after the age of 70. Therefore, by maintaining the healthy natural skin pH condition, probiotics are believed to be able to provide potential changes to the skin environment that benefit aging skins (Hachem et al. 2003; Cinque et al. 2017; Kober and Bowe 2015).

Besides therapeutic evidence derived from studies in molecular biology and animal level, human studies shed some lights on its anti-aging effect, however, mostly in oral dosage. To the best of our knowledge, the anti-aging effect hasn't been widely examined in topical usage.

5.3.7 Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) is an antigen-induced skin disease trigged by exposure to causative substances; the occurrence of ACD is usually followed by skin inflammation induced by innate or adaptive immune response (Mowad et al. 2016; Tan et al. 2014).

Considered as a delayed-type hypersensitivity, ACD is affecting approximately 7% of the population, the pathogenesis of which is associated with epidermal immune response activation initiated by allergens (Gittler et al. 2013). Two etiological stages of ACD were identified (Gittler et al. 2013):

1. Sensitization and activation of antigen-specific T cells, primarily Th1 and Th2 cells (known as CD4+ T-cells), together with release of inflammatory cytokines by keratinocytes including TNF-alpha (tumor necrosis factor alpha) and interleukins.
2. Elicitation of immune response which leads to inflammatory cascade when antigens are re-encountered.

Indicated by the pathology of ACD as well as the immune regulation and anti-inflammatory profile of probiotics, it is natural for scientists to connect probiotics with potential treatment for ACD. However, most of the emerging studies were only carried out mostly in vitro, yet clinical evidence that directly evaluates the effect of topical usage of probiotics on ACD hasn't been accumulated.

In one of the studies, oral administration of probiotics strain *Lactobacillus casei* is proved to moderate the severity of allergic contact dermatitis via molecular pathways in CD8+ T cell-mediated skin inflammation mice model. Study showed that during the elicitation stage of delayed type of hypersensitivity, *L. casei* treatment reduces the recruitment of effector T cells into the skin. Moreover, it is demonstrated that *L. casei* inhibits skin inflammation via MHC class II-restricted CD4+ T cells that are involved in development of ACD (Hacini-Rachinel et al. 2009).

Although there is indirect evidence which demonstrate the anti-hypersensitivity effect of oral administration of probiotics, the lack of straightforward clinical evidence requires further investigation for the topical treatment of allergic contact dermatitis.

5.3.8 Skin Cancer

Cutaneous neoplasms have been associated with the imbalance of skin microbiota which leads to proliferation of cancerous cells, whereas a healthy skin microbiota has the potential to repress cancerous cells by controlling the production of inflammatory agents. Among different strains of microbes responsible for maintaining skin bionomics, *Staphylococcus epidermis* is one of the diverse communities of microbes inhabiting a healthy human skin (Nakatsuji et al. 2018). A recent study was carried out in SKH-1 hairless strain of mouse model having skin neoplasia due to UV-B exposure (Dwivedi et al. 2006; Dinkova-Kostova et al. 2006). While the mice were being exposed to UV radiations, topical administration of *Staphylococcus epidermis* reduced the tumor occurrence and its number. Upon identification, it was observed that *Staphylococcus epidermis* produces 6-HAP, a compound that is responsible in restricting the synthesis of DNA and also suppresses the hyperproliferation of cancerous cells. Therefore, the application of probiotics could be advantageous in skin tumors and can be further explored (Nakatsuji et al. 2018).

5.4 Future Challenges

Although a deeper insight on the functional aspects of probiotics has been uncovered, a number of questions regarding their exact pathophysiological mechanism still remain ambiguous. One of the biggest issues faced with probiotic treatment is the shortly lived habituation of probiotic bacterial colonies. Upon termination of treatment, no probiotic bacterial colony is detectable after a period of 10–12 days. In order to have a successful concentrated probiotic bacterial colony, it is necessary that probiotics adjust to the environment, which in turn depends on phylogenetic diversity of the microflora and the individual's accessible resources (Firmesse et al. 2008; Maldonado-Gomez et al. 2016; Szanto et al. 2019). Another possible concern could be the diversity of skin microbiota. There are some set of variations between distinctive sites in human skin which permit only specific colonies for the colonization of probiotics (Grice et al. 2008). A number of other factors such as age, gender, and maintenance of proper hygiene are likely to be responsible for different therapeutic responses to probiotics observed in individuals. Hence, personalized medication/treatment could be recommended in such cases of skin disorder (Dimitriu et al. 2019).

Although this chapter has covered almost all topical probiotic treatment options prevalent, its impact as a treatment option for skin disorders still remains unsure. Even though a number of human as well as animal studies have shown positive effects, numerous questions still exist. The probiotic strain and optimum dosage required as a treatment option is still yet to be discovered. Also, some of the probiotics have not shown enough compatibility with other constituents present in creams, leading to a problem for preparation of topical formulations. However, it has been discovered that upon combining oral as well as topical probiotic therapy, a solution for effective treatment strategy could be found (Marcinkowska et al. 2018). Furthermore, administration of antibiotics along with topical/oral probiotic therapy is recommended as former is responsible for its targeted action against the pathogen while latter flourishes the microbiome in human body (Teughels et al. 2008).

The current categorization by the FDA distributes probiotics under several categories such as cosmetic products, food or food additives, nutritional supplements, or drugs (case-by-case basis). However, there is still no official definition that explicitly describes the term topical probiotics.

Although the use of topical probiotics in improving skin conditions in various skin diseases is uncovered by a number of studies, still FDA does not classify probiotics as drugs but as "cosmetics." Since the marketing of cosmetic products does not need any FDA approval, it therefore makes it easier for the manufacturers to label probiotics to be used as therapeutics, even though it is not substantiated. Hence, further examination on efficacy and safety of topical probiotics in ameliorating skin diseases needs to be evaluated.

5.5 Conclusion

The chapter highlights the topical probiotic treatment options available for the amelioration of certain skin disorders. Although for all the above-discussed skin disorders, an imbalance in the microbiome is not the sole contributing factor behind their pathophysiology, an improvement of the imbalance could be considered as a plausible option. A healthy microflora might help reduce the symptoms associated with the skin disorders. Thus, topical probiotics could be considered as one of the treatment options for treating skin conditions.

Although being low-cost and highly available, topical probiotic formulation has faced complications due to severe side effects observed in patients who are immunocompromised. Apart from having an exceptional safety data, no comments have been laid upon its efficacy in the long run. Thus, it is necessary to have a thorough investigation into this area, and more meta-analyses are desired to characterize the risk-benefit profile of this therapy. The clinical data of human subjects upon treatment with topical and oral probiotic therapy should be collected with larger sample size. Also, the dosing frequency, dose itself and the combinations should be further evaluated to offer a better insight of probiotics treatment in order to substitute the prevailing antibiotic era.

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Status of Using Probiotic Supplementation in Acne

6

Aishwarya Hattiholi, Shivani Tendulkar, and Suneel Dodamani

Abstract

Skin conditions like acne vulgaris have been linked to hormonal imbalance as well as microbiota pertaining to the brain-gut-skin axis. Having acne and distressed skin conditions also have links to cognitive and behavioural indisposition, often due to deficiency of certain nutrients and thereby giving rise to the approaches discussing gut-brain-skin axis. This has been addressed widely by the supplementation of diet with probiotics. This keeps the human skin microbiome in check, thereby eliminating the growth of commensal skin bacteria and maintaining skin microbiome homeostasis. The use of probiotics has proven beneficial in acne conditions facing antibiotic resistance and inhibiting inflammation in severe cases. This has increased the demand for oral probiotics to modulate and treat skin conditions. This section will focus on different approaches in the use of such probiotic supplements for treating acne, various interplaying factors, effects, and advancements.

Keywords

Acne · Antibiotic resistance · Brain-gut-skin · Immunomodulation · Inflammation · Microbiome · Probiotics

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6.1 The Pathogenesis

Acne is a dermatological condition that is associated with the pilosebaceous units of the skin, mainly occurring on the face and neck but spreading to upper chest, shoulders, and back in some, and is caused by *Propionibacterium acnes* (also known as *Cutibacterium acnes*). Due to the wide variation and degree of occurrence as well as differences in clinical reports, it is difficult to actually determine the prevalence. Mostly occurring in adolescents, it continues or even initiates in young adults (late onset). Moreover, female prevalence is higher than that of males in young adults, but is the opposite for adolescent males (Cunliffe and Gould 1979; Williams et al. 2012). *P. acnes* is an anaerobic gram-positive pathobiont, meaning it is generally present in the skin microbiota as symbiotic bacteria in the pilosebaceous follicles but acts as pathogen when present in a higher number during dysbiosis (Goodarzi et al. 2020a). *Proteobacteria, Corynebacterium, Cutibacterium*, and *Staphylococcus* are also found in the sebum-rich areas of the skin (Woo and Sibley 2020). The lipid-rich environment created by microcomedones helps the growth of the bacteria. The bacteria might exist as a biofilm which would otherwise be absent on normal skin due to the host defense and develops a resistance to the antimicrobial treatments (Williams et al. 2012; Sfriso et al. 2020).

About a 1000 species of bacteria have been associated with the human skin microbial niche, with the commensal species co-existing as pathobionts. In the wake of dysbiosis, these commensal bacteria compete against the beneficial species and outgrow them (Yu et al. 2020). The major phyla of this niche are *Actinobacteria, Firmicutes, Proteobacteria*, and *Bacteroidetes.* It is interesting to note that the microbial colonisation on skin surface may vary from that of the follicles, which again varies in individuals (O'Neill and Gallo 2018). Skin microbiota differs by geographical location, topography, age, sex, etc. (Khayyira et al. 2020). *P. acnes* strains: ribotype 4 and 5 are present in people with acne only, when compared to people with normal skin. Other strains and species are present in both the groups; only the dysbiosis makes the difference (Knackstedt et al. 2020).

The pathogenesis involves (1) follicular proliferation (hyperkeratinisation), (2) excess sebum production (hyperseborrhea), (3) inflammation, and (4) colonisation of *Propionibacterium acnes*. This varies in individuals, based on the type of lesion, and includes papules, comedones, pustules, nodules, and cysts. Early onset is usually dominated by the comedones due to the under-development of sebum. This doesn't provide for the growth of the pathogen, *Propionibacterium acnes.* It progresses only when sebocytes mature and produce enough sebum to harbour the pathogenic bacteria. During the inflammation, markers like IL-1 and CD4+ lymphocytes and macrophages are expressed. Sebaceous glands are crucial for producing antimicrobial peptides, neuropeptides, and antibacterial lipids that are active in the innate immune system (Williams et al. 2012). Non-inflammatory acnes include open comedones known as *whiteheads* and closed comedones known as *blackheads* (Berry et al. 2020). The persisting scarring in some individuals might also lead to psychological and emotional disturbances in some (Collier et al. 2008) and will be discussed in the following sections.

6.2 Factors Influencing Acne Pathogenesis

Humidity, pH, temperature, diet, exercise, lipid concentration, medications, surgeries, physical, and mental stress affect the skin microbiota (Knackstedt et al. 2020). This dysbiosis gives room for the pathogenic or commensal species to outgrow the beneficial ones, thereby triggering pathogenesis. Factors like picking, hormonal changes during menstrual cycle, and emotional stress exacerbate the condition. In people with polycystic ovarian syndrome, increased insulin resistance and high serum dehydroepiandrosterone might be responsible. Certain acnes like the acneiform eruptions are as a result of drug side-effects. Other external factors include smoking, sweating, humidity, pollution, use of greasy skin products and cosmetics, clothing material, diet and skin hygiene, etc. Acne often affects the individual by irritating physical symptoms like pain, itching, and irritation but is more likely to cause emotional harm. The skin blemishes affect the confidence and self-esteem, resulting in a complex. This affects the psychology of the affected individual and might be the underlying cause of depression and anxiety in some. The quality of life is severely affected as it induces social inhibition and psychosomatic symptoms (Williams et al. 2012; Dessinioti and Dreno 2020).

6.2.1 Microbiota and Dysbiosis

6.2.1.1 The Microbiota

The skin microbial niche like any other in the human body varies in diversity across the age groups. *P. acnes* has been observed to be more abundant in individuals of ages above 20 than in those below 20. This is largely attributed to the development of the sebaceous glands, as its maturity and secretion facilitates the colonisation of the bacteria. Species richness and higher diversity is seen in children, adolescents, and elderly age groups than in young adults and middle-aged group. Although no concrete evidence is yet available, gender is also a factor in the microbial diversity and acne prevalence, which may be largely due to the hormonal effects on sebum production. *Cutibacterium granulosum* is also known to co-localise with *P. acnes* in sebum-rich parts of the skin and may be important for maintaining the normal skin barrier. It has been observed in healthy individuals as well as in comedones and pustules of acne patients and are known to exhibit lipase activity (O'Neill and Gallo 2018; Park et al. 2020). *P. acnes, Staphylococcus epidermidis,* and *Snodgrassella alvi* are the major species in an acne affected skin microbiota in both males and females, while *Pseudomonas putida* is abundant only in females. *P. acnes* and *Lawsonella clevelandensis* have been associated with acne severity. *S. epidermidis* like other bacteria uses the glycerol present in the skin cells to produce short-chain fatty acids (SCFA) that help in their competitive survival (Park et al. 2020). This is backed by the fact that the bacteria produces antimicrobial peptides like modulins, epilancin, epidermin, and Pep5 (Lee et al. 2019a). The succinic acid produced by the species blocks the surface Toll-like receptors (TLRs) which might also be responsible for controlling the proliferation of *P. acnes*. In vivo studies showed that this also

reduces the expression of macrophage inflammatory protein-2 (a CXC chemokine) (Park et al. 2020). *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* pose higher risks of disturbing skin microbiota and the consequent attraction of opportunistic or commensal bacteria that increase the severity of infections. Moreover, GI tract bacterial growth is known to accelerate through stress which increases the bowel transit time and compromises the protective barrier (Goodarzi et al. 2020a).

6.2.1.2 Microbial Secretions and Induced Secretions

P. acnes exhibits virulence factors like lipase, protease, endoglycoceramidase, neuraminidase, hyaluronate lyase, and Chrisitie-Atkins-Munch-Petersen (CAMP) that take part in inflammation and tissue damage. Proteases, neuraminidase, endoglycoceramidase, and hyaluronate lyase help *P. acnes* to break down the components of extracellular matrix, which triggers inflammation. Lipases are involved in hydrolysis of sebum triglycerides and convert them into free fatty acids, which in turn induce inflammation and hyperkeratosis. Genetic analysis shows the involvement of linear plasmids in formation of biofilms, colonisation, and virulence. Such biofilms are observed in skin biopsies of acne patients. The extracellular polysaccharides delay the influx of antimicrobial agents and develop resistance against them (Lee et al. 2019a). The TLR2 ligands on *P. acnes* help in inducing inflammation and stimulate IL-1α and granulocyte macrophage-colony stimulating factor, while in some they stimulate IL-6, IL-8, TNF-α, and human β-defensin 2 (GM-CSF) (O'Neill and Gallo 2018; Thompson et al. 2020). Monocytes and sebocytes also secrete IL-1β and involve the expression of NLRP3, an inflammasome gene. This mechanism involves proteases and reactive oxygen species. *P. acnes* also induces TH17/Th1 responses and secretion of IL-17A and IFN-γ, indicating the involvement of TH17 pathway in acne pathogenesis.

The species secrete extracellular polysaccharides that enhance its adherence and form biofilms. This biofilm is a close association of bacteria from same or similar species through quorum sensing and enables it to strongly compete against other species, including the beneficial one. This also allows the commensal species to reduce antibiotic susceptibility and develop resistance against them as well as the host immune response and antimicrobial agents. The bacteria secretes propionic acid that forms irregular keratinocytes and microcomedones (Dréno 2017). Polymicrobial biofilms consisting of species like *Staphylococcus* are present with *Propionibacterium* in severe acne conditions in the pilosebaceous glands of skin and are often responsible for antibiotic resistance and increased inflammation. These biofilms restrict the sebum movement, trapping it in the skin and accumulating corneocytes and further increasing the comedones.

As for specific bacterial distribution, *Propionibacteria* and *Staphylococci* are predominantly present in sebaceous area, *Corynebacteria* in moist skin areas and *β-Proteobacteria* along with *Flavobacteriales* and some other species in the dry skin area (Mottin and Suyenaga 2018). *Staphylococci* and *Streptococcus* in skin microbiome induce TNF-α, IL-17A, IL-17F, and IL-22 that exacerbate the skin

lesions (Okada et al. 2020). Gut microbiota in acne patients enhance the circulation of endotoxins and increase intestinal permeability (Deidda et al. 2018).

6.2.2 Lipids

Bacterial colonization in the sebocytes results in the production of free fatty acids and short chain fatty acids (SCFA) that results from fermentation of the glycerol present on skin. The free fatty acids produced by sebaceous glands are however observed in absence of any bacterial activity and might trigger acne lesions and expression of IL-1α (Zouboulis et al. 1998). Lipid synthesis takes place during the proliferation and differentiation of sebocytes.

The SCFAs help the survival of the metabolite producer bacterial species by balancing the growth of other species, which controls pathogenic invasion. SCFAs are also involved in activity against Methicillin-resistant *S. aureus* (MRSA) and *Streptococcus* strains (Huang et al. 2020). Therefore, constant availability of the carbon source is important in maintaining the microbial homeostasis.

6.2.3 Hormones

Sebum responds to the androgen 5α-dihydrotestosterone and triggers its production. The enzyme 5α-reductase converts testosterone to this androgen and has been detected to have a higher activity in acne skin. Thyroid-stimulating hormone is also known to stimulate sebaceous glands and thyroxine increases lipid synthesis (Zouboulis et al. 1998). Insulin-like growth factor (IGF)-1 signalling affects the production of androgens, sebaceous lipogenesis, and formation of comedones. Modulation of this pathway, restoring skin dysbiosis, and *P. acnes* phylotype diversity are potential acne treatment strategies (Dessinioti and Dreno 2020). Androgens activate the hormonal DHT receptor that affects sebocytes and sebum production. Hyperandrogenia in women, which is also known to flare up before menstrual cycle, exacerbates the acne conditions.

Oestrogen controls glucose and lipid metabolism as well as inflammatory responses. Testosterone takes active part in lipid production and is heavily influenced by insulin resistance. Insulin resistance is known to elevate the oil production and exacerbate acne. Oestrogen levels fluctuate throughout the menstrual cycle and the reproductive age of women. Gastroesophageal reflux and acidity are also linked to increased oestrogen levels. Other factors like the leaky gut syndrome are associated with hormonal imbalance that affects the pathogenesis of cystic acne psoriasis (Maguire and Maguire 2020). Endocannabinoids are also believed to be involved in skin cells proliferation, apoptosis, maintenance of sebaceous glands, etc. and has attracted an interest in acne treatment (Dréno 2017).

6.2.4 Diet and Nutrition

The famous brain-gut-skin theory by Stokes and Pillsburry explains a link between the gut microbiota and skin health which can be altered by emotional and nervous state. According to the hypothesis, the gut microbiota would migrate from colon to distal small intestine and alter the intestinal microflora, when stomach acid level changes. The intestinal microflora engages in the mTOR pathway to control fat metabolism and is also known to be involved in controlling cell expansion and intestinal barrier. This is a potential target in acne pathogenesis as mTORC1 is suggested to be involved. As for the involvement of psychological stressors, intestinal microbiota produces neurotransmitters that can cross the intestinal mucosa barrier and induce systemic inflammation. *L. acidophilus* has shown modulation of inflammation in the brain-gut-akin contest (Stokes and Pillsbury 1930; Bowe and Logan 2011; Lee et al. 2019a).

High glycaemic diet is known to exacerbate the acne conditions. Upon increased intestinal permeability, lipopolysaccharide endotoxins are known to be released into intestine which induces inflammation, oxidative stress, and insulin resistance, which then triggers hyperinsulinemia and gut microbiota dysbiosis (Kumar et al. 2014; Dréno 2017; Maguire and Maguire 2020). The high glycaemic diet increases IGF-1/insulin-like growth factor binding protein-3 ratio and cause hyperinsulinemia that in turn increases the androgens and the consequent sebum production. Milk products increase IGF-1 absorption, and therefore, dairy products are associated with acne vulgaris pathogens, while fermented milk products having lactobacilli do not aid this pathogenesis (Ismail et al. 2012). Free fatty acids and cholesterol induce the peroxisome proliferator-activated receptors, and fats induce leptin receptors which affect sebocytes, as they take place in lipid formation. Leptin is also known to induce IL-6 and IL-8 (Dréno 2017).

When it comes to dietary intakes, chocolate is said to be a contributor of acne development and exacerbation but lacks any evidence. Vitamins and minerals play essential roles in acne development as well as prevention. Vitamin D, for instance, is important for immune system regulation and has target receptors in sebocytes and is known to be active in acne vulgaris. Vitamin B12 supplements induce porphyrin in acne patients, which induces skin inflammation and might be responsible for exacerbation of acne. Zinc, on the other hand, is essential for proper functioning of skin and has bacteriostatic properties. It is known to act against *P. acnes* and reduce the expression of inflammatory cytokines like TNF-α (Williams et al. 2012; Goodarzi et al. 2020a, b; Nørreslet et al. 2020).

6.2.5 Other Factors

Acne rosacea is known to coexist with certain autoimmune diseases, as indicated by the expression of specific human leukocyte antigen alleles, and is also associated with GI diseases, coeliac disease, Type 1 diabetes, multiple sclerosis, and rheumatoid arthritis. All these have been attributed to an imbalance or dysbiosis of the

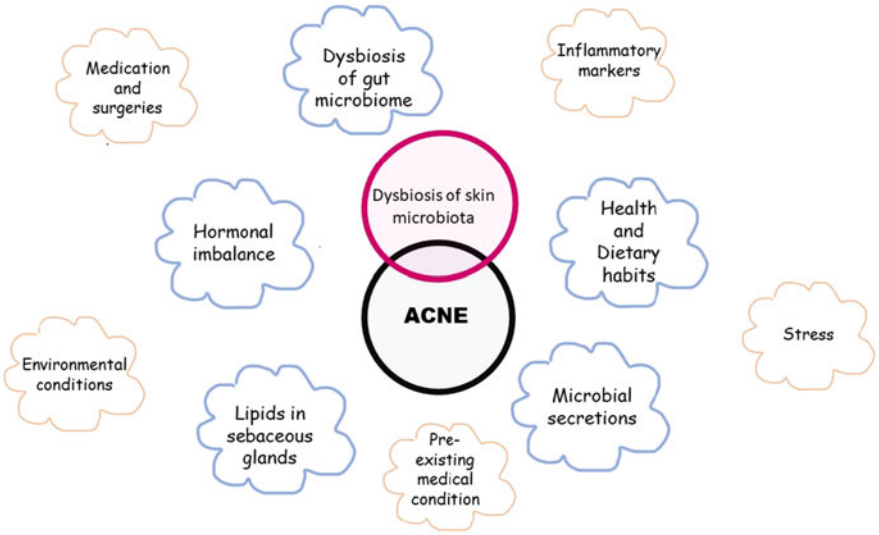


Fig. 6.1 Factors influencing acne pathogenesis

enteral microbiota. This association is often related to an increase in zonulin levels, which is a human protein involved in regulating intestinal permeability (Yüksel and Ülfer 2020). However, the significance of this in dysbiosis needs to be evaluated. While it is seen that inflammation is induced upon pathogenesis, it exacerbates the pre-existing acne conditions.

Stress is known to express neuromodulator receptors like substance P and corticotrophin-releasing hormone in sebocytes and affect the sebum production. Histamines triggering the histamine receptors are also known to affect similarly (Dréno 2017). Reactive oxygen species (ROS) exacerbates inflammation in acne, and thus antioxidants are of interest in this respect (Goodarzi et al. 2020a). Figure 6.1 summarises various factors influencing acne pathogenesis.

6.3 Acne Associated Psychological Effects

Studies show that female patients with acne are more prone to anxiety and depression and have given rise to a sex-specific stress depressive disorder. Apart from the general pressure and complex females face in this respect in the society, the associated emotional and hormonal fluctuations elevate the stress. Suicidal tendencies, however, are irrespective of the gender in acne patients (Uhlenhake et al. 2010; Altunay et al. 2020). It is necessary to note that social media influence adolescents and young adults, mostly women during the treatments. This might heavily imperil their treatment and moreover negatively influence their psychology about the condition (Yousaf et al. 2020). Depression and anxiety are more likely to occur in adults than in adolescents and according to a study, the emotional cost of

acne is more in Middle Eastern regions when compared to other regions of the world (Samuels et al. 2020).

To assess and monitor the psychological aspects, generic questionnaires like the Generic Index Measure and Dermatology Life Quality Index as well as specific ones like Cardiff Acne Disability Index, Assessment of the Psychological and Social Effects of Acne (APSEA), and the Acne Quality of Life are used by clinicians diagnosed with acne and associated psychological distress. The facial blemishes and inflamed spots harm the individual's self-esteem and confidence to begin with, not to mention the jeopardising effects on their social life and relations. With this emotional distress, they tend to seep into severe depression and anxiety at par with patients having epilepsy, arthritis, asthma, diabetes, and lumbar pain and even having suicidal tendencies. Although these psychological and emotional effects of acne on the individual are profound, it still lacks general awareness in the society (Follador and Campelo 2006). Emotional stress in turn also triggers gut dysbiosis, increase permeability, and induce systemic inflammation (Kumar et al. 2014).

6.4 Widely Used Treatment Strategies

Benzoyl peroxide, tropical retinoids and antibiotics, hormonal therapy, lasers, and photodynamic therapies are conventionally used for treatment, depending on the severity (Williams et al. 2012). Topical retinoids like adapalene and tretinoin are mainly vitamin A derivatives and show anti-inflammatory and comedolytic activities. 0.1 and 0.3% adapalene gel or tretinoin formulations ranging from 0.01 to 0.1% are generally prescribed for mild to moderate cases. In case of antibiotics, moderate to severe cases are prescribed doxycycline with a dose of 100 mg once or twice daily. Alternate treatment for this involves minocycline. Cyclins and tetracyclines are the broad-spectrum antibiotics prescribed for treatment and have antibacterial and immunomodulatory effects. Salicylic acid is also prescribed as a mild comedolytic agent. All these formulations are also available in combination with benzoyl peroxide, which is a free radical creating antimicrobial. It is also anti-inflammatory and a mild comedolytic agent. Although it has been used frequently in skin care and acne maintenance in the past, it is no longer recommended by the clinicians due to irritating reactions and bleaching action. It is nevertheless used in some combination prescriptions with antibiotics to counter antibiotic resistance (Berry et al. 2020; Park et al. 2020).

For hormone-based isotretinoin treatments, oral contraceptive pills are prescribed alone or in combination with other. These pills control androgen production, which eventually control sebum production. Progestins and antiandrogens like spironolactone are used in adult females. These however pose risks mainly in adolescents due to hormonal fluctuations; complicate the menstrual cycle and can also impair the kidneys, cause hyperlipidaemia, liver dysfunction, and hyperleukaemia; and show other teratogenic activities (Thompson et al. 2020). Along with the prescribed antibiotics and skin creams for acne treatment, skin care routine to control oil and sweat on the skin is also usually recommended. But agents

suppressing sebaceous glands often have the reverse effect and alleviate the condition (Zouboulis et al. 1998).

Many studies have reported antibiotic resistance faced by acne-associated bacteria like *P acnes* and *S. epidermidis* which hinder the antibiotic treatment strategy (Moon et al. 2012). Bacteriophage therapy, prebiotics, and probiotics are the new face of treatments in the wake of this antibiotic and antimicrobial resistance (Woo and Sibley 2020). Along with probiotic administration, a high protein and low glycaemic diet with fermented food is encouraged for homeostasis of gut microbiota and good skin health (Thompson et al. 2020). Bacterial formulations that ameliorate the acne conditions through their metabolites are attractive in this industry. A topical, intranasal formulation of ammonia-oxidising bacteria has been developed that converts ammonia and urea on skin to nitrite and nitric oxide. Nitric oxide is active against acne pathogens and modulates IL-1β, IL-8, and TNF-α levels (O'Neill and Gallo 2018). The bacteria *Nitrosomonas eytropha* has also been formulated into probiotic mist has shown to reduce wrinkles (Notay et al. 2020). Heat-killed *P. acnes* vaccines have also been suggested as a treatment strategy to reduce inflammation, and in vivo model-based studies show induction of regulatory T cells and type 1 T-helper cells (O'Neill and Gallo 2018).

6.5 Probiotics

Gut microbial species like *Bacteroides, Lactobacilli*, and *Bifidobacterium* use non-edible compounds like xyloglucans and fructo-oligosaccharides and convert them into vital nutrients and supplements for the host. These also help them keep the pathogenic population in check, maintain glucose, lipid and protein homeostasis, etc. The gut microbiota is also known to transport nutrients like vitamin K and vitamins of group B in the host system (Salehi et al. 2021). The gastrointestinal (GI) microbiota also influences the skin homeostasis and modulates the immune responses. This is attributed to the migration of the gut microbiome and its metabolites to skin. In vitro studies show epidermal cell differentiation upon treatment with lactobacillus-fermented milk whey. The GI bacteria can improve a disrupted cutaneous barrier and reduce inflammation (Kober and Bowe 2015; Notay et al. 2017). *S. epidermis* is naturally found on skin and is crucial for defence against *P. acne* which acts by glycerol fermentation. Increasing their number through probiotic administration can help to increase defenxe against acne by outgrowing the causative bacteria. *Lactobacilli*-containing lotions and *S. salivarius* found in the oropharyngeal system show activity against *P. acnes* (Goodarzi et al. 2020a). These strains are not only useful in acne treatment but also in skin regeneration. *Enterococcus faecium* administration to intestinal stem cells enhances proliferation and self-renewal as well as increased expression of pluripotency marker (Kim et al. 2020).

Probiotics have seen a wider range of influence in the gut-brain-axis in humans and is being exploited for various treatments. Probiotics promote the growth of beneficial microbiota in the human gut and keep a check on the growth of

pathobionts. They are also known to take active part in host immunomodulation, reduce inflammation, and regulate insulin levels and triacylglycerol levels, etc. The skin microbiota ferment glycerol to control the growth of commensal bacteria (Fuchs-Tarlovsky et al. 2016). Probiotics are known to decrease the production of sebum and colonization of *P. acnes* in the follicles which also reduces the inflammation. Studies have shown inhibition of CD8+ T cell differentiation into cytotoxic cells and reduction in their migration to skin by probiotic administrations. Apoptosis-mediated inflammation has also been observed to be reduced through the recruitment of FoxP3+ Treg cells (Atabati et al. 2020). They also inhibit proinflammatory cytokines like IL-8 in keratinocytes and epithelial cells and show anti-inflammatory properties. *Lactobacilli* in probiotics have also shown increased skin regeneration and repair of skin barrier. Fermented dairy beverages having *lactobacilli* have shown to decrease lesions and sebum production (Goodarzi et al. 2020a). Probiotic *Bifidobacterium* administrations reduce stress, while *lactobacilli* enhance IgA production through exopolysaccharide immune-stimulation and inhibit NF-κB and IL-8 expression. IgA traps pathogenic microorganisms in the mucus barrier and protects the epithelial cells of the intestine from enteric toxins. *Bifidobacterium breve* probiotics enhance IL-6 and IL-10 production that help in reducing the inflammation and decrease TNF-α. In most of the probiotics, *Lactobacillus acidophilus, Lactobacillus plantarum, Enterococcus faecalis*, and *Bifidobacterium bifidum* are present along with other lactic acid bacteria producing strains and species (Knackstedt et al. 2020).

Probiotic *Staphylococcus epidermidis* strains also display these activities by bacterial interference (Huang et al. 2020). Topical bacteriotherapy has been in question for cutaneous disease treatment for a long time, and studies have shown improvements in acne and seborrhoea. In case of *Roseomonas mucosa* therapy in patients with atopic dermatitis, it improved epithelial barrier, innate and adaptive immunity apart from acting against *S. aureus*. This course has been made easy ever since the development of probiotics and its oral administration, which have repeatedly shown improvement of gut microbiota and immunomodulation, as well as amelioration of acne and other skin conditions. *Lactobacilli* exhibit activity against skin-related commensal bacteria (Lee et al. 2019b).

While probiotics are generally administered orally either in the form of pills and capsules or drinks and beverages, novel formulations like skin patches, gels, creams, and mist are being developed for skin applications in both clinical and cosmetic industries. Apart from reduction in inflammation, fermented milk having probiotic strains show reduced skin surface triacylglycerols while maintaining the normal moisture and pH levels (Mottin and Suyenaga 2018). Lactobacilli in oral probiotics enhance insulin sensitivity, modulate inflammation by inducing anti-inflammatory cytokines like IL-10, and promote T-reg cells. Sphingolipids are known to be produced by probiotics act against *P. acnes* and improve acne conditions, while other ceramides improve skin barrier. Substance P, which takes place in sebum production and skin inflammation, is suppressed by probiotics (Lee et al. 2019a). Membrane-encapsulated probiotics like *Staphylococcus epidermidis* with enhanced glycerol fermentation which may be available as skin patches exhibit antimicrobial

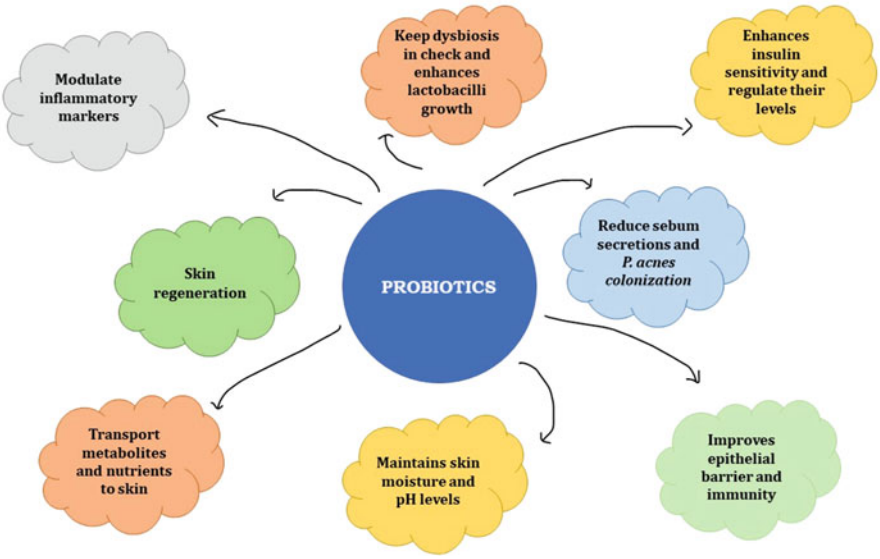
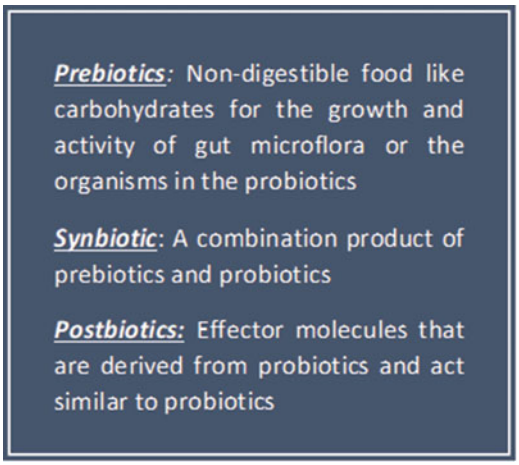


Fig. 6.2 Effects of probiotics

activity against *P. acnes* and reduce the levels of macrophage inflammatory protein-2 (Yang et al. 2019). Probiotic administrations in combination with antibiotics also significantly reduce acne as seen in randomized studies (Notay et al. 2017). Nanomaterials carrying antibacterial agents have been constructed that efficiently act against *P. acnes* and show anti-inflammatory properties. One such formulation, Perfluorocarbon nano-droplets loaded with rifampicin and indocyanine green are also proposed to carry probiotics (Hsiao et al. 2020).

Bacteriocins are majorly attributed to the antimicrobial and immunomodulatory effects in probiotics (Corr et al. 2007; Khayyira et al. 2020). These bacteriocins act against a wide range of bacteria, biofilms, and multi-drug resistant strains, while suppressing inflammation in host body, and are therefore known as host-defence (antimicrobial) peptides (Cavera et al. 2015; Arumugam et al. 2019). *Streptococcus salivarius* inhibits *P. acnes* by secreting a bacteriocin-like inhibitory substance and inhibits inflammatory pathways (Bowe and Logan 2011). Improvement of inflammation and intestinal microbiota through probiotic administration has also been observed in patients affected with polycystic ovarian syndrome (PCOS) and associated acne. A study showed enhanced anti-inflammatory and anti-oxidant activity of probiotics in synergy with anti-oxidant rich pomegranate juice (Esmaeilinezhad et al. 2020). *Prebiotics* are also gaining attention in acne treatment and managing gut dysbiosis due to the enhancement of probiotic activity. A study showed Glucomannan hydrolysate stimulates the growth of probiotic lactobacilli and inhibits the commensal bacteria, thereby enhancing the probiotic activity (Al-Ghazzewi and Tester 2010). Figure 6.2 summarises effects of probiotics.



6.6 Concluding Remarks

Acne development and exacerbation depends on skin type, topography, the environmental conditions, diet and lifestyle, and other factors discussed in this chapter. Nevertheless, the major factor governing here is the gut and skin microbiota. The gut microbiota affects the skin microbiota which together affects the sebum production. Hormones play an important role in this aspect as they control the lipid synthesis too. Presence of sebum is crucial for the colonization and proliferation of acne-causing *P. acnes* and therefore is an important mechanism for treatment strategies. While many conventional treatments like antibiotics focus on reducing the population of pathogenic bacteria, some others look at sebum control through regulating hormones and sebocyte function. This however has seen many limitations and is not sufficient to effectively treat acne. Probiotics have been getting the attention due to their antibacterial and anti-inflammatory properties.

Despite having many evidences of their usage for acne treatment, it is not conclusive enough. More research needs to be done to fix the blind spots. For one, the sample sizes in clinical trials and studies are small, and the parameters are not consistent and universal. Most importantly, the mechanism of probiotics in antibacterial activity and modulation of host immunity remains unknown. Equal importance needs to be given to the associated complications like psychological effects. The emotional state of the patients triggers stress, exacerbating the pre-existing acne conditions which will slow down the treatment and compromise the strategy. Clinicians need to assess and monitor the psychological state of individuals with acne more frequently to assure their mental health. This should be closely monitored and addressed by the clinicians.

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Probiotic Effects on Skin Health: The Case of Photoprotection as a Model of Gut-Skin Dialog

7

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Abstract

The skin is the most external organ of our body, and it is constantly exposed to environmental challenges. In this way, ultraviolet radiation (UVr) contained within sunlight is one of the most important insults that our skin deals with. UVr deeply affects skin cells, promoting DNA damage, oxidative stress, and mitochondrial alterations, all of which lead to local inflammation after acute exposures and to skin carcinogenesis after chronic ones. Additionally, UVr can also alter our immune system, suppressing adaptive immune responses.

Probiotic microorganisms are very well known for their capacity to modulate the immune system, mainly at the gastrointestinal tract. However, orally administered probiotics also affect skin immunity. It has been demonstrated that different oral probiotic formulations may impact skin health. Whole

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microorganisms, as well as isolated molecules, have proven to be effective in skin photoprotection, avoiding detrimental effects of UVr on the skin. Some of these effects have been shown, such as a protection against UV-induced immunosuppression, carcinogenesis, loss of Langerhans cells, and other effects that will be summarized in the present chapter.

The mechanisms responsible for the translocation of the effects from the gut epithelium to the skin and its draining lymph nodes are still poorly understood, but some hypotheses will be set out here, considering the effects of probiotics on the gut-associated lymphoid tissue.

Keywords

Skin diseases · Skin cancer · Dendritic cells · UV radiation · Lipoteichoic acid

7.1 Introduction

Probiotic microorganisms are worldwide known for the beneficial effects they produce on human health. Precisely, probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." The most common use of probiotics is linked to food, especially to milk-derived products. Studies about the consumption of fermented milk and its relationship with longevity were the origin of the probiotics' description and studies.

More than a century ago, the Russian Nobel Prize awardee Élie Metchnikoff correlated the consumption of soured milk with gastrointestinal health and longevity observed in people from different regions, including the Caucasus region and Asian Russia (Metchnikoff 1908). In that fermented milk, the presence of various bacteria was described, which had the ability to produce lactic acid. Moreover, bacteria isolated from the Bulgarian "yahourth" were largely studied at the Pasteur Institute, demonstrating that they were capable of producing large amounts of lactic acid; consequently, they were called *Bacillus bulgaricus*. Additionally, French doctor Henry Tissier described the presence of *Bacillus bifidus* (due to their "Y" form) in the gastrointestinal tract of healthy individuals and their reduction in children with diarrhea, suggesting the beneficial effects of these bacteria in the gut microflora (Tissier 1906).

It is not surprising, then, that large experimental pieces of evidence studying and describing the beneficial effects of probiotic consumption on human health have been published during the last century. Those studies were designed, in the beginning, to study the role of beneficial microbes against pathogenic ones. In this way, Metchnikoff stated that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Tissier 1906). Later, the effects on different pathologies, including cancer and inflammatory diseases, were also analyzed. But the focus of those studies was mainly related to gastrointestinal health:

diarrhea and other symptoms caused by infections and microbiota alterations, gastrointestinal cancer (including colon cancer), and chronic inflammatory diseases such as Crohn's disease and ulcerative colitis. The gastrointestinal effects of probiotics have been extensively treated in different chapters from the present book series. In this chapter, we are going to discuss probiotics' impact on the skin, as well as the involvement of the immune system on those effects.

Probiotics' effects on the immune system are mainly related to molecular interactions between the microorganism and cellular receptors, presented in gastrointestinal immune cells as well as in epithelial ones (Sánchez et al. 2017). The recognised molecular targets are surface molecules, which are exposed to the host, including lipoteichoic acid (from Gram-positive bacteria), peptidoglycan, surface proteins, and different glycans that may modify different surface molecules. These molecular targets are sensed by immune receptors, such as toll-like receptors (TLRs) 2 and 6, which mediate particular effector responses, depending on the gut environment. These responses tend to be tolerogenic under typical situations, but they also may impact on effector T-cell responses, as the antitumoral immune attack of local and distant cancer cells (de Moreno de LeBlanc et al. 2007). Probiotics may also produce soluble molecules that too impact on the gut immune system. In this way, the role of lactic acid production as a significant assessment for probiotic capacity has been described since the very first description of this group of bacteria. As an example of the role of this molecule on gut immunity, lactate may decrease inflammatory responses on dendritic cells and macrophages in vitro and prevent intestinal inflammation in a murine colitis model (Iraporda et al. 2015, 2016). But besides lactic acid, probiotic (as well as gastrointestinal microbiota) may produce large amounts of short-chain fatty acids (acetate, propionate, and butyrate), which affect the immune cell metabolism and the final immune response (Tan et al. 2014). Overall, probiotics affect the balance of gut microbiota and modify the global availability of molecular targets for immune receptors and soluble metabolites, leading to modulation of adaptive immune responses.

No impact of probiotic consumption on other tissues was being considered at first, but it has been shown recently that beneficial effects are not limited to the gastrointestinal tract. In 2016, the "International Scientific Association for Probiotics and Prebiotics" workshop aimed to establish evidence that proves probiotics' effects on distant organs, such as the skin. A review was published after this workshop, and the following questions were stated: "Is the skin the most improbable site for orally administered probiotics to affect?" (Reid et al. 2017).

Hereafter, we present pieces of evidence and comments on the role of probiotic consumption on the amelioration of skin affections. First, we comment on the role of probiotic consumption on skin disorders that involve immune function alterations (such as atopic dermatitis and psoriasis). Next, we go deeper into the effects of probiotics against the damage triggered after exposure to ultraviolet radiation, as a way to demonstrate the connection between gut and skin immunity.

7.2 Probiotic Effects on Skin Health

The human microbiota is a large and complex community of microorganisms that include bacteria, fungi, protozoa, and viruses with critical value in maintaining a healthy state. Possibly, the microbiome and the implications in the human pathology research area are one of the hottest spots in the biomedical field currently.

During different skin conditions, the alteration of gut or skin microbiota, known as dysbiosis, has been documented. The most studied of these conditions are atopic dermatitis (AD), acne vulgaris, and psoriasis. Some of these pathologies have shown an increased gut permeability what is believed to be a critical step in its pathogenesis. With this in mind, efforts have been made to modulate gut and skin microbiota mainly by probiotic consumption to treat these patients.

Different experiments and trials have been made to evaluate the capacity of ingested probiotic to safely modulate gut microbiota. Notably, also the topic probiotic administration has been used to induce skin microbiota restoration. Both strategies could be useful in the prevention or treatment of skin pathologies (Fig. 7.1).

In this section, we will focus briefly on the microbial alterations at both gut and skin levels that are found in AD, as an example of these cutaneous pathologies.

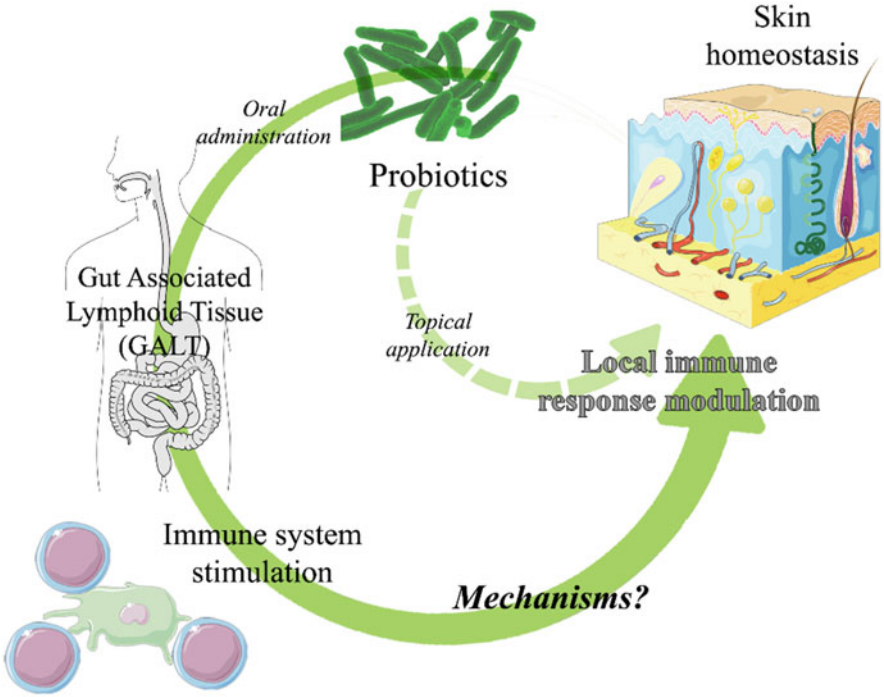

Fig. 7.1 Probiotics affect skin health by oral or topical application. Oral route activates the immune system promoting activation of different cell types, including dendritic and T cells

Moreover, we will summarize the current evidence of oral probiotics as an interesting therapeutic approach for the treatment of diverse skin conditions.

7.2.1 Changes of the Gut Microbiota in Skin Disorders

Atopic dermatitis (AD) is a chronic, inflammatory skin disease characterized by dry skin, intense pruritus, and eczematous cutaneous lesions that often appear with other atopies (allergic rhinitis, bronchial asthma). AD prevalence is increasing, reaching 10–20% in the pediatric population and up to 3% of adults; commonly the onset occurs during the first year of life (Nutten 2015).

The nature of gut microbiota in AD patients has been profusely studied in order to assess if they present a reduced bacterial diversity and to determine particular bacterial growth alterations. This may be important for understanding AD pathophysiology and establish possible probiotic treatments. Regarding microbial diversity, the evidence is contradictory. Out of 11 observational studies reviewed by Petersen and collaborators (Petersen et al. 2018), in 5 of them, there were no significant differences observed regarding gut microbiota, while other 5 AD patients showed a lower bacterial diversity compared to healthy individuals. In only one study, an increase in bacterial diversity was observed in AD patients' fecal samples. On the other hand, feces of AD patients present significantly less *Enterococcus* (Mah et al. 2006) and short-chain fatty acid (SCFA)-producing bacteria, like *Bifidobacterium*, *Blautia*, *Coprococcus*, *Eubacterium*, and *Propionibacterium*, than feces of healthy individuals. Some *Staphylococcus*, *Bifidobacterium*, *Corynebacterium*, and *Bulleidia* were completely absent in AD patients (Reddel et al. 2019). On the opposite, *Staphylococcus* (Watanabe et al. 2003), *Faecalibacterium prausnitzii* (Song et al. 2016), *Escherichia coli* (Penders et al. 2006), and *Faecalibacterium*, *Oscillospira*, *Bacteroides*, *Parabacteroides*, and *Sutterella* (Reddel et al. 2019) are increased in AD patients' feces. Furthermore, *Bifidobacterium* count and percentage differed by the disease state: lower numbers were found in those with severe AD but not in patients with mild atopic symptoms (Watanabe et al. 2003). In the same direction, a negative correlation between fecal *Clostridium* numbers and AD severity has been established (Kirjavainen et al. 2001).

One may argue that the gut microbiome dysbiosis is just an associated event in AD pathophysiology and not a major cause of this condition. However, not only the administration of oral probiotic bacteria could reduce the risk of AD development, but they also were effective in treating AD patients and improve the symptoms. Apparently, gut microbiota plays a pivotal role in AD. From a mechanistic point of view, gut microbiota dysbiosis has been shown to precede the onset of AD, supporting the theory of "microbial deprivation syndromes of affluence." According to this theory, diminished intensity and diversity of microbial stimulation impair proper immune maturation during early childhood. In fact, limited microbial pressure results in insufficient Th1 cell induction and the failure to suppress Th2 responses. The switching of the immune stimulation toward a pronounced Th2 phenotype is suggested to be a major mechanism to explain allergy development

and maintenance. Besides, it is now clear that normal levels of SCFA in the intestinal lumen are critical to promoting regulatory immune responses, since a decrease in these compounds, such as butyrate, promotes intestinal epithelium inflammation and is partially responsible for the increased intestinal permeability observed in AD patients. As mentioned before, AD patients' feces contain less SCFA-producing bacteria and therefore showed reduced levels of SCFA in feces compared to healthy individuals.

Changes in gut microbiota were also observed in acne vulgaris (Deng et al. 2018) (a chronic disease of the pilosebaceous unit that manifests clinically as non-inflammatory comedones or inflammatory papules, pustules, and nodules) and in psoriasis (Codoñer et al. 2018). The detailed description of these alterations exceeds the aim of this chapter.

7.2.2 Changes of the Cutaneous Microbiota in Skin Disorders

The interactions of the bacteria with the epithelium and the immune system are complex and dynamic. It depends on a wide variety of factors and may be disturbed from the basal, normal, non-inflammatory state. These global concepts may be applied to the gut as well as to the skin. The complexity of the immune system mechanisms may explain the connection between the gut and the skin. AD patients present, besides gut microbiota alterations, changes in skin microbiota as well.

A reduction of the cutaneous microbiome diversity was reported in AD patients (Wollina 2017; Paller et al. 2019), being the disease associated with early colonization with *Staphylococcus aureus* (Byrd et al. 2017). The cutaneous presence of *Staphylococcus epidermidis* and *Staphylococcus cohnii* (Cogen et al. 2010) has proven to have a protective effect against AD in children. Increased colonies of *S. aureus*, *S. epidermidis*, *Propionibacterium*, *Corynebacterium*, and *Streptococcus* (Cogen et al. 2010) were isolated in the AD lesions. Another study has found an association between AD severity and the abundance of the genus *Corynebacterium* and the phylum *Proteobacteria*. The presence and chronicity of eczema appear to be more important determinants of skin microbiome configuration (Grice 2014).

Changes in skin microbiome composition were also observed in other dermatological conditions such as rosacea, acne, psoriasis, or seborrheic dermatitis (Grice and Segre 2011).

Immune tolerance to skin microorganisms is supposed to be established soon after birth, as it has been reported in mice models (Scharschmidt et al. 2015). This tolerance is crucial to avoid immune attack to the skin microorganisms, initiating an inflammatory response. Moreover, the cross talk between different bacterial strains through quorum sensing signals also contributes to avoiding the skin colonization by pathogen bacteria, such as *S. aureus* (Williams et al. 2019). However, the balance can be broken, even though the events that initiate the alterations in the microbiota are still unknown.

Treatments that efficiently restore normal balance between species in the cutaneous microbiota are needed. The use of probiotics for skin conditions is a promising new field of research.

7.2.3 Probiotics in the Treatment of Skin Disorders

Probiotics are an interesting therapeutic strategy for clinical skin conditions. For that purpose, probiotics may be administered directly on the skin, or they may be ingested. The safety of these products is a matter of discussion, and even though they have been consumed worldwide for centuries, the safety in their use depends on the frequency of administration as well as in the route. As they are live microorganisms, their safety depends on the intended use (Sanders et al. 2010).

Probiotic consumption is one of the most important modulators of the gut microbiota, together with antibiotic intake. In the case of probiotics, the modulation involves the production of different molecules, such as metabolic compounds and antimicrobial agents, which suppress the growth of other microorganisms (Spinler et al. 2008; O'Shea et al. 2012), and competition with other intestinal microbes for binding sites or receptors on the intestinal mucosa (Collado et al. 2007).

Topical cutaneous probiotic applications have been poorly investigated; however, there are pieces of evidence in vitro and in vivo about the efficacy of probiotics on cutaneous health. In vitro, *L. rhamnosus* and *L. reuteri* were used to induce keratinocyte proliferation and to avoid *S. aureus*-induced apoptosis (Mohammedsaeed et al. 2015; Prince et al. 2012). Besides, lysate from *Lactobacillus* and *Bifidobacterium* effectively reinforce the barrier function of the skin, through modulation of tight junction proteins (O'Neill et al. 2013). These effects fortifying the epidermal layers correlate with *L. plantarum* ability to avoid wound infection and the consequent dead of mice, in a model of wound infection by *Pseudomonas aeruginosa* (Argenta et al. 2016). Unfortunately, there is a lack of clinical trials to clearly determine the usefulness of topical probiotics in the treatment of chronic skin conditions.

On the other hand, the oral administration of probiotics has been studied for different skin conditions. Atopic dermatitis is probably the pathology for which most clinical trials have been set. Because AD is more prevalent in children than adults, there are more studies performed on this population. Meneghin and colleagues gather together clinical trials aimed to prevent or treat AD in children using different oral probiotics (Meneghin et al. 2012). Regarding the prophylactic administration of probiotics to prevent the development of AD, they revised 17 studies in which 13 showed different grades of effectiveness. It is important to emphasize that some of the trials were performed on pregnant mothers and continued in the newborn children, showing some efficacy in highly predispose individuals to develop AD (Enomoto et al. 2014; Kukkonen et al. 2007). On the other hand, different *Lactobacillus* species (Drago et al. 2011), as well as *Bifidobacterium* (Lin et al. 2015), were effective in treating AD patients and improve the symptoms. The revision of Meneghin and colleagues presents the overall effectiveness of 15 out of 20 clinical

trials showing some positive effects on the disease management due to the use of some probiotic formulation. Regarding adult patients, there are some trials published demonstrating the efficacy in the treatment of established AD. The treatment with *Bifidobacterium animalis* subsp. *lactis* LKM512 showed a decrease in the itch of adult AD patients (Matsumoto et al. 2014). Moreover, the treatment increases the levels of kynurenic acid, an antipruritic and antinociceptive metabolite. Interestingly, the probiotic viability does not seem to be essential for the treatments. Heat-killed *L. acidophilus* L-92 showed to be effective in decreasing the scores of severity and the eosinophilia and increasing the serum levels of a regulatory cytokine, TGF-β, in treated AD patients compared to placebo (Inoue et al. 2014).

An updated revision, including the analysis of clinical trials performed in pediatric and adult AD patients, has been recently published by Rusu and colleagues and is recommended for the readers who want a piece of detailed information about this topic (Rusu et al. 2019).

Regarding the use of probiotics for other skin conditions, the evidence is less solid (Notay et al. 2017). In the treatment of acne vulgaris, there is just one clinical trial published on female patients using a mixture of *L. acidophilus*, *L. delbrueckii* subspecies *bulgaricus*, and *B. bifidum* alone and minocycline (the study included three groups: probiotics alone, antibiotic alone, or a mixture of both). Patients were monitored in weeks 4, 8, and 12, regarding the number of their lesions. By week 4, all the experimental group reduced the lesions compared to baseline, but without significant differences between them. The same effect was observed in week 8. However, at week 12, there was a significant decrease in the number of lesions in the group of patients treated with the mixture of probiotics and antibiotic compared to the other two groups (Jung et al. 2013). Interestingly, in the group of patients treated with the antibiotic alone, there was a 13% of incidence of candidiasis, probably due to a dysbiosis that was compensated in the group treated with the antibiotic plus probiotics. It is important to highlight that probiotics alone worked as well as antibiotic alone.

For the treatment of psoriasis with probiotic, there is also just one clinical trial to comment. In this case, *B. infantis* 35624 was used. The aim of the trial was to evaluate the ability of the probiotic to reduce the inflammatory mediators present in these patients. After 8 weeks of treatment, the serum levels of C-reactive protein and TNF-α were reduced significantly compared to baseline, without affecting the levels of IL-6 (Groeger et al. 2013). Unfortunately, the authors did not evaluate the cutaneous effects of the treatment on the severity of the lesions.

To sum up, there is evidence showing the connection between the gut immune system and the skin. Patients may take advantage of this connection by supplementing their alimentation with probiotics, which ultimately could impact positively in their pathologies.

7.3 Ultraviolet Radiation Effects on Skin Health

The effects of probiotic treatment of the ultraviolet (UV)-induced skin damage have some experimental shreds of evidences, published during the last years. In order to adequately explain these protective effects at such a distant organ, we need first to explain the alterations caused by skin exposure to UV radiation. These alterations may be triggered after a single exposure, which we are going to present as acute effects of UV radiation, or after multiple exposures along with life, which is related to chronic effects. Both acute and chronic UV-induced alterations can be ameliorated by probiotic ingestion.

The UV radiation is part of the electromagnetic spectrum emitted by the sun. It comprises a range of wavelengths between 100 and 400 nm and can be subdivided into three categories: UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm). Although the sun emits large amounts of UV radiation, only 5% of it reaches the Earth's surface. UVC rays are completely absorbed by ozone and scattered by O_2 and N_2 molecules, while only 10% of UVB and 100% of UVA reach the Earth's surface. Therefore, human skin is only exposed to UVA and UVB rays. UV radiation has been shown to affect the skin in different ways according to its wavelength: while UVA penetrates deeper into the dermis, UVB only reaches the epidermis (the most superficial part of the tissue). Short wavelengths have higher energy and are, thus, more potentially damaging (Svobodová and Vostálová 2010). UVA is responsible for sunburn, skin photoaging, and wrinkle formation, whereas UVB produces direct damage to the skin macromolecules, such as DNA and proteins, and indirect damage through the production of reactive oxygen species, leading to lipid peroxidation and protein modifications (Matsumura and Ananthaswamy 2004). Exposure to UV radiation is essential for life and presents some beneficial effects on human health. However, this radiation can also have acute and chronic harmful effects on the skin, from sunburn and photoaging to photoinduced carcinogenesis.

7.3.1 UV Radiation Acute Effects

The skin is the main target of UV radiation. Its effects on target cells and tissues include molecular and cellular damage produced when the energy of the radiation is absorbed by chromophores present in the skin. UV damage occurs in two different ways, direct and indirect damage, depending on the molecule that absorbs the radiation and the damaged one. The skin contains endogenous chromophores (photosensitizers) such as nucleic acid bases, aromatic amino acids, NADH, NADPH, quinones, flavins, porphyrins, carotenoids, 7-dehydrocholesterol, eumelanin, and urocanic acid; many of them induce the formation of reactive oxygen species (ROS). On the one hand, when photosensitizers are activated to an excited state, they interact with molecular oxygen and directly transfer the energy to produce singlet oxygen ($^1O_2$), known as type I photosensitization mechanism. This can also produce oxygen free radicals ($O_2^{\bullet -}$) by transferring an electron, which can then

result in the formation of H_2O_2, known as type II photosensitizing mechanism. In addition, $NO^{\bullet}$ could be generated through UV light-induced decomposition of endogenous nitrite anion, which results in reactive nitrogen species. All these species can directly damage protein or lipid peroxidation and also DNA (Pillai et al. 2005). In the skin, ROS are constantly generated by keratinocytes and fibroblast and are rapidly removed by different antioxidant systems, nonenzymatic ones, such as ascorbic acid, tocopherol, ubiquinol, and glutathione, and enzymatic ones, such as catalase and superoxide dismutase, in order to maintain the prooxidant/antioxidant balance.

The phototoxic effect of UVA radiation is much lower than UVB radiation. The effects of UVB radiation on DNA are mostly caused by the formation of dimeric photoproducts between adjacent pyrimidine bases on the same strand. There are two main types of dimers: cyclobutane pyrimidine dimers (CPDs), between adjacent thymine (T) and cytosine (C) residues, and pyrimidine-pyrimidone (6–4) photoproducts (6–4 PPs), among adjacent pyrimidine residues. Both lesions occur most frequently in areas of tandem pyrimidine residues, which are known as "hot spots" of UV-induced mutations. These photodimers might produce DNA duplication failures, leading to punctual mutations randomly distributed in cellular DNA, recognizable by CC→TT (UV-specific) and C→T (non-UV specific) transitions. However, cells present several DNA repair systems that remove DNA lesions. The most important mechanism is the nucleotide excision repair (NER) system, which removes bulky DNA damage (Matsumura and Ananthaswamy 2004). NER failure or exceeding of its repair capacity results in the accumulation of mutations in skin cells, which can lead to the development of skin cancer (Svobodová et al. 2012). Upon DNA damage by UV radiation, there are other mechanisms triggered to prevent the transmission of mutations to daughter cells: (1) cell cycle arrest followed by DNA repair and (2) induction of apoptosis of damaged cells (Müllauer et al. 2001). UV radiation induces the accumulation of p53 protein, which, in turn, induces the production of p21, which inactivates the CDK-cyclin complex inducing a cell cycle arrest at the G1 phase, before its replication (Decraene et al. 2001). If the DNA damage is too severe to be repaired, the cell goes into the apoptotic pathway by the disruption of the balance between the proapoptotic protein Bax and the anti-apoptotic protein Bcl-2, upregulating the expression of Bax gene (Boise et al. 1993). Bax induces mitochondrial outer membrane permeabilization, which induces, on the one hand, the release of cytochrome c, activating the caspase apoptotic pathway, and, on the other hand, mitochondrial depolarization, oxidative phosphorylation uncoupling, and $O_2^{\bullet -}$ production (Düssmann et al. 2003).

As the skin is the outermost organ of the body, it is the main target of UV radiation. For this reason, keratinocytes are central players in the establishment of the inflammatory response. UV radiation causes a disruption of the epithelial barrier, and consequently, keratinocytes trigger a coordinated immune response in order to maintain skin homeostasis. The inflammatory process includes a cascade of events, which involves the production of inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6, IL-18, chemokines IL-8 and CCL-20, antimicrobial peptides α and β defensins, cathelicidin, S100 proteins, and ribonucleases) and growth factors (GM-CSF and

VEGF-α). The last ones increase vascular permeability leading to edema formation and infiltration of inflammatory blood leukocytes (neutrophils, macrophages, and lymphocytes) to the irradiated area.

However, when the skin is exposed to UV radiation, keratinocytes and other immune cells, such as mast cells, neutrophils, and monocytes, also produce soluble regulatory mediators such as IL-10, IL-4, prostaglandin E_2 (PGE_2), and platelet-activating factor (PAF) (Shreedhar et al. 1998; Zhang et al. 2008). Direct damage to keratinocytes induces PAF production, stimulating the expression of cyclooxygenase 2 (COX-2) necessary to produce PGE_2. Besides, PAF activates mast cells and induces their migration to draining lymph nodes. In addition, trans-urocanic acid (UCA), which is present in the stratum corneum and is able to absorb the energy contained in UV radiation, isomerizes to the cis isoform. Cis-UCA interacts with the serotonin receptor present in keratinocytes, Langerhans cells (LCs), and mast cells, stimulating the production of TNF-α from keratinocytes, which avoids LC migration and prevents antigen cell presentation. Cis-UCA also stimulates histamine and PGE_2 production by LCs and mast cells. Consequently, multiple signaling pathways and cells are activated, and more soluble mediators are produced (Fig. 7.2).

As a consequence of the release of the molecular intermediates mentioned before, cutaneous immune responses are highly conditioned. The normal cutaneous response against a foreign antigen, like those from infectious pathogens or tumoral cells, is initiated by Langerhans and dermal dendritic cells, which are activated and migrate to lymph nodes to prime-specific T cells. The UV radiation is capable of suppressing these immune responses, due to alterations in the activation state of dendritic cells and a suppressor phenotype induced in specific T cells during priming in the lymph nodes. Moreover, it has also been described a loss in the number of epidermal Langerhans cells after UV irradiation. Plenty of mediators are involved in this differentiation, including many mechanisms mentioned before: mast cells, IL-10, PGE_2, and others. In this way, the abolishing of the IL-10-mediated mechanism leads to a decrease in the UV-induced immunosuppression, as it has been demonstrated in the IL-10 knockout mouse model (Ghoreishi and Dutz 2006) and human subjects due to IL-10 promoter polymorphisms (Alamartine et al. 2003). Moreover, pharmacological inactivation of cyclooxygenase 2, which ultimately inhibits PGE_2 productions, leads to a decrease in UV-induced immunosuppression (Prasad and Katiyar 2013).

The impact of UV-induced immunosuppression on skin pathology development or alterations in the immune system-microbiota cross talk is still a matter of research. However, its role in carcinogenesis has been very well studied and presented in the next section.

7.3.2 Chronic Effects

As it was mentioned before, UV radiation promotes DNA damage, leading to mutations and consequent malignant transformation of epidermal cells. As the most numerous cell types in the epidermis, keratinocytes are the main target of

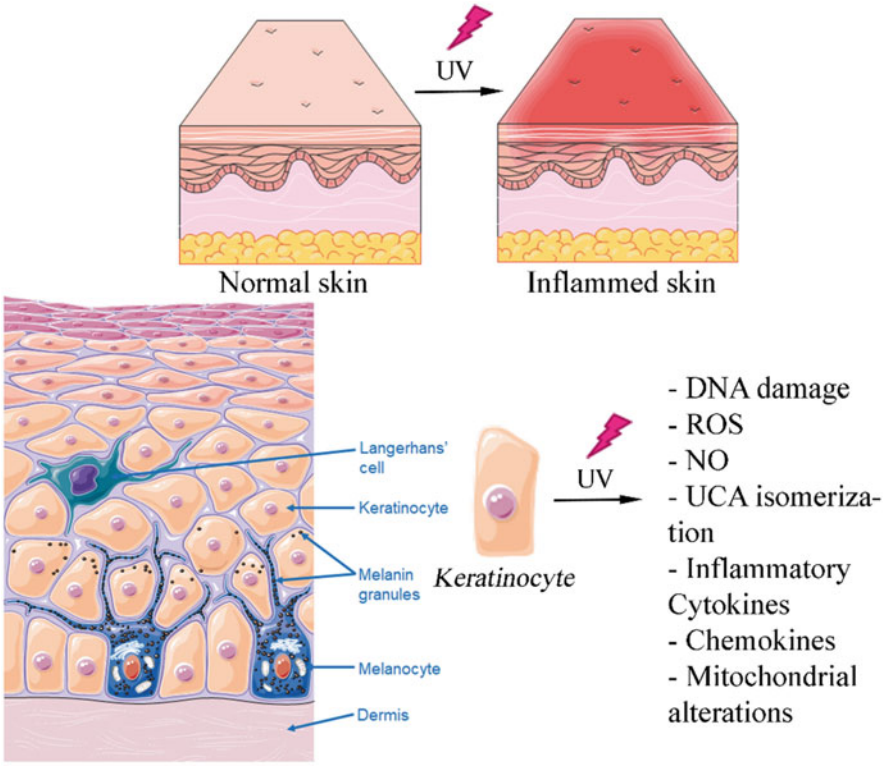

Fig. 7.2 Skin response induced after UV exposure is complex and includes local inflammation and cellular and molecular alterations. Keratinocytes, the main cell type in the epidermis, are highly affected and secreted different immune mediators as a response to radiation

UV radiation. Their malignant transformation leads to the development of basal cell carcinoma (BCC) or squamous cell carcinoma (SCC). Moreover, another cell type presented in the epidermis may suffer alterations and malignant transformation, such as the melanocytes that lead to the development of cutaneous malignant melanoma (CMM or melanoma). The development of these three skin cancers is correlated with exposure to UV radiation (Armstrong and Kricker 2001). In these tumors, it is common to find mutations on specific cell cycle control genes, such as the abovementioned p53 (Nakazawa et al. 1994). However, more than four decades ago, Margaret Kripke described that UV-induced tumors in mice were highly immunogenic, meaning that they were easily rejected when transplanted on a healthy mouse (Kripke 1974). At that moment, it was considered that UV radiation must have been promoting more effects than just mutagenesis, affecting the immune system. Actually, Dr. Kripke was the first one to describe the suppressive effects of UV radiation on mammals, using SCC transplantation mouse models. These tumors, commonly rejected in normal mice, were able to establish and grow when implanted in UV-exposed hosts, even though the animals were exposed just to one

dose of radiation (Fisher and Kripke 1977; Ullrich and Kripke 1984). Many of the molecular mechanisms described before were studied in UV-induced tumor models. However, to analyze the mechanisms involved, contact hypersensitivity (CHS) models were employed. CHS is an acute inflammatory response triggered by specific T cells against a topically applied antigen. Different molecules and mechanisms mentioned to be involved in UV-induced immunosuppression have been studied as targets in antitumoral treatments: PGE_2 and COX-2 (Pentland et al. 1999; González Maglio et al. 2010), IL-10 production (Nagano et al. 2008), vitamin D (Dixon et al. 2011), and reactive oxygen species (Duncan et al. 2009).

However, the complex pathways activated by UV radiation on the skin and immune organs can also be modified by the administration of probiotics. These treatments are summarized in the next section, as the main message of this chapter.

7.4 Probiotic Modulation of UV-Induced Skin Damage

The use of different agents for oral photoprotection has emerged as a topic of interest in the last years. There are different components with the capacity of ameliorating some of the harmful effects of UV radiation. Some examples are vitamins, plants, and botanical actives and nonbotanical foods (Parrado et al. 2018). Among the first group, it is interesting to mention the classical antioxidant molecules, such as β-carotene, which protect against ROS-induced damage but also derivatives, such as nicotinamide (an amide form of Vit B3), which showed antitumoral effects in human trials (Snaidr et al. 2019). On the other hand, the use of botanicals in oral photoprotection has been described many years ago. Plenty of plant and fruit extracts present beneficial effects against UV-induced damage, from inflammation and immunosuppression to carcinogenesis. Some good examples, supported by the bibliography, are the use of green tea polyphenols and *Polypodium leucotomos* extract. Finally, the last group of compounds for oral photoprotection, the nonbotanical foods, includes some polyunsaturated fatty acid and probiotics. Even though there are few published works in probiotic-mediated photoprotection, the evidence shows that it is a field of importance that deserves our attention and more basic research and clinical trials in the future (Baquerizo Nole et al. 2014).

7.4.1 Live Probiotics in Photoprotection

The first paper reporting the use of probiotics in photoprotection, to the best of our knowledge, was published by Audrey Guéniche and colleagues in 2006 (Guéniche et al. 2006). They treated hairless mice (SKH1 strain) with 1×10^8 CFU/day of *L. johnsonii*, for 10 days, prior to exposing them to a single dose of UV radiation. They reported prevention in the UV-induced immunosuppression with the probiotic treatment. Moreover, they also observed a loss of epidermal Langerhans cells and an increase in serum IL-10 induced by UV radiation that was prevented by the

administration of *L. johnsonii*. As expected, the erythema induced in the skin, 24 h after irradiation, was not affected by the probiotic.

Another paper published using mice models studied the effects of *Bifidobacterium breve* on UV-induced damage (Sugimoto et al. 2012). The treatment was also initiated before exposure to UV radiation, but in this work, the irradiation was applied in 4 consecutive days. As a measure of the damage induced, the appearance of the skin and the elasticity, elastase activity, and interleukin (IL)-1β levels were determined. UV radiation promotes significant increases in elastase activity and IL-1β levels; both effects were prevented by the *B. breve* administration.

The use of fermented milk, as well as the exopolysaccharides purified from it, was effective in treating the UV-induced DNA damage, by upregulating the transcription of enzymes involved in DNA repair. Moreover, the treatment also prevented UV-induced hyperplasia, keratinocyte proliferation, and erythema. The relationship between anti-inflammatory and pro-inflammatory cytokine transcription (IL-10/IL-12 and IL-10/IFN-γ) was increased by both treatments after UV exposure (Morifuji et al. 2017). Probiotics used to produce fermented milk and to isolate exopolysaccharides were *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1247 and *Streptococcus thermophilus* 3078, and the administration of both treatments began 1 week before exposure to a single dose of UV radiation.

More evidence in the usefulness of probiotics for photoprotection was analyzed in clinical trials. In 2008, Peguet-Navarro and colleagues performed a randomized placebo-controlled trial recruiting 54 healthy volunteers that were supplemented with *L. johnsonii* for 6 weeks prior to expose them to UV radiation. They observed the number of Langerhans cells and their capacity to prime allogeneic T cells 1, 4, and 10 days after exposure to UV radiation. The probiotic pretreatment didn't have an effect 24 h after irradiation since the decrease in Langerhans cell number and function observed in the placebo group was not affected. However, 4 days after the irradiation, a higher number of these cells were observed in *L. johnsonii* treated subjects. Moreover, these cells recovered their ability to prime T cells (Peguet-Navarro et al. 2008). To the best of our knowledge, this recovery in Langerhans cells' function was the first photoprotective effect reported for oral probiotics.

In a different study, also performed with *L. johnsonii*, a mixture of the probiotic with carotenoids was administered to healthy individuals, before they were exposed to UV irradiation. In this study, three different sources of radiation were employed: non-extreme UV with high UVA level (CT1, relationship UVA irradiance/UVB irradiance = 24), solar-simulated UV radiation (CT2, relationship UVA irradiance/UVB irradiance = 10), and sunlight during summer vacations (CT3) (Bouilly-Gauthier et al. 2010). Each subject of the trial was exposed to UV radiation prior to supplementation in order to obtain basal responses; then, they were supplemented for 6 weeks and challenged again with the same irradiation scheme (in a different zone of the body). In the CT1 cohort, a reduction in inflammatory dermal cells (CD45+) was observed after supplementation. Moreover, a UV-induced decrease in Langerhans cells was avoided by oral treatment. In CT2 cohort, an increase in the minimal erythemal dose of radiation was observed after supplementation, meaning that a higher exposure to UV radiation was necessary to promote the same erythemal

response in supplemented subjects. Finally, the CT3 cohort was evaluated by dermatologists. They observed that supplementation prevented sunburn, sun intolerances, and the appearance of sunspots. In accordance with the opinion of the professionals, the involved subjects reported an improvement of skin resistance to sun exposure, improved skin color, and better skin condition. These results are very interesting, but it is impossible to distinguish if the effect reported is due to the probiotics, to the carotenoids, or both.

As a final comment to this section, it has been mentioned that probiotic supplementation impacts the gut microbiota, modifying its balance. Changes in gut microbiota have been associated with the efficacy of the immune response against cutaneous melanoma in mice models. In a simple experiment, Sivan and colleagues proved that genetically identical animals (obtained from different laboratories) that were colonized by different bacteria responded differently to B16 melanoma cell line implanted in their skin. The administration of fecal suspensions or the cohousing of the mice equalized the tumor growth between animals. Moreover, this enhanced immune response allowed anti-PD1-PDL-1 therapy to be more effective. The effect was identified to be induced by *Bifidobacterium*, showing that the administration of probiotics may enhance antitumoral immune responses, also in the case of skin cancers (Sivan et al. 2015). On the other hand, UV radiation may affect skin microbiota. Cutaneous microorganisms have different susceptibilities to UV radiation, also depending on the dose of the radiation. Moreover, they also can be differentially affected by the cutaneous immune response (including inflammatory factors and antimicrobial peptides) triggered by UV radiation. The alterations that may suffer the microbiota in exposed individuals remain to be elucidated (Patra et al. 2016).

7.4.2 Effects of Isolated Probiotic Components

Probiotics, as well as pathogen bacteria, present a wide variety of molecules that can be recognized by immune receptors. Some of these molecules are presented in the bacterial cytoplasm, and others are exposed on the cell surface. These superficial molecules may trigger immune mechanisms even if bacteria are dead. Even though there are large pieces of evidence demonstrating the need for probiotic viability to produce the beneficial effects, there are some others that demonstrate that single molecules, isolated from the bacterial surface or secreted by the bacteria, are enough to produce benefits on health. Actually, it has been introduced the term "postbiotic" to refer to any molecules with beneficial effects on health (Aguilar-Toalá et al. 2018). In our laboratory, we have experienced using lipoteichoic acid, a surface molecule, to treat UV-induced skin damage. This experience will be summarized after a brief description of some characteristics of the lipoteichoic acids.

7.4.2.1 Functional and Structural Characteristics of LTA

Gram-positive bacteria have a particular and distinctive component on their cell surface with respect to Gram-negative bacteria: teichoic acids (TAs), which are

specific polymers that comprise approximately half of the cell wall dry weight. Although the term TAs derives from the Greek word *teichos*, meaning "wall," two different types of TAs have been found: a literally wall teichoic acid (WTA)—attached to the cell wall—discovered by Armstrong and colleagues in 1958 (Armstrong et al. 1958) and a lipoteichoic acid (LTA)—anchored to the cell membrane—discovered by Kelemen and colleagues in 1961 (Kelemen and Baddiley 1961).

The structural characteristics of WTA and LTA are centered in a backbone usually formed by anionic polymers, which in LTAs are mostly poly-glycerol phosphate but in WTA are more variable, with the possibility of having poly-glycerol phosphate or poly-ribitol phosphate as the backbone. These polyol phosphate units are then covalently anchored either to the cell wall N-acetylmuramic acid (WTA) or to hexose residues of cell membrane glycolipids (LTA) (Araki and Ito 1989).

These TAs have been found to play relevant physiological roles for bacterial survival and also contribute to the interaction between bacteria and host. Regarding this interaction, LTA from probiotic bacteria has been proven to have immunomodulatory effects in some hosts. Therefore, LTA is one of the most studied molecules in these probiotic bacteria, and it is considered equivalent to LPS in Gram-negative bacteria, due to its immunogenicity and capacity to interact with TLR receptors, specifically TLR2/TLR6 (Schwandner et al. 1999; Schröder et al. 2003).

LTA is structurally diverse in different strains; thus, the structural heterogeneity of LTA in probiotics may have an impact on various responses observed regarding the modulation of host immune response and its beneficial or harmful roles.

Particularly, LTA from most of the microorganisms of the *Lactobacillus* genus is formed by a glycerol phosphate backbone, with D-alanine and N-acetylglucosamine residues, anchored by two glucose molecules linked to diacylglycerol. But even within the genus *Lactobacillus*, there are substantial differences in the percentage of D-alanine present in the LTA molecule, in the length of the glycerol phosphate backbone, and in the glycosidic anchors (Shiraishi et al. 2016). Consequently, different *Lactobacillus*' LTA exerts dissimilar effects. For example, LTA from *Lactobacillus plantarum* does not induce the secretion of TNF-α in macrophages in vitro (Ryu et al. 2009), while LTAs from *Lactobacillus fermentum* and from *Lactobacillus casei* are able to induce the secretion of this cytokine, not only in macrophages in vitro but also in murine splenocytes (Matsuguchi et al. 2003).

7.4.2.2 Effects of LTA Isolated from *L. rhamnosus* on Skin Damage

One of the main advantages of working with isolated molecules is to state a relationship between the target and the effect clearly. In this way, probiotic effects are extremely difficult to analyze, since they present multiple targets to the immune system, each of them triggering different effector responses. These complex responses may be orchestrated to produce a global effect on the immune system that, as has been presented, impacts on not only gut health but also distant organs such as the skin. Even though probiotic effects are not only restricted to the recognition of target molecules by immune receptors and limiting the growth and

colonization by harmful bacteria is also considered an important effect, we decided to focus our work in the analysis of a specific molecule from a probiotic bacteria: LTA from *Lactobacillus rhamnosus GG* (ATCC 53103). The study of this molecule may contribute to the knowledge in the probiotics-host interactions, but it may also have an impact on the development of new pharmacological strategies based on the immunomodulation promoted by these microorganisms since LTA can be delivered in different formulations than live probiotics.

L. rhamnosus LTA, purified in our laboratory, have shown to be active, capable of activating macrophages and bone marrow-derived dendritic cells but can promote a softer inflammatory response than LPS from *E. coli*. This effect was analyzed by the cytokine production after 24 h of stimulation, observing less production of TNF-α, IL-6, IL-12, IL-1β, and IL-10. Notably, the relationship between a pro-inflammatory cytokine (TNF-α) and an anti-inflammatory one (IL-10) is higher in LTA-stimulated cells, showing a tendency toward a regulatory response. When administered orally in mice, *L. rhamnosus* LTA can activate dendritic cells in lamina propria that ultimately activates T cells with a Th1 phenotype (Fig. 7.3). This modulation is not limited to the gut-associated lymphoid tissue (GALT); using this oral treatment, we efficiently decreased UV-associated skin alterations.

Our first approach was to analyze epidermal alterations (hyperplasia and apoptotic cells), T-cell number and phenotype (in the skin and skin-draining lymph nodes), and dendritic cell activation (lymph nodes) after 20-day and 6-month exposures to UVB radiation, in LTA orally treated mice (Weill et al. 2013). We observed increased T-cell numbers in lymph nodes, both CD4+ and CD8+, that produced larger amounts of IFN-γ in animals exposed during 20 days to UV radiation. This enhanced immune cellular response, with a Th1 profile, correlated with a stronger antitumoral response in the 6-month-exposed animals. In this group of mice, SCC development can be observed approximately 4 months after initiating chronic irradiation. *L. rhamnosus* LTA treatment, applied along with the whole irradiation

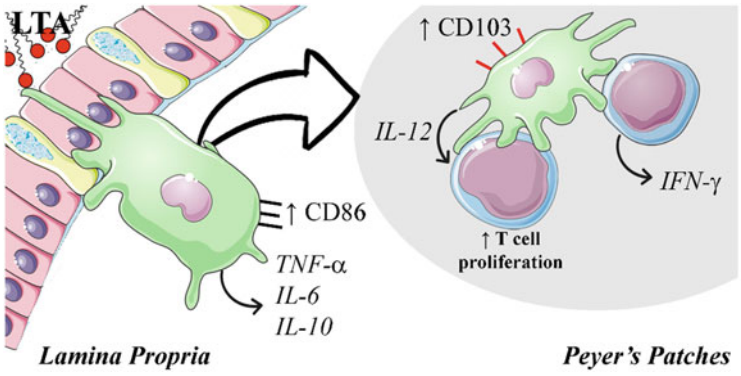

Fig. 7.3 Orally administered *L. rhamnosus* LTA is sensed by dendritic cells in the lamina propria. These cells migrate to Peyer's patches and induce T-cell activation and differentiation to IFN-γ-secreting T cells

scheduled before each exposure, promoted a significant decrease in the number of cutaneous lesions. Moreover, in these animals, a delayed in the onset of the tumors was also observed (Weill et al. 2013). Before our work, the efficacy of whole live probiotics against UV-induced immunosuppression after acute exposures was already published (Guéniche et al. 2006). The findings from our lab demonstrated that the immune system modulation was initiated in the gut that later reaches the skin. Further, the skin-draining lymph nodes produce long-term effects and can be triggered by a single, isolated molecule. The antitumoral effect of *L. rhamnosus* LTA was also evaluated as a therapeutic strategy in mice, by treating the animals after the tumors developed and the irradiation ceased. In this approach, LTA also showed efficacy in reducing the number of lesions as well as the total tumoral area (Friedrich et al. 2019), showing not only a prophylactic activity but also a therapeutic one.

L. rhamnosus LTA was also evaluated for its ability to reduce UV-induced immunosuppression after short exposures to the radiation source. For this aim, we treated the animals with eight administrations of the molecule by gavage before UV exposure. Twenty-four hours after irradiation, we sensitized the animals with oxazolone in unirradiated skin to induce a strong T-cell response. The efficacy of the T-cell effector response was evaluated by challenging the ears of the animals 6 days after sensitization. The inflammatory reaction, measured as the ear swelling 24 h after challenge, was abrogated by UV radiation, as it is very well known. *L. rhamnosus* LTA, as well as for *L. johnsonii* NCC 533, was highly effective in preventing immunosuppression (Friedrich et al. 2019).

To understand the cellular mechanisms involved in the described antitumoral and anti-suppressive effects of *L. rhamnosus* LTA, we further analyzed the CHS reaction to oxazolone with the LTA treatment before irradiation. First, we analyze three populations of skin dendritic cells that reach the lymph node 2 days after sensitization: Langerhans cells (LCs), CD103+ dermal dendritic cells (CD103+ dDCs), and CD103- dermal dendritic cells (CD103- dDCs). LC migration to lymph nodes was decreased by UV radiation, but LTA treatment prevented it. We also observed a decrease in the activation state of LCs (analyzed by CD80 expression), but this alteration was not modified by LTA treatment. Alterations produced by the radiation in dDCs, both CD103+ and CD103-, were not affected by LTA treatment. However, we observed a reversion in the activation state of lymph node resident DCs, which was decreased by UV radiation. Overall, we observed different shreds of evidence showing that UV radiation diminishes the global ability of different DC subtypes to present antigens. LTA treatment initiated before the exposure was effective in restoring some of the normal responses.

The global DC alterations induced by UV radiation led to a decrease in CD4+ and CD8+ T-cell activation, evaluated through CD44 expression 4 days after oxazolone sensitization. This decreased T-cell activation in the skin-draining lymph node is the reason for the absence of inflammatory response after challenge in UV-exposed animals. As expected, *L. rhamnosus* LTA treatment prevented the alterations in CD4+ and CD8+ T cells, restoring normal levels of activation after oxazolone sensitization.

The effect of *L. rhamnosus* LTA seems to be related to local skin inflammation during the antigenic challenges (considering both oxazolone and tumor cells as antigenic challenges). Inflammation in oxazolone-sensitized unirradiated skin is slightly downmodulated by UV radiation but restored to normal responses in LTA-treated mice. Moreover, using a different non-inflammatory antigenic stimulus as ovalbumin and reporting T-cell responses using OT-II mice, we observed that T-cell responses were also decreased by UV radiation, but the response was not affected by LTA treatment (unpublished data).

To summarize, *L. rhamnosus* LTA triggers immune mechanisms in the GALT (molecules or cells) that disseminate to the body, probably through blood, and reaches the inflamed skin. The LTA-triggered effector component (molecule or cells) reaches the skin and promotes a reinforce antigen presentation by DCs, leading to stronger T-cell activations and, finally, an enhanced local T-cell response. This enhanced response can be seen as a normal inflammation after challenge in CHS reaction, as well as an effective antitumoral immune response that leads to SCC size reduction. All of these effects are induced by an isolated molecule from the surface of a probiotic, and the use of the whole live microorganisms may promote even better responses, but they need to be further studied (Fig. 7.4).

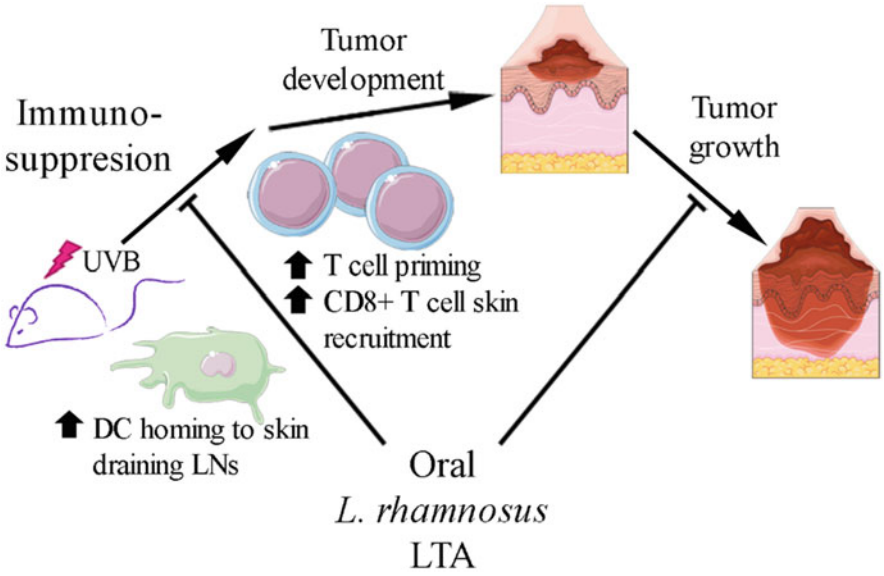

Fig. 7.4 UVB radiation induces systemic immunosuppression and skin cells' mutations, leading to tumor development and growth. Oral administration of LTA from *L. rhamnosus* inhibits immunosuppression through inducing dendritic cell (DC) migration to lymph nodes, triggering T-cell priming, and, finally, CD8 T-cell recruitment to the inflamed skin

7.5 Gut-Skin Dialog: Possible Mechanisms Involved

The question about the connection between the gut and the skin immunity remains largely unknown. Different hypothesis can be set, but it looks certain that probiotic (as well as prebiotics or postbiotics) oral administration impacts the skin immune responses (Friedrich et al. 2017). This can be observed in probiotic-induced dendritic cell and effector T-cell modulation both in the skin and in the skin-draining lymph nodes in different pathological scenarios (inflammatory condition, tumors, dysbiosis, etc.).

Here are some ideas that need to be explored in the next years, and we are certain that many others should rise soon:

1. The same probiotic bacteria strain is capable of reducing the cutaneous inflammatory response, such as in established AD, and promoting antigen-specific effector T-cell responses, such as in antitumoral and in anti-suppressive responses. How is this possible? Are there different mechanisms which get activated depending on the scenario? Or is it the same mechanism that impacts in a different way depending on the type of preactivated cells?
2. The immune mechanisms that are activated in the gut by probiotics include both antigen-specific and nonspecific cells. Which one of these targets is being recognized on the skin? Is it possible that antigens from damaged skin are recognized by gut-activated T cells?
3. In skin-draining lymph nodes, there are changes in dendritic cell phenotype and the corresponding T-cell differentiation. Are these cells coming from the GALT? Or are these effects justified by a wave of cytokines that reach the skin from the GALT?
4. Immune cells are differentiated from precursors in the bone marrow. Are there molecular signals from the GALT that induce the differentiation of inflammatory dendritic cells from the bone marrow?
5. What is the role of the microbiome in the global response to probiotics? Are there changes induced by probiotics that ultimately impact on the cutaneous immune response?

Regarding the last question and considering the topic of skin exposure to sunlight, there are two recent publications that we would like to address, as a final comment.

On the one hand, cutaneous microbiota is capable of modulating the harmful effect of UV radiation (Patra et al. 2019). In germ-free mice, responses to UV radiation were more suppressive and less inflammatory than in microbiota-colonized mice. These differences were observed in the CHS response (more immunosuppression in germ-free mice than in colonized ones), epidermal hyperplasia and dermal neutrophilic infiltration after UV exposure (both parameters increased in colonized mice), and a cytokine milieu that tends to an inflammatory response in colonized mice and an immunosuppressive response in germ-free mice.

On the other hand, skin exposure to UV radiation deeply affects the gut microbiota balance (Ghaly et al. 2018). Mice were exposed to UV irradiation and

were divided into groups according to vitamin D supplementation (three levels of Vit D: high-level Vit D with 10,000 IU/kg, low-level Vit D with 2280 IU/kg, and control without Vit D). At different time points after treatment, fecal samples were taken to analyze microbial diversity. Results show that whereas Vit D did not affect microbiota, skin exposure to UV irradiation promoted changes in the beta-diversity of feces microbiota (vs. control nonirradiated mice). These changes were independent of the level of Vit D supplementation. Besides microbiota alterations, skin irradiation promoted changes in the expression of colonic IL1β, showing that microbiota modifications also correlated with immune response alterations.

7.6 Conclusions

The interactions between the microbiota and the host condition the health balance. The gut and the skin microbiota are crucial to maintaining the balance between microbial signals and immune responses. Established cutaneous chronic diseases that affect skin immune response involve dysbiosis.

Moreover, exposure to UV radiation deeply affects the immune response balance possibly impacting on the microbiota. The use of probiotics tends to restore the balance between the microorganisms and the immune response, to assure an adequate response against different antigenic stimuli. There is a strong need for knowledge in these complex interactions, but the research work has already begun.

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Relationship Between Probiotics and Gut-Skin Axis in Skin Wound Healing: A Recent Update

8

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Abstract

There is an intricate relationship between human skin health and gut microenvironment, and both are equally influenced using probiotics. In recent years, there is growing evidence suggesting the role of probiotics in metabolism, immunomodulation, wound healing, and various inflammatory and infectious conditions. Both the skin and gut are morphologically different but share some common physiological features. The gut and skin interact mainly through this microbiota and the metabolites secreted by them that interfere with a cascade of biological pathways regulating metabolism, immunity, inflammation, oxidative stress, and neuroendocrine function. Understanding the mechanism of action by which gut influences skin health (inside-out) is essential to define the cross talk between the two compartments. Probiotics can be exploited as modern therapeutics or as an adjuvant to classical therapies in the management of a variety of human diseases. However, limited data is available on the clinical potential of oral and topical probiotics in the treatment of skin- and gut-associated diseases. Although probiotics are considered safe, a comprehensive investigation is also required to establish the safety measures in immunocompromised persons. The

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present review highlighted the significance of probiotics in the gut-skin axis with a special reference to gut microbiota, skin homeostasis, and skin wound healing.

Keywords

Probiotics · Gut-skin axis · Microbiota · Microbiome · Skin wound healing

8.1 Introduction

Nutrition determines the status of the health of an individual, and the gut microbiota is largely impacted by the kind of nutrition consumed by an individual. The human body harbors trillions of microorganisms with the most diverse group of microbiota found in the gastrointestinal tract, particularly small and large intestine. These microorganisms are commensals and protect the gut microenvironment from the colonization with pathogenic organisms, thereby maintaining the gut homeostasis. There is growing evidence suggesting the role of gut commensals in regulating the vital biological process such as host metabolism, inflammation, and immune regulation (Martin et al. 2019; Clarke et al. 2014; Bravo et al. 2011; Belkaid and Hand 2014). The imbalance in gut microbiota due to invasion by pathogenic microorganisms leads to various disease conditions, and the association of gut microbiome with diseases like atherosclerosis, metabolic syndrome, obesity, inflammatory bowel diseases, diabetes mellitus, infectious diseases, etc. has been reported in recent studies. Extensive studies were performed to understand the complex network of gut microbiota and its effect on host health. Several beneficial microorganisms were identified in the gut such as *Lactobacillus* (*L*) and *Bifidobacterium* (*B*) that confer health benefits. The discovery of beneficial gut microorganisms led to the evolution of the concept of probiotics. In recent years, probiotics have gained considerable attention owing to their health benefits. According to the latest guidelines of the United Nations (Food and Agriculture Organization) and the World Health Organization, probiotics are described as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the hos" (Hill et al. 2014). At present only a few bacterial species such as *Lactobacilli* and *Bifidobacteria* have been extensively studied, and limited information is available on other beneficial microbes, thereby limiting the scope of probiotics. Probiotics should not be confused with the term prebiotics that is defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit (Gibson et al. 2017). Both probiotics and prebiotics complement each other as prebiotics act as food/substrate for probiotics and other commensals of the gut. Prebiotics mostly involve dietary fiber enriched glucans and fructans (e.g., fructooligosaccharides) that are not digested by human endogenous enzymes (Gibson et al. 2017). Recently, a new term "synbiotics" was coined which involves a concoction of probiotics and prebiotics, and the strategy has improvised thereafter

to use the combined application of probiotics-prebiotics in a targeted manner (Schrezenmeir and de Vrese 2001). Therefore, in today's scenario of the evolving landscape of probiotics, understanding of prebiotics and synbiotics is also essential. However, this chapter will focus only on probiotics and their impact on skin diseases involving the gut-skin axis phenomenon.

8.2 Gut Microbiome Physiological Role

8.2.1 Gut Microbiome Diversity in the Human Body

It is known that the human body is a habitat for trillions of microbes in specific niches in different parts of the body (Savage 1977). The number of microorganisms present in the human body is ~10 times the entire cells of an individual (Savage 1977; Berg 1996) and can weigh around 2 kg. The revised estimates were demonstrated in detail in a recent study (Sender et al. 2016). The majority of microbiota populates the gastrointestinal tract, especially the small and large intestines. The colon makes the top of the list, with about 10^{14} microbes, followed by the skin with around 10^{12} microbes (Berg 1996; Sender et al. 2016). The gut microbiota is highly conserved among the individuals but having genomic composition ~150 times of human genome that is predominantly bacterial (99.1%), 0.1% viral/eukaryotic, and the remaining is of archaeal origin (Qin et al. 2010). This microbiota begins to colonize the gut since birth, and as a result of coevolution with host cells, a symbiotic relationship is established between the host and gut microbiome. The gut microbiota is sometimes considered a separate organ (Baquero and Nombela 2012) owing to its functional role in human physiology.

A metagenomic study revealed that there are around 1000 different bacterial species present in the gut, and the majority of them are specific to host. Since only ~150 species (out of 1000) are shared among individuals, the gut microbiota may act as blueprint identity of the host. By the age of 3 years old, the gut microbiome becomes fully developed (Qin et al. 2010; Baquero and Nombela 2012; Raveh-Sadka et al. 2015; Yatsunenko et al. 2012). Studies have shown a vast diversity in gut microbiota depending on age, food, habits, and environmental factors (Yatsunenko et al. 2012; Dominguez-Bello et al. 2010; Senghor et al. 2018; Zhong et al. 2019). A metagenome and metabolome study carried out in the Indian population revealed that the North Central cohort (having a plant-based diet) is enriched in *Prevotella* species with predominant biosynthesis pathways of branched-chain amino acid and lipopolysaccharide. However, the Southern cohort (having an omnivorous diet) shows enrichment of *Ruminococcus* (*R*), *Bacteroides,* and *Faecalibacterium* (*F*) species having short-chain fatty acid (SCFA) biosynthesis pathways and branched-chain amino acid transporters (Dhakan et al. 2019). The study results are consistent with the notion that gut microbiota is greatly influenced by the composition of the diet. There is growing evidence suggesting a significant impact of the gut microbiome in regulating vital physiological processes such as metabolism, neuroendocrine function, immune regulation, and inflammation (Martin

et al. 2019; Clarke et al. 2014; Bravo et al. 2011; Belkaid and Hand 2014). In the gut ecosystem, the host-microbe association is highly symbiotic. Certain food components, mainly dietary fibers, can only be digested by gut bacteria (e.g., xyloglucans by *Bacteroides*, oligosaccharides and fructooligosaccharides by *Bifidobacterium* and *Lactobacillus*) as the host body is lacking specific enzymes. The SCFAs of less than six carbon atoms (e.g., acetate, propionate, butyrate, and valerate) are known to be synthesized by gut bacteria, and it is estimated that SCFAs contribute 10% of the energy supply of the body.

Besides acting as an energy reservoir, SCFAs also play a vital role in immune regulation, inflammation, and neurological function by modulating the signaling pathways of the gut-brain-immune axis. There are many bacterial species producing neurotransmitters like gamma-aminobutyric acid (*Lactobacillus* and *Bifidobacterium*), noradrenaline (*Bacillus subtilis*, *Bacillus mycoides*, *Proteus vulgaris*), dopamine (*Bacillus* spp., *E. coli* (K-12), *Klebsiella pneumonia, Proteus vulgaris,* etc.), acetylcholine (*Lactobacillus plantarum*), histamine (*Lactobacillus* spp., *Lactococcus*, *Streptococcus thermophiles*), and serotonin (*E. coli*, *Lactobacillus* spp., *Lactococcus*, *Streptococcus thermophilus*, *Klebsiella*) in the gut (Strandwitz 2018) working along a gut-brain circuit. The role of neurotransmitters is well defined in normal mental health. Vitamins are essential micro-nutrients that play a diverse physiological role, and gut microbiota contribute significantly to the host body by actively producing water-soluble vitamins (vitamins B1, B2, B3, B5, B6, B7, folate, B12, and vitamin K) (Said and Nexo 2018).

In recent years, accumulating evidence suggests that the impact of the gut microbiome on the host immune response begins very early in life. The gastrointestinal microbes and their secretory metabolites act directly and indirectly on the immune system, thereby regulating both the adaptive and innate components, particularly mucosal immunity. The gut microbiome gives the first lesson of tolerance to the cells lining the intestine in the early phase of life and plays a decisive role in maintaining the gut-immune homeostasis by discriminating between "self" and "nonself" microbes (Janeway 1992). However, the mechanism of "tolerance" development is not known. Toll-like receptors and nucleotide oligomerization domain receptors present on gut epithelium play an imperative role in the recognition of microbes and their associated components (lectins, defensins, cathelicidin, muramic acid, lipopolysaccharides, peptidoglycans, flagellin, bacterial genetic materials, etc.). Activation of these pattern recognition receptors triggers the innate immune response that involves granulocytes (neutrophils, eosinophils, and mast cells), natural killer cells, and antigen-presenting cells (dendritic cells and macrophages). Upon activation, these receptors initiate a cascade of downstream signaling molecules including the nuclear factor-kappa B pathway, thereby triggering the release of pro- and anti-inflammatory cytokines followed by T-cell activation (Liu et al. 2017). Therefore, the pattern recognition receptors act as a bridge between innate and adaptive immune responses in the gut. The activation of gut mucosal immunity is evaluated by the frequency of immunoglobulin A (IgA) producing B cells and IgA titers. The IgA index is regarded as a hallmark effector component of mucosal immunity as higher IgA titer (40–60%) was reported in the small intestine but low

titer (<30%) was found in the colon (Bunker et al. 2015). Peyer's patches are found in the small intestine, and they are considered as an inductor site of mucosal immunity in the gut, evident by the IgA index which is higher in the small intestine (40–60%) but lower in the colon (<30%). Among the population of the gut microbiota, the segmental filamentous bacterium of the *Clostridiaceae* family plays an important role in eliciting the CD4+ T-helper cell (Th1, Th17, and regulatory T cells) response (Gaboriau-Routhiau et al. 2009). In mice, administration of live *Lactobacillus* (*L*) *casei* bacteria enhances the proliferation of IgA+ and IL6+ cells in Peyer's patches producing detectable antibodies against the bacterium, whereas *L. paracasei* demonstrated increased stimulation of dendritic cells by CD4+ T cells, proliferation of lymphocytes, and secretion of pro-inflammatory cytokines in Peyer's patches (Galdeano and Perdigón 2006; Tsai et al. 2010). *B. adolescentis* is intimately associated with intestinal epithelium and is capable of inducing a Th17-cell response (Tan et al. 2016).

Recent studies have shown that lymphoid tissue-resident commensals (LRCs) (e.g., *Alcaligenaceae* family members and *Achromobacter xylosoxidans*) colonized the Peyer's patches, mesenteric lymph nodes, and lymphoid follicles of primates and mice (Fung et al. 2016). These LRCs induce a toll-like receptor-dependent tissue-specific immune response in the gut that is Th17-mediated and ILC3 (type 3 innate lymphoid cell)-mediated leading to production of their cytokines such as interleukin (IL)-17 and IL-22. IL-22 plays a dual role, as it is critical in favoring the colonization of LRCs, and at the same time, it plays a role in limiting the LRC population to avert LRC-driven systemic inflammation (Fung et al. 2016; Sonnenberg et al. 2012). The aforementioned literature defined the fundamental significance of the gut microbiome and its impact on a broad spectrum of biological processes. The dysbiosis of gut microbiota leads to alteration in gut permeability (leaky gut), inflammation, and immune dysregulation triggering the development of infectious, inflammatory, metabolic, and autoimmune diseases. Development of gastric, liver, pancreatic, and colorectal tumors and modulation of the chemotherapeutic response have also been linked to gut dysbiosis (Arthur et al. 2012). Modifying the gut microbiome will have future therapeutic potential, but this approach will require a thorough understanding of the composition of individual gut microbiota and delineating the characteristic features of a healthy gut microbiome. Nevertheless, the concept of using probiotics that aim to enrich and restore the population of commensal microorganisms in the gut is a promising avenue to maintain a healthy gut ecosystem.

Ample evidence indicates the beneficial role of probiotics in metabolism, in immune modulation, and in preventing systemic inflammation, as discussed in detail in the later sections. The impact of probiotics on the skin-gut axis is summarized in Fig. 8.1.

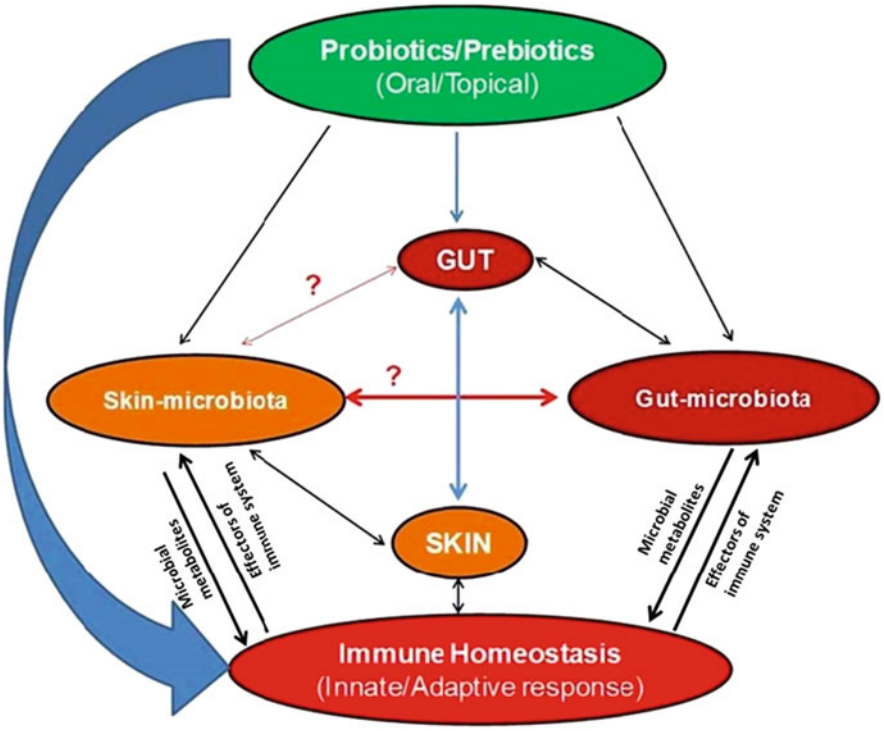

Fig. 8.1 Schematic presentation of a complex network of host-microbiome (gut and skin), its interaction with the gut-skin-immune system, and implication of probiotics**.** Probiotics play an important role in host gut-skin dysbiosis conditions by restoring the healthy gut-skin ecosystem. Probiotics can directly or indirectly influence the gut-skin microbiota and modulate the gut-skin-immune axis. The understanding of the dialogue between skin-microbiota vs. skin and gut-microbiota vs. gut is evolving, and their substantial evidence suggesting the impact of intriguing interaction on the body's physiological processes including immune regulation. However, the microbial diversity and interaction of gut and skin microbiota is not understood. The effect of skin microbiota on the gut ecosystem is not explored yet. Variety of microbial metabolites is secreted by gut-skin microbiota, and it directly/indirectly affects the immune- and tissue-specific homeostasis. Effector molecules secreted by immune cells (e.g., cytokines, chemokines, growth factors, and other biologically active compounds) also affect the gut-skin-associated microbial ecosystem. A fine-tuning exists between the immune system and host-microbiome, and imbalance may lead to pathological conditions. (? = Not/poorly defined)

8.3 The Implication of Probiotics in Skin Diseases

The gut microbiome in context with skin health and skin diseases is an emerging area in dermatology, but the knowledge of the gut-skin axis and its relevance to disease pathogenesis is lacking. However, there is substantial evidence indicating the beneficial effects of probiotics and prebiotic supplementation in humans, ranging from phenotypic improvement in skin texture, composition, and hair growth to changes at

the molecular level (Table 8.1). A randomized double-blind clinical trial demonstrated the antiaging effect upon the administration of *L. plantarum* HY7714 (10^{10} CFU/day for 12 weeks), as shown by a significant improvement in skin hydration, gloss, elasticity, and decrease in wrinkle depth (Lee et al. 2015). Another trial with *L. casei* (1×10^{11} bacteria/day for 8 weeks) revealed a significant reduction in transepidermal water loss (TEWL) and skin flakiness (Saito et al. 2017). Oral administration of heat-killed *L. lactis* (60 mg/day for 8 weeks) significantly modulated various skin properties such as decreased cheek melanin content and elasticity, as well as increased sebum content with a prominent effect in younger age groups (Kimoto-Nira et al. 2012). A double-blind trial that involved combinations of probiotic and prebiotic (*B. breve* strain Yakult + galactooligosaccharides) supplemented to fermented milk (100 mL/day for 4 weeks) resulted in maintaining an optimum level of skin hydration, decreased cathepsin L-like endopeptidase activity, and phenol content in serum and urine, exerting dual beneficial effects on the gut and skin (Kano et al. 2013).

Besides oral probiotic formulations that act along the gut-skin axis, there are emerging topical probiotic formulations that can be applied directly onto the skin with promising outcomes for skin disorders like atopic dermatitis, acne, seborrheic dermatitis, and nonhealing ulcers (Rosenfeldt et al. 2003; Myles et al. 2018; AOBiome n.d.; Guéniche et al. 2008; Peral et al. 2010). It is to be noted that skin health is influenced not only by the gut microbiome but also by the skin microbiome. A topographical analysis of the skin microbiome revealed the presence of 19 bacterial species, but the majority of the bacterial population are confined to 4 phyla, more specifically 51.8% of *Actinobacteria, 24.4% Firmicutes*,*16.5% Proteobacteria*, and *6.3% Bacteroidetes* (Grice et al. 2009). These bacterial populations colonize specific skin sites consistently among individuals. Recent studies have revealed the importance of quorum sensing—a unique phenomenon of microbial communication among the bacterial species (Williams et al. 2019; Brandwein et al. 2016). In atopic dermatitis (AD), it is reported that *Staphylococcus* (*S*) *aureus* invades the skin barrier by releasing proteolytic enzymes and phenol-soluble modulin α leading to inflammation, but the autoinducing peptides secreted by normal skin bacteria (coagulase-negative staphylococci) inhibit *S. aureus* activity via the quorum-sensing regulated accessory gene regulator (agr) system. Such a regulatory communication mechanism may also exist between the skin and gut microbiomes, although it has remained unexplored (Brandwein et al. 2016). Therefore, delineating the composition of both gut and skin microbiomes is essential for understanding the underlying quorum-sensing-mediated regulatory mechanism (if any) of microbiota between the two compartments, with the potential to lead to new therapeutic approaches. However, the current chapter is limited to oral probiotics and their role in skin diseases.

In humans, the gut microbiome ecosystem is continuously being challenged by external environmental factors that range from daily food intake (often concentrated with pesticides, lack of dietary fibers, nutritional deficiency), water (often contaminated with pathogenic microbes and chemicals), and antibiotics. The same is true for the skin microbiome that is exposed to harmful ultraviolet radiation and extensive use of skincare cosmetic products (including a potentially harmful

Table 8.1 Summary of human and animal studies: probiotic intervention and skin diseases

Author/year	Disease/ model	Probiotics/prebiotics	Route (oral/ topical)	Participants (human/ animal)	Remarks	References
Isolauri et al. 2000	AD	*L. rhamnosus* GG	Oral	$N = 27$ (human)	Marked improvement in AD severity and decreased levels of inflammatory markers	Isolauri et al. (2000)
Pessi et al. 2002	AD	*L. rhamnosus* GG	Oral	$N = 09$ (human)	Enhanced the production of anti-inflammatory cytokine (IL-10)	Pessi et al. (2000)
Rautava et al. 2002	AD	*L. rhamnosus* strain GG	Oral	$N = 62$ (human)	Probiotics increased the immunoprotective potential of breast milk in mothers by increasing TGF-β2	Rautava et al. (2002)
Navarro-Lopez et al. 2018	AD	*Lactobacillus* + *B. bifidum strain*	Oral	$N = 50$ (human)	Reduction in SCORAD index	Navarro-López et al. (2018)
Chapat et al. 2004	CD	*L. casei* (DN-114001)	Oral	Allergen-induced CD (mouse)	Inhibits antigen-specific IFN-γ-producing CD8+ effector T cell	Chapat et al. (2004)
Hacini-Rachinel et al. 2009	CD	*L. casei* (DN-114001)	Oral	Allergen-induced CD (mouse)	Decrease skin inflammation by increasing the number of FoxP3+ Tregs in the skin	Hacini-Rachinel et al. (2009)
Shah et al. 2012	CD	*L. acidophilus* strain L-92	Oral	Allergen-induced CD (mouse)	Increased number of FoxP3+ Tregs in spleen and skin draining lymph nodes	Shah et al. (2012))
Vijayashankar et al. 2012	Psoriasis	*L. sporogenes*	Oral	$N = 1$ (human)	Pustular psoriasis showed complete remission in 6 months	Vijayashankar and Raghunath (2012)
Groeger et al. 2013	Psoriasis	*B. infantis* 35624	Oral	$N = 26$ (human)	Reduced systemic inflammation and decreased plasma CRP and TNF-α	Groeger et al. (2013)

Chen et al. 2017	Psoriasis	*L. pentosus* GMNL-77	Oral	Imiquimod-induced psoriasis (mouse)	Reduced the levels of pro-inflammatory cytokines	Chen et al. (2017)
Kim et al. 2010	Acne	Lactoferrin-enriched fermented milk	Oral	$N = 18$ (human)	Amelioration of inflammatory acne lesion counts with a parallel decrease of triacylglycerols in skin surface lipid content	Kim et al. (2010)
Jung et al. 2013	Acne	*L. acidophilus* + *L. delbrueckii bulgaricus* + *B. bifidum*	Oral	$N = 45$ (human)	Marked improvement of skin lesion count	Jung et al. (2013)
Fabbrocini et al. 2016	Acne	*L. rhamnosus* SP1	Oral	$N = 20$ (human)	Improves acne lesion by normalizing expression of genes involved in insulin signaling	Fabbrocini et al. (2016)
Sugimoto et al. 2012	Skin aging	*B. breve* strain Yakult	Oral	UV-irradiated (mouse)	Prevent UV-induced skin changes by suppressing elastase activity and IL-1β levels	Sugimoto et al. (2012)
Satoh et al. 2015	Skin aging	*B. breve* B-3 (MCC-1274)	Oral	UV-irradiated (mouse)	Prevent TEWL, epidermal thickening, damage to tight junction, and basement membrane	Satoh et al. (2015)
Hong et al. 2015	Skin aging	Galactooligosaccharide (prebiotics) *B. longum*	Oral	UV-irradiated (mouse)	Increased expression of CD44, TIMP-1, Col1, and the water-holding capacity of the skin reduce TEWL and erythema	Hong et al. (2015)

chemical in soaps, shower gels, deodorants, detergents, etc.) leading to disequilibrium in skin microbiota (Prescott et al. 2017; Berne et al. 2008; Warshaw et al. 2009). The abovementioned environmental insults and extensive use of skin products make both the gut and skin microbiomes vulnerable to attack by pathogenic bacteria, thus causing various gut-skin-associated disorders.

8.3.1 Atopic Dermatitis (AD)

AD (eczema) is a type of IgE-mediated hypersensitive allergic reaction characterized by alteration in the skin barrier function, increased TEWL, and pH alterations. Deregulated cell-mediated immune responses due to alteration in T-helper 1 (Th1) and Th2-cell functions have also been reported in AD along with genetic association with filaggrin mutation. Additionally, dysbiosis was observed in lesional and non-lesional AD skin in the form of decreased microbial diversity and increased abundance of *S. aureus* (Bjerre et al. 2017). Oral intervention with *L. rhamnosus* GG (LRGG) for 1 month caused a significant improvement in AD severity scoring of atopic dermatitis (SCORAD) index with decreased levels of inflammatory markers such as tumor necrosis factor (TNF-α) and fecal α1-antitrypsin (Isolauri et al. 2000). The anti-inflammatory property of LRGG was also reported with significant increased levels of IL-10 and transforming growth factor-β2 (TGF-β2) in AD patients receiving LRGG (Pessi et al. 2000; Rautava et al. 2002). The preventive effect of *Lactobacillus* (odd ratio = 0.7) and *L.* + *B. bifidum* (odd ratio = 0.62) against the development of AD is evident by a reduction in SCORAD and odds ratio (Panduru et al. 2015; Navarro-López et al. 2018). The above studies indicated that probiotic effects are species-specific, confer both preventive and therapeutic benefits by immune modulation, and restore skin hydration capability.

8.3.2 Contact Dermatitis (CD)

CD is an allergen-induced CD+ T-cell-mediated immune response that arises when the skin encounters an irritant/allergen leading to the development of itchy rashes, blisters, etc. with signs of inflammation. In an allergen-induced mouse model of CD, *L. casei* (DN-114001)-enriched probiotic supplements mediate its anti-inflammatory action either by attenuating the expansion of antigen-specific IFN-γ-producing CD8+ effector T cells or by increasing the number of FoxP3+ (forkhead box P3) Tregs in skin/IL-10 secretion by Tregs in skin draining lymph nodes (Chapat et al. 2004; Hacini-Rachinel et al. 2009). A similar mechanism was observed in a mouse model treated with *E. coli* Nissle 1917, with decreased epidermal thickness and immune cell infiltration along with the increased frequency of Tregs in the skin (Weise et al. 2011). Oral administration of heat-killed *L. acidophilus* strain L-92 also resulted in an enhanced number of FoxP3+ Tregs in spleen and skin draining lymph nodes with a parallel increase in the expression of Foxp3, TGF-β, and IL-10 in the skin (Shah et al. 2012).

Besides, probiotic and prebiotic supplementation has also revealed a reduction in contact hypersensitivity in the mouse model of allergen-induced contact dermatitis. Prebiotic (fructooligosaccharide) supplementation significantly increased the *Bifidobacteria* (*Lactobacilli* remained unchanged) species, i.e., *B. pseudolongum* (lactobacilli remained unchanged), and it is negatively correlated with skin inflammation (Watanabe et al. 2008).

8.3.3 Psoriasis

Psoriasis is a multifactorial inflammatory disease caused by immune and environmental triggers in genetically susceptible individuals. Clinically, it is characterized by erythema, hyperproliferation of keratinocytes, and acanthosis that appear as silver scaly red patches (plaques) on the skin. There are studies associating psoriasis with inflammatory gastrointestinal diseases {e.g. inflammatory bowel disease (IBD), Crohn's disease, and ulcerative colitis}, and shared immune and genetic pathways have been reported in these diseases (Hampe et al. 1999; Fu et al. 2018; O'Neill et al. 2016). Gut bacterial DNA was detected in the circulation of psoriatic patients (O'Neill et al. 2016). A similar gut microbiome profile has been reported in psoriasis, psoriatic arthritis (PsA), and IBD with lower abundance and diversity of common gut commensals (Scher et al. 2015). The reduced relative abundance of *Bacteroidetes* and *Coprobacillus* in psoriasis and *Akkermansia* and *Ruminococcus* in PsA were the characteristic gut microbiome features (Scher et al. 2015). All these studies indicated the possible link between the gut microbiome and skin diseases supporting the gut-skin homeostasis. The effect of probiotics on psoriasis was evaluated in many studies including mouse model and human subjects. In a mouse model of imiquimod-induced psoriasis, oral administration of *L. pentosus* GMNL-77 significantly reduced the levels of proinflammatory cytokines (TNF-α, IL-6, IL-17, IL-22, IL-23) in the skin and IL-17$^+$ and IL22$^+$ producing T-helper cells in the spleen (Chen et al. 2017). However, the exact mechanism of active suppression of pro-inflammatory cytokines is not known, but it was suggested that probiotics may modulate regulatory T cells (Tregs) via decreasing the gut-resident CD103$^+$ dendritic cells. Another randomized double-blind trial revealed modulation of systemic inflammation in psoriasis subjects with *B. infantis* 35,624 supplementations by decreasing the plasma C-reactive protein and TNF-α (Groeger et al. 2013). In a case report of pustular psoriasis, supplementation of *L. sporogenes* (1 sachet, 3 times/day) and biotin (10 mg/day) in a 47-year-old female patient (unresponsive to methotrexate, steroids, and dapsone) showed marked improvement within 4 weeks, and 6-month follow-up revealed almost complete remission (Vijayashankar and Raghunath 2012). Sodium butyrate is commonly produced by gut microbes with a regulatory effect on keratinocyte proliferation and TGF-β. Sodium butyrate in combination with epidermal growth factor receptor inhibitor (PD153035) was shown to induce differentiation in human keratinocytes suggesting the use of sodium butyrate as prebiotics in the treatment of psoriasis (Leon Carrion et al. 2014).

8.3.4 Acne

Acne is a very common skin condition that is caused by infection/blockade of the pilosebaceous gland leading to the formation of papules, pustules, nodules, or comedones on the skin. The underlying etiology involves increased production of sebum, follicular hyperkeratinization/keratinocyte desquamation, hormonal (androgens) imbalance, and infection by *Propionibacterium acnes* (or *Cutibacterium acnes*). The importance of probiotics in acne treatment is evident from the study that compared the efficacy of probiotics (mix of *L. acidophilus*, *L. delbrueckii bulgaricus*, and *B. bifidum*) with and without minocycline (Jung et al. 2013). Significant improvement was observed in subjects receiving probiotics, which was comparable to the minocycline group; however, the combination of probiotics + minocycline was more efficient. Consumption of lactoferrin-enriched fermented milk (200 mg/day for 12 weeks) revealed a decrease in total acne count and grade and inflammation along with reduced sebum and lipid content (Kim et al. 2010). An oral dose of a probiotic strain of *L. rhamnosus* SP1 (75 mg/day for 12 weeks) demonstrated a 30% improvement in acne lesions by maintaining the normal expression of genes involved in insulin signaling, i.e., IGF1 (insulin-like growth factor 1) and forkhead box protein O1 (FOXO1) (Fabbrocini et al. 2016). It is to be noted that many other studies utilize topical probiotics in treating acne, which are not discussed in the present chapter.

8.3.5 Skin Aging

Skin aging is a general phenomenon that is influenced by both endogenous and exogenous factors and which is marked by loss of moisture, lipid and collagen content, elastosis, discoloration, and thick wrinkle formation. Exogenous factors such as exposure to ultraviolet light (photoaging), lifestyle, and food habits accelerate the biological clock of aging. There are few studies available suggesting the efficacy of probiotics in regulating the skin aging process; however, evidence stems from mouse models (Sharma et al. 2016; Sugimoto et al. 2012; Satoh et al. 2015). Probiotics containing the live *B. breve* strain Yakult (BBY) demonstrated photoprotective effects on the skin of hairless mouse that is evident by decreased levels of elastase enzyme activity and ultraviolet (UV)-induced IL-1β production (Sugimoto et al. 2012). Similar effects were observed with oral administration of *B. breve* B-3 in a model of UV irradiation-induced photoaging in hairless mice with marked suppression in TEWL and epidermal thickening (Satoh et al. 2015). The effects of prebiotics (galactooligosaccharides), probiotics (*B. longum*), or a combination of both were also investigated in UV-induced mouse models of photoaging. Significant reduction in TEWL (37.8%, 34.9%, and 33.7%), erythema, increased CD44 (regulator of keratinocyte proliferation), TIMP-1 (tissue inhibitor of metalloproteinase 1), and Col1 (collagen type 1) mRNA expression in the skin of mice treated with prebiotics, probiotics, and their combination, respectively, were observed compared to controls (Hong et al. 2015).

8.3.6 Probiotics and Chronic Wounds

Cutaneous wounds are injuries to the epithelial lining of the skin. These wounds are caused due to disruption of the skin and may be influenced by predisposing factors like diabetes, obesity, peripheral vascular insufficiencies, and age (Mustoe 2004). A well-coordinated wound repair mechanism involves four overlapping stages of hemostasis, inflammation, proliferation, and tissue remodeling (Gurtner et al. 2008; Siciliano and Mazzeo 2012). Wounds that are arrested at the inflammatory-proliferative stage for more than 4 weeks often become nonhealing and are defined as chronic wounds. The number of people suffering from chronic wounds is increasing at an alarming rate and is a cause for a socio-economic burden for both the patients and healthcare providers. Almost all chronic wounds are colonized with 12–20 different species of pathogenic microorganisms per wound, mainly *S. aureus* and *P. aeruginosa* forming chronic wound biofilms (Cowan 2011). These biofilms are polymicrobial with colonies enclosed in an extracellular polymeric substance (EPS). Along with considering the bacterial load on the wound, it is important to know the different strains of pathogens present and whether these species coexist to synergize or compete for nutrients. Bacterial cells growing in the biofilms are highly resistant to antibiotics, making it difficult to sanitize these wounds. Sustainability of the biofilm and the chronic wound microenvironment is a highly complex and dynamic process that partly contributes to the prolonged delay in the wound healing repair mechanism (Scales and Huffnagle 2013).

The use of probiotics to counteract the effect of pathogenic bacteria in wound biofilms is an emerging area for treating chronic wound infections and is summarized in Table 8.2. The mechanism by which probiotics aid in the healing process is still unclear but includes possibilities like competing with pathogenic biofilm bacteria for nutrients, producing antimicrobial compounds, restoring immunomodulatory mediators, lowering the pH of the wound bed, and promoting reepithelization and collagen formation (Kadam et al. 2019). Together, these probiotics help get rid of pathogenic bacteria and restore normal wound flora.

Lactobacillus species have been shown to have beneficial effects for the treatment of chronic wound infections. Several in vitro assays have demonstrated better adherence of *L. acidophilus*, *L. delbrueckii*, and *L. paracasei* to human keratin which weakens the adhesion of pathogenic bacteria to the wounds. The supernatants of lactic acid bacteria were tested for antimicrobial activity. Not only do these bacteria lower the pH of the culture media, but they also produce substances called bacteriocins that have antibacterial effects against pathogenic bacteria (Sikorska and Smoragiewicz 2013). *Lactobacillus* species are usually propagated and grown in Wilkins-Chalgren agar media in anaerobic conditions at 37 °C. The density is determined spectrophotometrically, and 10^8 CFU/mL of the bacteria is usually cultured to study the effects of probiotics on skin wounds for 20 days (Sultana et al. 2013). This is followed by centrifugation, washing with phosphate buffer saline (PBS), lysing using bead beater, and concentrating in cell culture media. A final filtration step is required to remove any bacterial debris or whole bacteria. This dose

Table 8.2 Antibacterial effect of probiotics and skin wound healing

Author/year	Pathogenic bacteria	Bacteria type	Model	Probiotic	Remarks	References
Shahandashti et al. 2015	*S. marcescens*	Gram (−)	In vitro: • Planktonic • Biofilms	*L. plantarum* (ATCC 4356) *L. acidophilic* (ATCC 8014)	Effect of bacteriocin from probiotics tested on planktonic and biofilm forms of *S. marcescens* Bacteriocins act as an antagonist to pathogens	Vahedi Shahandashti et al. (2016)
Lopes et al. 2017	*E. coli* *P. aeruginosa* *S. aureus*	Gram (+) Gram (−)	In vitro: • Planktonic • Biofilm forms	*P. innocua* (DSMZ 8251) Several *L.* sps.	Most *Lactobacillus* sps. show antimicrobial activity against the pathogens mentioned. *P. innocua* can break down the biofilm	Lopes et al. (2017)
Onbas et al. 2018	*P. aeruginosa* PAO1 (ATCC 27853) *S. aureus* (ATCC 43300)	Gram (+) Gram (−)	In vitro: • Planktonic • Biofilm forms	*L. plantarum* F-10	Reduction in wound biofilm was observed by scanning electron microscopy. Quorum-sensing controlled virulence factors of *P. aeruginosa* were also inhibited. Cell-free extract of the strain shows an inhibitory effect on the formation of wound biofilm	Onbas et al. (2019)
Mohammedsaeed et al. 2015	–	–	In vitro: • Proliferation • Migration assays	*L. rhamnosus GG* *L. reuteri* *L. plantarum* UCC118 *L. fermentum*	Scratch assays were performed on keratinocyte monolayer with lysates of the probiotics. An increased level of chemokine receptor gene was observed. *L. rhamnosus* and *L. reuteri* accelerate the reepithelization of keratinocytes	Mohammedsaeed et al. (2015)
Sultana et al. 2013	–	–	In vitro: Keratinocytes cell culture	*B. longum* (ATCC 51870) *L. plantarum* (ATCC 10241)	Keratinocytes were grown on cell culture inserts and treated with lysates of the probiotics. Except for *L. fermentum*, other probiotics	Sultana et al. (2013)

				L. reuteri (ATCC 55730) *L. rhamnosus GG* (ATCC 53103) *L. fermentum* (ATCC 14932)	enhance tight junction barrier function via modulation of protein components	
Huseini et al. 2012	*P. aeruginosa* (ATCC 27853)	Gram (−)	In vivo: Burn injury rat model	Kefir gel with *L. kefiri Leuconostoc, Lactococcus Acetobacter* Non-lactose yeast	Burn injuries were made on rats, and the wounds were infected with *P. aeruginosa* after 24 h. Kefir gel is an effective treatment for burn wounds as compared to silver sulfadiazine treatment (standard)	Huseini et al. (2012)
Guéniche et al. 2006	–	–	In vivo: Mice model	*L. johnsonii* NCC533	Hairless mice were exposed to an acute dose of UV radiations. IL-10 levels and epidermal Langerhans cell density were measured. Orally administered probiotic has enhanced immunomodulatory effects in mice	Guéniche et al. (2006)
Oryan et al. 2018	–	–	In vivo: Burn injury rat model	*S. cerevisiae* MYA796	Collagen hydrogel scaffold was administered with and without *S. cerevisiae* on rats. Histopathological and biomechanical investigations showed that the application of *S. cerevisiae* with collagen scaffold suppresses inflammatory responses making it a more effective treatment for burns	Oryan et al. (2018)
Gan et al. 2002	*S. aureus* (NCTC 6571)	Gram (+)	In vivo: Rat model of infection	*L. fermentum* RC 14	The rat model of surgical implant infection was studied with the probiotic. Co-inoculation of *L.* with *S. aureus* inhibited the development of subcutaneous abscesses.	Gan et al. (2002)

(continued)

Table 8.2 (continued)

Author/year	Pathogenic bacteria	Bacteria type	Model	Probiotic	Remarks	References
					L. fermentum and its secreted biosurfactant have anti-staphylococcal activity	
Gudadappanavar et al. 2017	–	–	In vivo: Rat model excision wound	*L. acidophilus* *L. plantarum*	*L. acidophilus* promotes wound healing and may support the hypothesis that links the gut-brain-skin	Gudadappanavar et al. (2017)
Shu et al. 2013	*S. aureus* (USA 300)	Gram (+)	In vivo: Mice model	*P. acnes* (ATCC 6919)	The probiotic suppresses the growth of *S. aureus*	Shu et al. (2013)
Jones et al. 2012	–	–	In vivo: Rabbit model	*L. fermentum* (NCIMB 7230)	*L. fermentum* improved wound healing with better neovascularization, keratinocyte proliferation, and reduced inflammation due to production of NO induced by probiotics	Jones et al. (2012)
Peral et al. 2009	*S. aureus* *P. aeruginosa* *S. epidermidis* *E. cloacae* *K. Pneumonia* *E. faecalis*	Gram (+) Gram (−)	Clinical trial $N = 8$	*L. plantarum* (ATCC 10241)	The probiotic topically applied to male and females with second- and third-degree burns. The results showed that *L. plantarum* can act as an alternative treatment to silver sulfadiazine (standard)	Peral et al. (2009)
Valdez et al. 2013	*S. aureus* *P. aeruginosa* *S. epidermidis* *E. cloacae* *K. pneumonia* *E. faecalis*	Gram (+) Gram (−)	Clinical trial $N = 20$	*L. plantarum* (ATCC 10241)	Treatment with probiotic shows twice as an increase in wound healing and bacterial load reduction	Peral et al. (2009)

of bacteria has been shown to improve tight junction barrier function and promotion of migration of keratinocytes in scratch wound healing assays (Sultana et al. 2013).

Serratia marcescens is a gram-negative opportunistic bacterium found in wound infections, urinary tract infections, and nosocomial infections. Increased resistance to broad-spectrum antibiotics has led researchers to explore probiotics for the treatment of such infections. Previous studies have shown gram-positive bacteria to be more susceptible to probiotics due to the absence of the outer membrane. However, in this study, bacteriocin-like substance (BLS) from cell-free extracts of *L. plantarum* and *L. acidophilus* showed outstanding inhibitory effects toward *S. marcescens*, which suggests outer membrane permeability of BLS in gram-negative (−) bacteria (Vahedi Shahandashti et al. 2016). Bacteria use a complex cell-cell interaction called quorum sensing (QS) for the formation and expansion of wound biofilm which makes it more difficult to eliminate in the organized biofilm form than in the planktonic form. Destruction of biofilm in the study suggests QS inhibitory activity of certain strains of lactic acid bacteria.

Propioniferax innocua showed considerable destruction of preformed wound biofilm consisting of *E. coli*, *Propionibacterium aeruginosa*, and *S. aureus* (Lopes et al. 2017). *L. plantarum* was also shown to be effective as an anti-biofilm probiotic against *P. aeruginosa*, methicillin-resistant *S. aureus*, and some hospital-derived strains (Onbas et al. 2019). Along with biofilm destruction, certain strains of lactic acid bacteria like *L. rhamnosus* act as protective probiotics that compete with *S. aureus* for the integrin binding site of keratinocytes. This induces better wound reepithelization by promoting keratinocyte proliferation and migration. Microarray analysis of the scratch assays revealed an increase in the gene for chemokine CXCL2 and its receptor. Thus, the acceleration of the epithelization of keratinocytes can be attributed to chemokine-induced migration (Mohammedsaeed et al. 2015). In addition, lysates from *B. longum* and *L. rhamnosus* GG enhanced tight junction barrier formation which correlates with an enhanced level of tight junction proteins claudin 1, occludin, and ZO-1 (Sultana et al. 2013).

The most common approach to test the efficacy of probiotics in vivo is by topical application simultaneously with infection. In vivo analysis revealed probiotic extracts like kefir gel on rats improved reepithelization, angiogenesis, and collagen formation leading to faster wound closure and healing (Huseini et al. 2012). Kefir is the fermented product of milk that contains *L. kefiri*; species of the genera *Leuconostoc, Lactococcus,* and *Acetobacter*; as well as some non-lactose fermenting yeast (Gibson 2006). Probiotics were shown to have immunomodulatory effects on mice where orally administered probiotics were able to modulate Langerhans cells and IL-10 levels in response to UV-induced injury (Guéniche et al. 2006). Yeast probiotics *Saccharomyces cerevisiae* were tested on burn wounds in rats with and without collagen hydrogel scaffold. The results with scaffold showed enhanced morphological wound healing with higher collagen content as the scaffold helped in holding moisture for the yeast (Oryan et al. 2018). *S. aureus* bacteria are found in most wounds and are a common cause of surgical infections. Some studies have identified *L. fermentum* as an anti-staphylococcal agent. Both the lactic acid bacteria and its cell-free supernatant inhibit staph infection on rat models with surgical

implant infection (Gan et al. 2002). Similarly, *L. acidophilus* showed significantly improved wound closure on three different wound models in male Wistar rats (Gudadappanavar et al. 2017).

Skin infections are populated with multidrug-resistant bacteria especially *S. aureus,* which is resistant to several antibiotics like meticillin, oxacillin, and nafcillin. This makes management and treatment of infection difficult (Sikorska and Smoragiewicz 2013). Several studies show the antagonistic effects of *Lactobacillus* species on *S. aureus*. Probiotics inhibit pathogen growth by either competition for nutrients or attachment sites. *L. reuteri* and *L. rhamnosus* showed reduced keratinocyte cell death when applied to *S. aureus* infection (Charlier et al. 2009). The production of bacteriocin produced by probiotics has also been related to an antagonistic effect on multidrug-resistant bacteria (Voravuthikunchai et al. 2006). Some bacterial species like *Lactobacillus paracasei* reduce the attachment and adherence of multidrug-resistant bacteria, thus showing antimicrobial activity. One such study showed that a strain of *L. paracasei* produced a bacteriocin paracasein A which had antimicrobial activity against 32 strains of pathogenic bacteria that includes several multidrug-resistant organisms as well (Bendjeddou et al. 2012).

Propionibacterium acnes are commensal bacteria accounting for more than half of the human skin microbiome. Wounded mice topically treated with *P. acnes* and glycerol showed reduced levels of pathogenic *S. aureus* (Shu et al. 2013). The mechanism of action is hypothesized to be the fermentation of glycerol to propionic acid. Low pH caused by the production of propionic acid is known to inhibit various strains of *S. aureus*. Nitric oxide (NO) is a signaling molecule that acts as a chemoattractant to stimulate the production of cytokines like IL-1 and recruit monocytes and neutrophils during the inflammatory stage of wound healing. In a study, *L. fermentum* immobilized in alginate beads were placed in between two layers of Tegaderm™ with glucose (Jones et al. 2012). The fermentation of glucose produces nitrate plus protons forming gaseous NO. This patch was applied to full-thickness wounds on white male rabbits. Histopathological analysis revealed improved wound healing with better neovascularization, keratinocyte proliferation, and reduced inflammation due to the production of NO induced by probiotics (Jones et al. 2012). Evaluation of in vivo models for developing strategies involving treatment with probiotics is encouraging. However, the biofilm phenotype in the murine model is formed in vitro and then applied to the wound which can alter the host response. The current research lacks in vivo models with biofilm formation that can be reflective of human wounds and microbiome. Clearly, more work needs to be done in optimizing treatment strategies that can be applied for chronic wound therapies.

While there is a gap in research studies of animal models with probiotics, some promising clinical trials have been performed (Watters et al. 2015). In one study, eight males and females with second- and third-degree burns were treated with topical application of *L. plantarum* (Peral et al. 2009). The purpose of this study was to evaluate the effectiveness of lactic acid-producing bacteria in comparison to standard treatment of wounds with a microbicidal agent like silver sulfadiazine. In one group, the probiotic was applied directly to the wound for 10 days, and the other

group received daily antiseptic 0.5% chlorhexidine and treatment with silver sulfadiazine cream. There were no significant differences in the wound healing rates and bacterial load in both groups. This study demonstrates the use of *L. plantarum* as an alternative to silver sulfadiazine treatment. Another study conducted by Valdez et al. aimed at investigating the use of *L. plantarum* for the treatment of diabetic foot ulcers. Twenty patients were topically administered with *L. plantarum* and 10 patients in the control group received standard debridement treatment over 30 days followed by posttreatment checkups after 20 days (Peral et al. 2009). Researchers reported a twofold increase in wound healing rates and bacterial load reduction. In another study, chronic leg ulcers of 34 patients showed 43% improved wound healing in diabetic and 50% in nondiabetic patients in 30 days (Peral et al. 2010). For this experiment, whole cultures of *L. plantarum* ATCC 10241 were grown in MRS broth at 37 °C. Gauze containing the bacteria was applied on cleaned wounds every day for 10 days. A dose ranging from 10^6 to 10^7 CFU is usually administered on skin wounds per gram of tissue and has shown to be effective in skin wound healing. After daily application of bacterial lysates on skin wounds for 10 days, reduced wound area was observed till day 30.

Besides, gut microbiome research has taken a leap beyond probiotic-prebiotic supplementation in treating gastrointestinal diseases by the introduction of fecal microbiota transplantation (stool transplant) therapy where stool from healthy donors is transplanted to the affected recipients (Allegretti et al. 2019). As discussed in this section, several in vitro and some in vivo studies have shown probiotics to be an effective treatment against pathogens. To explore these studies, it would be critical to evaluate more clinical data and accurately representative in vivo models. The use of such nonconventional therapeutics holds promise as a potential treatment regime.

8.4 Conclusion

A better understanding of the phenomenon of quorum sensing in the gut microbiome, gut-skin microbiome interactions, and their impact on host cellular and physiological processes will require further extensive research, which will eventually enable us to harness the beneficial effects of the inside-out gut-skin axis. The utility of probiotics and prebiotics in modulating the gut and skin microbiota is currently limited due to the lack of knowledge of the benefits of the gut microbiome and the precise role of these commensals in the body's physiology. Gut microbiome research has leaped probiotic-prebiotic supplementation in treating gastrointestinal diseases by the introduction of fecal microbiota therapy. The idea may sound promising for skin-associated diseases, keeping in view the mechanism across the gut-skin axis. Although there are some preclinical and clinical studies using probiotics as an antibacterial agent, thereby enhancing the skin wound healing. However, future investigations are required in this direction to advance the understanding of the relationship between probiotics and the gut-skin axis.

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Probiotics for Atopic Dermatitis: An Update

9

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Abstract

Atopic dermatitis (AD) is a chronic, inflammatory disorder associated with dry, scaly, and extremely itchy skin having complex pathophysiology. Mostly, symptomatic treatments are offered to AD that include skin moisturizers, antihistaminic, topical steroids, etc. Although exact pathophysiology of AD is not clear, it was reported that along with genetic factors, epidermis dysfunction, and immune system abnormalities, microbiome in the gut-skin axis also contributes for the progression of AD. The alteration in gut flora results in dysbiosis, an imbalance within natural microbiota which causes many health complications including AD. Probiotics are live microorganisms which support gut microbiota and elicit several health benefits when consumed. However, viability and stability of probiotics are the major concerns for their effective usage. Probiotics are available in various conventional dosage forms like tablet, capsules, sachets, oral liquids, etc. Additionally, they are also incorporated into controlled delivery systems using microencapsulation and lyophilization techniques which not only deliver required amount of probiotic at a targeted site but also significantly

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improve their sustainability. At present, several clinical trials are ongoing for different strains of probiotics and their relevance to microbiota. In the future, new routes and dosage forms can be explored for effective delivery of probiotics in management of AD.

Keywords

Atopic dermatitis · Microbiome · Probiotic delivery · Viability · Probiotic strains

9.1 Introduction

9.1.1 Gut Microflora

Human intestine/gut serves as the largest interface between external environment and mucosal tissues. In particular, the large intestine hosts abundant microbes showing highly complex nature of ecosystem (Witte 2000). Due to drastic varying conditions in gastrointestinal (GI) tract, bacterial abundance is different in different parts. In the small intestine (jejunum and ileum), it is 10^4–10^8 CFU/mL which goes up to 10^{14} CFU/mL in the large intestine. The total number of microorganisms colonized in the human body is ten times more in number than human cells (Bäckhed et al. 2005). Recent studies undertaken on gut microbiome have shown that there are about 2172 different species of bacteria divided into 12 different phyla and over 7000 different strains (Thursby and Juge 2017). Most common anaerobic genera found in the intestine are *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Clostridium*, *Lactobacillus*, etc. Around 386 species of bacteria are strictly anaerobic in nature. Many aerobes are also abundantly present such as Gram-negative enteric bacilli (*E. coli*, *Salmonella* sp.) and Gram-positive cocci (*Enterococcus*, *Staphylococcus*, *Streptococcus*, etc.). In addition, some strains of fungi are also found (*Candida* sp.). This composition of microbes in human gut is much simpler in infants and children, and its complexity increases as age of individual increases. It remains stable for most of the adulthood. Method of delivery of baby also plays an important role in the development of gut microflora as vagina-delivered baby shows higher concentration of lactobacilli in early stages of development, whereas C section-delivered baby shows abundance of *Clostridium* species (Thursby and Juge 2017).

9.1.2 Functions of Gut Microflora

The relationship between human host and microbes, though mutualistic, is often explained as communal relation. Gut microflora helps in regulation of many functions which leads to better health. It plays important role for many physiological functions such as:

Table 9.1 Bacterial strains for maintaining skin health (Thursby and Juge 2017)

Strain of bacteria	Function
Bifidobacterium sp.	Folate production and vitamin synthesis
Lactobacilli sp.	Vitamin B12 production, aid in digestion of milk, production of lactic acid
L. rhamnosus	Promote cell renewal and aid in process of wound healing
Akkermansia muciniphila	Maintenance of epithelial integrity
L. plantarum	Maintenance of integrity of epithelial barrier
B. fragilis	Development of the immune system through decreasing $CD4^+$ T-cell population
F. prausnitzii	Activation of NF-kB pathway, decreasing proinflammatory responses

(a) Intestinal development and homeostasis.
(b) Protection against pathogenic challenges.
(c) Metabolism of various harmful components (non-dietary starches, conjugated bile acids).
(d) Degradation of oxalate-based complexes.
(e) Synthesis of various vitamins such as vitamins B12, B6, B5, B9, B1, B2, and K.
(f) Tropic effect on intestinal mucosa such as stimulation for synthesis of microvilli.
(g) Maturation of innate and acquired immunity.
(h) Harvesting of energy.

Many studies have shown importance of various bacterial strains in case of skin health. Table 9.1 depicts some important gut microbes playing important role in pathophysiology of skin disorders.

9.1.3 Gut Dysbiosis

Gut dysbiosis refers to imbalance caused in composition of microbiome and disturbance in their equilibrium state of existence which may reflect into disease conditions. There are many reasons which alter microflora temporarily or in certain cases irreversibly. These include:

(a) Change in diet.
(b) Change in environmental conditions.
(c) Prolong treatment with antibiotics.
(d) Surgery.
(e) Smoking.
(f) Immune system modification by external factors (therapy with immunosuppressant).
(g) Genetic mutation. etc.

Interestingly, connection between gut dysbiosis and atopic dermatitis (AD) is established. Fujimura et al. showed that patients with AD show higher abundance of fungal species *Candida* and *Rhodotorula* and relatively lower abundance of bacterial species such as *Bifidobacterium*, *Akkermansia*, and *Faecalibacterium.* In this study, patients were selected within the age of 1–11 months. Atopic kids showed increased levels of *Clostridium* species and decreased levels of *Bifidobacterium* levels. Penders et al. carried out KOALA Birth Cohort Study in which they conclude that children with AD showed higher colonization of *E. coli* and *C. difficile*. Gut microflora plays an important role in transformation of immature T cells into various specialized types of T cells such as Th1, Th2, Th17, and Treg cells. Treg cells prevent differentiation of naive T cells into other Th cells. It suppresses immune response by downregulation of eosinophils and mast cells and also decreases production of IgE (Akdis 2006). Bacteria like *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Clostridium*, and *Bacteroides* have propionic acid and butyric acid as metabolic products which serve as a trigger to regulate production of Treg cells (Furusawa et al. 2013; Round and Mazmanian 2010), whereas *Cutibacterium* is involved with the formation of propionate and acetate derivatives of short-chain fatty acids (SCFA), responsible for controlling and suppressing growth of methicillin-resistant *S. aureus* (MRSA) commonly found in atopic lesion infections (Schwarz et al. 2017). Significant dysbiosis of *F. prausnitzii* species was also reported by Song et al. in the fecal samples of AD patients which plays an important role in the production of SCFA (butyrate derivative). This study of dysbiosis of various bacterial strains further advocates the use of probiotics for treatment of AD.

9.2 Atopic Dermatitis (AD)

The word "atopy" is derived from the Greek word *atopia*, meaning "different" or "out of place." AD is commonly defined as a chronic, pruriginous, inflammatory dermatosis characterized by dry, scaly, erythematous skin. It is the most common chronic inflammatory skin disease with multifaceted pathophysiology (Kalamaha et al. 2019). AD is considered as the subset of eczema and more severe type of eczema. AD was added to the group of atopic disorders in the year 1933 on the basis of association of this form of eczema along with asthma and allergic rhinitis as well as with allergies associated with food (Spergel and paller 2003). Atopic march is referred to a series of allergy-driven diseases such as AD, asthma, rhinitis (Hay fever), and food allergies. Atopic march is developed due to various underlying factors such as immune system dysfunction and genetic mutations. AD marks initiation of atopic march which usually shows clinical manifestations in early age of life (6 months to 1 year of age). Depending upon onset of AD, it is usually classified into three different classes such as infantile AD, childhood phase, and adulthood phase (Spergel and paller 2003).

(a) *Infantile AD*: Marks its beginning within 2 years of age. The erythematous papules and vesicles typically begin on the cheeks, forehead, or scalp and are

intensely pruritic. Lesions might remain localized to the face or might extend to the trunk or particularly the extensor aspects of the extremities in scattered, ill-defined, often symmetrical patches.

(b) *Childhood phase*: Observed from 2 years of age till puberty. Exhibit more thick skin papules and plaques representing more chronic disease. The classic areas of involvement in children are the hands, feet, wrists, ankles, anterior elbow, and knee pit regions.

(c) *Adulthood phase*: Detected from puberty to throughout adulthood. Predominant areas affected include the flexural folds, the face and neck, the upper arms and back, and the dorsa of the hands, feet, fingers, and toes.

9.2.1 Epidemiology of AD

Prevalence of AD in various ages and continents varies depending on various factors such as lifestyle, environmental factors, intercontinental genetic variations, etc. Commonly, prevalence of AD is seen in children although it shows significant occurrence in adulthood. AD affects up to ~20% of children during infancy and early childhood, 60% of which shows complete recovery from those symptoms by puberty (Takizawa et al. 2018).

The International Study of Asthma and Allergies in Childhood (ISAAC) carried out a total of three questionnaire-based surveys in periodic intervals. The first survey was carried out between 1994 and 1996 and covered 256,410 children aged 6–7 years in 90 cities and 458,623 children aged 13–14 years in 153 cities in 53 countries. The global average prevalence of eczema symptoms was 7.3% and 7.4% in 6–7- and 13–14-year-olds, respectively, wherein wide range of variations are observed within continents as well as countries (Williams et al. 1999). ISAAC phase II study was designed to focus on prevalence of various factors contributing to AD. It helped in establishing disease markers with genetic and environmental variations (Weiland et al. 2004). ISAAC phase III was performed from 2001 to 2003 and covered 60 countries. The overall global prevalence was 6.1% and 8.8% in 6–7- and 13–14-year-olds, respectively. The lowest prevalence was 2.7% in India, and the highest was 22.5% in Ecuador (Odhiambo et al. 2009).

In a survey carried out by Barbarot et al. in 2018 among participants by region, the prevalence of adult AD in the overall/treated populations was 4.9%/3.9% in the USA, 3.5%/2.6% in Canada, 4.4%/3.5% in the Europe, and 2.1%/1.5% in Japan. The prevalence was found lower in males as compared to females and also showed lower population of higher age groups. This study was performed using online survey method with statistical significance of 95% confidence interval.

9.2.2 Causes of AD

The exact cause for AD is still unknown, and many studies have been carried out for the determination of its causative factors. Many scientists have tracked back the

causes to genetic mutation, immunological dysfunction, as well as epidermal barrier abnormalities. Various environmental factors lead to development of AD or complications in AD. These factors are chemical irritants (detergent, soap, shampoo, etc.), allergens (dust, pollen grains, pet dander, molds, and mites), sudden temperature fluctuations, and hormonal changes (National Eczema Association).

9.2.3 Symptoms of AD

AD is a severe type of eczema, is chronic in nature, and might worsen with time. It usually occurs on the face, neck, and cheek region but can affect other parts of the body too. AD has various clinical presentations which make diagnosis very critical. Common symptoms include dry/scaly skin; redness; itching; cracks behind the ears; rash on the cheeks, arms, and/or legs; open, crusted, or "weepy" sores (usually during flares); skin thickening; and pus-filled blisters (in case of infections) (Berke et al. 2012). Based upon the type of AD, its symptoms are different as shown in Table 9.2 and Fig. 9.1.

9.2.4 Diagnosis of AD

Due to the similarity between eczema and AD, it is very difficult to diagnose AD. The simplified criteria include itchy skin with at least three of the following characteristics, history of asthma or allergic rhinitis, history of flexural involvement, history of generalized dry skin, onset of rash before 2 years of age, and visible flexural dermatitis (Berke et al. 2012). There are various methods which are commonly used in determination of severity of AD. Some of the commonly used ones are mentioned below.

Table 9.2 Symptoms associated with different types of AD

Type of AD	Symptoms
Acute AD	Vesicular, weeping, crusting eruption of the skin
Subacute AD	Dry, scaly, erythematous papules, and plaques
Chronic AD	Lichenification from repeated scratching

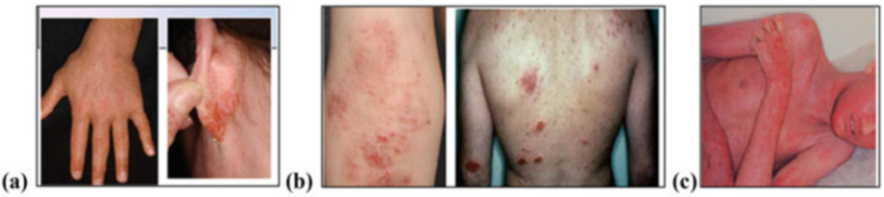

Fig. 9.1 Types of AD: (**a**) acute, (**b**) subacute, and (**c**) chronic (Bieber and Nestle 2015)

9.2.4.1 The Hanifin-Rajka Criteria of AD

The Hanifin-Rajka criteria is commonly used for the diagnosis of AD and requires occurrence of at least three major, commonly occurring symptoms such as thickening of the skin, early onset of disease pruritus, relapsing dermatitis, family history of allergic disorders, etc. Along with this, patient should also show at least three of the minor features such as xerosis, ichthyoids/palmar hyperlinearity/keratosis pilaris immediate (type I) skin test reactivity, increased serum IgE levels, sensitivity toward infections, dysfunctional cell-mediated immunity, tendency toward nonspecific hand or foot dermatitis, recurrent conjunctivitis, nipple eczema, cheilitis, Dennie-Morgan infraorbital fold, facial pallor, pityriasis alba, keratoconus, anterior subcapsular cataracts, orbital darkening, anterior neck fold itch when sweating, intolerance to wool as well as lipid solvents, etc. (Larsen and Hanifin 2002).

9.2.4.2 SCORAD Index

It accounts for body surface area measurements on the basis of intensity of AD, symptoms of itch, sleeplessness in patients, etc. It usually ranks in range of 0–103 for disease severity. The rule of nine is used for calculation of this index where affected areas are considered based upon measures stated below (Henry and Li 2016):

- Head and neck—9%.
- Upper limb—9% each.
- Lower limb—18% each.
- Anterior trunk—18%.
- Posterior—18%.
- Genital—1%.

9.2.4.3 Intensity and Severity Index

In this method, area affected by AD is taken as representative and is graded within scale range of 0–3 where 0 is none, 1 mild, 2 moderate, and 3 severe. Also, factors like redness, scaling and oozing of blisters, scratch marks, skin thickening, and dryness are considered (Henry and Li 2016).

9.2.4.4 Dermatology Quality-of-Life Index (DQLI)

This system usually consists of a questionnaire which covers all aspects of skin health right from itchiness and pain to choice of wardrobe as well as eating habits. It is usually scored in range of 0–30 (Henry and Li 2016).

9.2.5 Pathophysiology of AD

Focusing on pathophysiology of AD, exact mechanism is not completely understood. Numerous studies that have been carried out for the same demonstrated that complication with skin barrier regulation as well as immune system dysfunction contributes to majority of progression of AD. The epidermis forms physical and functional skin barrier, and abnormalities in this barrier are the main pathological

finding in case of AD. Disruption of epidermal layer leads to exposure of skin-resident immune cell to external antigens, triggering immune-based inflammatory reactions. Various proteins such as keratin, transglutaminases, filaggrin, and intracellular proteins play an important role in normal epidermal functioning. In discussion of pathophysiology of AD, various aspects of spread of disease such as its genetic variations, immunological variations, neuroimmunological mechanisms, epidermal dysfunctions, lipid composition alterations, and microbiome-related changes of skin environment should be considered (Kim et al. 2019a, b).

9.2.5.1 Genetic Variations

The stratum corneum (SC) forms first line of defense within the skin structure as an outermost part of the epidermis. The filaggrin (FLG) protein is predominantly present in SC and plays an important role in maintaining its barrier properties in association with keratin protein by formation of bundles of keratin cytoskeleton and microfibrils. It is also involved in the release of natural moisturizing factor (NMF), which is responsible for skin hydration and epidermal water retention. Gene responsible for expression of FLG protein is present on chromosome 1q21 which encodes for production of profilaggrin, further converted to its active monomers by proteolytic cleavage (serine proteases including channel-activating serine protease/Prss and matriptase/matriptase) and results into dephosphorylation. Metabolic degradation of FLG protein gives rise to uronic acid and pyrrolidine carboxylic acid and helps in maintaining skin pH. FLG haploinsufficiency (dominant phenotype in diploid organisms that are heterozygous for a loss-of-function allele) further contributes to AD in other aspects such as effects on skin pH proinflammatory cytokinin expression, *S. aureus* growth, etc. Two loss-of-function FLG mutations (R501X and 2282del4) are detected till date showing a semidominant pattern of inheritance of null mutation with incomplete penetrance. The number of FLG mutations identified in European populations is 20, of which 6 are prevalent and 14 are of low frequency. In Asian populations, additional 17 mutations, of which 8 are prevalent and 9 are of low frequency, have been identified. About 10% of European population is heterozygous carriers of FLG mutations, showing 50% reduction in expressed protein. Prevalence of these gene mutations is not that widespread in Asian and Korean patients compared to other western countries (Kaufman et al. 2018; O'Regan et al. 2008).

In addition, corneodesmosome, corneodesmosin, and desmoglein 1 deficiencies contribute to pathogenesis of AD. Cell-cell adhesion plays an important role in maintaining skin barrier integrity through formation of tight junctions which mainly forms second or internal line of defense of epidermal barrier. There are mainly two types of protein, namely, desmoglein 1 (DSG) and corneodesmosin (CDSN), present in the skin. Desmosomes serve as an adhesion material/glue for keratin cytoskeleton and help in maintaining barrier for mechanical stress. Loss-of-function mutation of genes encoding these proteins leads to epidermal barrier dysfunction and consequently results in AD along with other factors. Complete loss of CDSN expression results in skin peel syndrome type B, associated with severe pruritis and atopy. DSG-I mutation leads to severe dermatitis, multiple allergies, and metabolic wasting

(SAM) syndrome with elevated IgE levels, responsible for allergic immune responses (Samuelov and Sprecher 2014).

Polymerization or changes seen in certain set of immune system-related group of gene usually result in increasing risk of AD through modification in signaling pathways for helper T2 cells. It increases mRNA expression of IL-4 and IL-13. Sudden surge in production of these interleukins leads to decrease in production of FLG, shows loss of epidermal integrity, and leads to AD. Along with this, many chemokines are upregulated in AD to help recruit cells to sites of injury or irritation and promote the development of lesions. Generally, CCL5 (RANTES), CCL13 (monocyte chemoattractant protein 4), and CCL11 (eotaxin) are increased in AD skin lesions and possibly help in the recruitment of T cells, macrophages, and eosinophils into the skin. Further, immune-related genes that play a role in the development of AD include IL-31, IL-33, interferon regulatory factor 2, thymic stromal lymphopoietin (TSLP) and its receptors (IL-7R and TSLPR), signal transducer and activator of transcription (STAT) 6, Toll-like receptor 2, and high-affinity IgE receptor (Fc_RI) gene in specific populations (Thyssen and Kezic 2014).

Recent studies have provided an evidence for association of vitamin D receptors with AD. CYP450 subfamily 27 also plays an important role as it is involved in metabolism of vitamin D (active form D3). It is also useful for immune pathway regulation as well as epidermal barrier formation by regulating formation and differentiation of keratinocytes. Study results reveal that 5% of population suffering from AD showed vitamin D deficiency (Oren et al. 2008; Heine et al. 2013).

Epigenomic modification in DNA through environmental exposure has also shown its susceptibility toward AD. Methylation plays a crucial role in pathophysiology of AD through epigenetic modification. Methylation of umbilical cord blood at 5′-C-phosphate-G-3′ sites of *IL-4R* shows progression of AD at 1 year of age. Methylation of FLG gene adjacent CpG site also leads to increased risk of eczema (Barton et al. 2017; Bin and Leung 2016).

9.2.5.2 Immune System Abnormalities

Innate immunity and acquired immunity work in a complementary manner for maintaining overall immune responses in vertebrates. As per evolutionary developmental sequence, innate immunity forms oldest and first line of defense in individual, and acquired immunity develops in later stages. Immunological origin for AD pathogeneses lies within the fact that acquired immunity might be deregulating the innate immune responses. The innate immune system senses microbes through a group of germline-encoded proteins, named pattern recognition receptors (PRR). PRRs recognize pathogen-associated molecular patterns, which include bacterial cell wall components (such as lipopolysaccharide (LPS), peptidoglycan (PGN), and lipoteichoic acid), fungal cell wall (zymosan), viral double-stranded RNA molecules, and unmethylated CpG DNA, which are primarily found in bacteria. PRR activation results in the production of cytokines, chemokines, and AMPs, as well as the activation and recruitment of immune cells (immature dendritic cells (DCs), natural killer (NK) cells, and neutrophils (PMNs)).

Table 9.3 Abnormalities seen in PPR protein component in innate immunity-based pathogenesis of AD (De Benedeto et al. 2009)

PPR	Defects found in AD	Major function abnormalities
Toll-like receptors (TLR) NOD leucine-rich repeat-containing protein	TLR 2 and TLR9 1–4 N-nucleotide-binding domain	Antimicrobial peptide (AMP) chemokine and cytokine production
CD14		Production of cytokines and chemokines
Soluble PRRs	Mannose-associated lecithin	Opsonization or lysis of microbes Leukocyte chemotaxis

Table 9.3 contains information regarding types of PPR and problems associated with them in AD patients (De Benedeto et al. 2009; Medzhitov and Janeway 2002).

Acquired immune response mainly deals with type 2 signaling pathways. Modification in expression of these pathways forms the basis of pathophysiology of AD. Many studies showed IL-4 and IL-13 play vital roles in chemokine production, suppression of antimicrobial peptides (AMP), skin barrier dysfunction, allergic inflammation, and also suppression of innate immune responses. IL-31 was stated to enhance the release plus production of brain-derived natriuretic peptide (32 AA cyclic peptide, predominantly present in afferent set of neurons) and to coordinate cytokine as well as chemokine release from skin cells, thereby inducing itch in AD patients. IL-31 serves as a critical neuron-immune link between TH2 cells and sensory nerves (Meng et al. 2018). Another factor which plays an important role is thymic stromal lymphopoietin (TSLP) which is a pro-allergic cytokine. The TSLP receptor, a heterodimer of IL-7Rα and TSLPR, is expressed on Th2 cells, dendritic cells, mast cells, and type 2 innate lymphoid cells. Keratinocyte-derived TSLP expression is increased in acute and chronic lesions in patients with AD. TSLP production is triggered by exposure to environmental elements such as microorganisms, allergens, cigarette smoke, diesel exhaust, and chemical irritants (Jariwala et al. 2011; Turner and Zhou 2014). Although type 2 pathway plays a major role in AD progression, other factors also show AD progression. IL-17 has been reported to reduce expression of FLG and involucrin. More prominent Th17 activation was detected in blood and acute AD skin lesions in Asian patients compared with European-American patients. Tumor necrosis factor and TH2 altered the expression of primary and terminal differentiation products and reduced the level of long-chain free fatty acids, leading to epidermal water loss and decreased water retention of the skin, dryness, and subsequent itchiness (Tan et al. 2017; Leonardi et al. 2015).

9.2.5.3 Neuroimmunogenic Mechanism

Sensory neurons which are involved in expression of H1 receptor and H4 receptor activated by histamine are mainly involved in allergy-based inflammatory responses and itchiness. As mentioned above, IL-31 induces sensory nerve elongation and splitting which supports its role in sensitivity to negligible stimuli and sustained itch in patients with AD. Stimulation of STAT3 in the astrocytes of the spinal dorsal horn has been reported to be involved in chronic pruritus via the generation of lipocalin

2. Various types of neurotrophins and receptors regulated by them showed overexpression in AD, but exact mechanism is still unknown. Nerve growth factor (NGF) is a major mitogen for keratinocyte, and its concentration in SC serves as function of severity of AD. It acts by binding to two receptors with high affinity (tropomyosin-related kinase A (TrkA)) and others with low affinity (p75) whose expression are drastically increased in case of AD and may serve as evidence for indirect association between NGF and AD (Gaspar and Aidé et al. 2016).

9.2.5.4 Epidermal Dysfunction

Various interleukins (IL-4, IL-13, IL-17, IL-31, IL-33) which were discussed earlier show downregulation of epidermal proteins such as filaggrin, keratins, loricrin, involucrin, and cell adhesion molecules. This leads to disruption of barrier and onset of symptoms of AD. Sensitization to various external antigens is also prominent as it promotes unfiltered entry of antigens to contact with epidermal receptors. Brief summary of all factors contributing to epidermal dysfunction is given in Table 9.4.

9.2.5.5 Lipids

Lipids, such as ceramides, long-chain free fatty acids, and cholesterol, form the lipid matrix that is present in lamellar bodies and located between corneocytes in the stratum corneum. Precursors of these lipids stored in the upper lamellar region which further converted into its active form by progressive enzymatic activities help in maintenance of epithelial barrier. Alteration in these lipid compositions are common feature of AD. Long-chain ethylene oxide ceramides are essential because they are covalently bound to cornified envelope proteins and cover the surface of each corneocyte. Also, transepidermal water loss (TEWL) and *S. aureus* infection both show inverse relation with lipid composition in the skin (Tan et al. 2017; Leonardi et al. 2015).

Table 9.4 Epidermal dysfunctions contributing to AD (Kim et al. 2019a, b)

Epithelial dysfunction	Abnormality	Effects
Stratum corneum proteins	Filaggrin, transglutaminases, keratins, loricrin, involucrin, and intercellular protein deficiency	Dryness of the skin, skin pH elevation, skin sensitivity, often occurring inflammatory responses
Desmosomes	Diminished production of claudins	Lack of epidermal hydration, high transepidermal water loss, skin sensitization
Epidermal lipids	Decreased long-chain free fatty acids	Increased transepidermal water loss and increased infections
Antimicrobial peptides	Lowering of cathelicidin (LL-37) and human beta-defensins	Increased cytokinin production, increased skin infections

9.2.5.6 Microbiome

Microbiome is usually considered as one of the complications of AD. Due to epidermal dysfunction and integrity loss, the skin is very susceptible to infections. Main microbes involved in infections are *S. aureus*, *Corynebacterium*, and *S. epidermidis.* Abundance of *S. epidermidis*, *Streptococcus*, *Corynebacterium*, and *Propionibacterium* species was detected after AD treatment. *S. aureus* abundance leads to more severe form of AD, and *S. epidermidis* infection results in mild to moderate type of AD (Byrd et al. 2017). *S. aureus* induces T-cell-independent B-cell expansion which increases the release of proinflammatory cytokines such as TSLP, IL-4, IL-12, and IL-22. This further stimulates mast cell degranulation, which leads to skin inflammation and increased complications of AD. Patients suffering from moderate to severe AD show significant lowering of numerous intestinal common microflora, i.e., *Bifidobacterium*, and higher numbers of *Staphylococcus* compared to healthy individuals. Overgrowth of pathogenic bacteria, *E. coli*, and *Clostridium difficile*, is hypothesized for its connection with a decrease in beneficial bacteria, reduced induction of regulatory T (Treg) cells, diminished immunity, and increased intestinal permeability. These observations support the hypothesis that specific microbial composition in the gut prevented Th2-shifted immunity and stimulated regulatory immunity, producing regulatory dendritic cells and Treg cells (Watanabe et al. 2003; Penders et al. 2007).

9.2.6 Current Approaches in Treatment of AD and Their Drawbacks

AD is a chronic, recurrent cutaneous inflammatory disorder, which shows its origin in genetic and epidermal dysfunction as well as abnormalities in the immune system. Most population suffering from AD shows increasing severity of situation upon bacterial infections with *S. aureus.* Almost 60% of population dealing with AD shows further development of allergic rhinitis or asthma or food allergies as well. AD treatment mainly focuses on obtaining rapid control over the symptoms and improving quality of life. Current treatment acts in prophylactic manner rather than inhibiting the pathways of pathogenesis.

It mainly includes various classes of medications which are prescribed or included into the regimen depending on various factors such as (1) progression of the disease, (2) age of patient, and (3) severity of the condition based on SCORAD index, eczema area and severity index (EASI), etc. Due to the chronic nature of disease, it is advisable that selected therapy should be physically tolerable and should possess good compliance. Fundamental treatment of AD mainly focuses on various aspects of pathophysiology of AD as listed below (Nowicki et al. 2015):

(a) Proper skin care routine (emollient, humectants, moisture-retaining protein treatments).
(b) Anti-inflammatory treatments.
(c) Topical corticosteroids (TCS) and topical calcineurin inhibitors.
(d) Wet wrap treatment.

(e) Phototherapy.
(f) Oral antihistamines.
(g) Immunosuppressant therapy.
(h) Systemic treatments with corticosteroids.
(i) Avoiding triggering factor for atopic march.

9.2.6.1 First Line of Treatment (Basic Therapy)

This line of treatment is commonly advisable in case of children below 1 year of age and patients dealing with mild AD. This mainly includes the use of emollients and humectants, and their selection is based on patient-to-patient variation such as degree of skin dryness, nocturnal activities of the skin, contact allergy, etc. Retention of water in SC region of the epidermis and prevention of dryness of the skin are primary goals of this line of treatment. Selection of composition of emollient is key for better efficacy, and usually it is kept as similar to that of naturally available lipids in healthy skin which further results in active transport through receptors present in the skin and prolonged time of retention. Commonly used emollients in this class are ceramide, cholesterol and free fatty acid, tocopherols, etc. Compositions containing ceramide give better results as it is most deficient in the AD (Chamlin et al. 2001). Further advancement within first line of treatment is through the use of active emollients which facilitate lipid production in the skin. Derivatives of palmitic acid show stimulation of endogenous lipid production along with mild antimicrobial activities as well and antagonize the histamine-related itches by preventing degranulation of mast cells (Eberlein et al. 2008). Urea is another additive used along with other emollients as it is one of the components of NMF.

Wet wrap treatment is another emollient evolving first line of treatment which may or may not involve topical steroids. It is generally prescribed for patients within age group of 6 months to 10 years with severe type of AD (SCORAD >50). It mainly involves two layers of dressing, inner and outer. Inner dressing is always wet type of dressing saturated with emollient and/or medicament (0.05% fluticasone propionate/mometasone) in the ratio of 1:5 (body application) and 1:9 (facial application). This course of treatment is usually prescribed for a time span of 3–14 days, and treatment is required to be carried out under medical supervision. Wet dressing forms a physical barrier against external environment and provides cooling sensation to the skin. In addition, it prevents children from scratching irritated area of the skin. Outer dry dressing prevents adsorption of any dust on inner dressing and provides protection against leakage of emollient from inner dressing (Devillers and Oranje 2012; Devillers et al. 2002).

9.2.6.2 Second Line of Treatment (Mild Anti-inflammatory Treatment)

This line of treatment consists of topical corticosteroids, topical calcineurin inhibitors, oral antihistamine, antimicrobials, maintenance therapy, and avoiding contact to allergen.

Topical Steroids (TCS)

It is the most commonly used treatment for AD in the past 50 years. TCS exerts their effect mainly through forming complexes with receptor protein present in cytosol and further shows nuclear binding through steroidal receptors, leading to stimulation of production of glycoprotein by mRNA transcription changes. Glycoprotein (lipocortin) inhibits action of phospholipase A2 and decreases arachidonic acid release which shows reduction in inflammatory responses (Hirata et al. 1980). Commonly prescribed topical steroids are hydrocortisone acetate and hydrocortisone butyrate for children below age of 1 year. Mometasone furoate, fluticasone propionate, and methylprednisolone aceponate are approved for usage in children above 2 years of age. Other corticosteroids are approved for usage in children above age of 12 years (Darsow et al. 2010; Ring et al. 2012).

Topical Calcineurin Inhibitors

Calcineurin inhibitors exert their immunosuppressive effect by reducing IL-2 production and IL-2 receptor expression, which further leads to reduction in T2-cell activation. They also decrease production of IL-4 and IL-13 which are important mediators in AD pathogenesis (Greg et al. 2015). Pimecrolimus in the form of 1% cream is prescribed as first-line therapy in mild AD (Luger et al. 2013). 0.03% tacrolimus and 0.1% ointment are recommended in moderate to severe atopic eczema and AD. Compared to pimecrolimus, tacrolimus has a faster and more potent action. In comparison to topical steroids, topical calcineurin inhibitors show less adverse effects and do not have any age-related restrictions on its use (Schmitt et al. 2011).

Antimicrobial Treatment

Bacterial infections in AD cause major severity in treatment of the disease. The skin of patients with AD is colonized with this pathogen in 90% of cases. Infection with *S. aureus* usually gives rise to severe conditions of AD rather than infections with other species. Although the use of antibiotic is mentioned in line of treatment, it is not commonly used as it is associated with various complications of bacterial resistance development. Drugs prescribed under this category will depend mainly on strain of bacteria which is predominantly colonized in the AD. Certain drugs are recommended based on their efficacy index such as octenidine, chlorhexidine, mupirocin, fusidic acid, and retapamulin (Thum et al. 2013; Kircik 2012; Huang et al. 2009). Additionally, it is important to note that TCS and TCI categories of drugs also exhibit antimicrobial activity (mild) (Thum et al. 2013); thus, the use of antimicrobial agent is not generally prescribed.

Antihistamines

A first-generation antihistamine, hydroxyzine is the currently recommended drug of this category. It usually serves dual purpose as it inhibits degranulation of mast cell leading to inhibition of histamine-derived inflammatory responses and also shows sleep-inducing nature (sedative effect) which can be used as aid in AD patients as they commonly suffer from sleeping disorders. Antihistaminic activity shows

Table 9.5 Drug approval database as per patient age (Nowicki et al. 2015)

Drug	Approved age of patient
Fenistil	2 months
Hydroxyzine	12 months
Cetirizine	2 years
Levocetirizine	2 years
Loratadine	2 years
Desloratadine	1 year
Fexofenadine	12 years
Bilastine	12 years
Rupatadine	6 year

acceleration of epidermal barrier healing process as histamine shows reduction in filaggrin and keratin production (Gschwandtner et al. 2013). Second-generation antihistamines (AH2) are particularly useful in patients with AD accompanied by conjunctivitis or allergic rhinitis. Higher specificity of binding to histamine H1 receptor, longer half-life, and hydrophilic structure of AH2 contribute to improved efficacy and safety of these drugs (Simons and Early Prevention of Asthma in Atopic Children (EPAAC) Study Group 2007). Various second-generation antihistamines approved for use in AD are listed in Table 9.5 (Simons 1999, Simons and Early Prevention of Asthma in Atopic Children (EPAAC) Study Group 2007; Hoare et al. 2000; Ring et al. 2012).

9.2.6.3 Third Line of Treatment: Systemic Treatment

Patients suffering from severe type of AD in which first and second line of treatments do not work as efficiently as expected usually undergo systemic type of treatments. This mainly involves treatment with cyclosporine A, methotrexate, azathioprine, mycophenolate mofetil, systemic corticosteroids, and phototherapy as well as immunosuppressants.

Cyclosporine A (CsA)

It is recommended as first line of treatment in case of severe AD in adults although its use in children is restricted due to severe adverse effects. CsA reduces inflammation, size of lesions, and severity of pruritus and improves the quality of sleep. After obtaining improvement of skin lesions, it is recommended to reduce the CsA dose by 0.5–1.0 mg/kg b.w./day every 2 weeks. Withdrawal of the drug is associated with a risk of recurrence of skin lesions within several weeks after discontinuation of treatment. However, it is estimated that after treatment the skin condition does not return to the pre-CsA treatment state (Harper et al. 2000; Schmitt et al. 2007). The drug can be administered in continuous long-term therapy; however, administration in cycles lasting on average 12 weeks is recommended. It has been shown that CsA at a dose of 2.5–5.0 mg/kg/day given in cycles (cycle duration of 12–16 weeks) quickly leads to a significant improvement or disappearance of lesions in 80–90% of patients (Schmitt et al. 2007).

Methotrexate

It serves as last option of treatment, only recommended in AD complications which are unresolved by other line of treatments due to its severe side effects. Many studies have reported safety aspects of methotrexate in treatment of AD, but all these studies involved only adult subjects. Currently, methotrexate is recommended for the treatment of AD in adults at doses similar as in the treatment of psoriasis, i.e., 10–20 mg/week. It can be used in a single dose once a week, but it is more often applied in three doses of 2.5–7.5 mg every 12 h once a week (Nowicki et al. 2015). Other authors recommend the use of methotrexate at doses of 7.5–25 mg/week for adults and 0.2–0.7 mg/kg/week in children (Sidbury et al. 2014).

Azathioprine (AZA)

It has been used in the treatment of skin conditions including severe AD resistant to other treatments. The exact mechanism of action of AZA in treatment of AD is not yet understood. In vitro studies suggest that AZA has a suppressive and toxic effect on Langerhans cells (Liu and Wong 1997) and also lowers total serum IgE levels. It is recommended to use AZA at doses of 1–3 mg/kg b.w./day.

9.2.6.4 Phototherapy

Phototherapy was first reported in 1925 and since then it is used widely. All types of phototherapy available have been proven efficacious in case of severe AD. Most commonly, phototherapy is combined along with emollient therapy or TCS or TCI therapy. Below mentioned are commonly used types of phototherapy in AD (Nyamai et al. 2016):

1. Broadband UVB (290–320 nm).
2. Goeckerman regimen (5-methoxypsoralen, 8-methoxypsoralen—photosensitizing compounds taken orally 1 or 2 h prior to irradiation + broadband UVB).
3. Narrowband UVB.
4. Excimer laser (308 nm monochromatic).
5. Combination of UVA/UVB.

There is no evidence for the superiority of one method over another due to the unavailability of comparative studies. UVB is most commonly used among all (Sidbury et al. 2014). For phototherapy and photochemotherapy for children, NB-UVB is recommended as the treatment of choice for patients who have not responded to topical therapy (Ring et al. 2012). Dosing frequency for various phototherapies is totally depending on factors like minimal erythema, dose patient response to phototherapy, etc. Although currently used line of treatments work efficaciously, they have some adverse effects which give rise to harmful health conditions in the long run.

9.2.6.5 Drawbacks of Currently Available Treatments

Summarized in Table 9.6 are the undesired effects of currently used line of treatment for AD (Nowicki et al. 2015).

9.2.7 Emerging Therapeutic Treatments in AD

Current treatment for AD focuses on symptomatic relief. Due to various drawbacks of current treatments, they are not recommended for the long run. For chronic treatment, various pathways of pathogeneses of AD are explored, and new emerging treatments are designed in such a way that they will exert target-specific action which shows reduced incidences of side effects.

As discussed in pathophysiology of AD, IL-4 and IL-13 play an important role in disease progression. Specific targeting of this interleukin receptors and reducing their effect form the basis of treatment, which involve monoclonal antibodies. Dupilumab is the first humanized monoclonal antibody approved by the FDA as line of treatment for AD. Dupilumab blocks both IL-4- and IL-13-mediated signaling through type 2 receptor which requires heterodimerization of IL-4Rα and IL-13Rα1. Dupilumab also inhibits IL-4 signaling via type 1 receptor (Gooderham et al. 2018). Tralokinumab and lebrikizumab are two highly advanced IL-13 targeting antibodies that are still in clinical trials. Monotherapy and combination therapy of these three antibodies are still under studies, which showed better tolerability and safety index with respect to topical steroids (Moyle et al. 2019).

Dysfunction in multiple immune pathways (types 1, 2, 17, and 22) are also seen in AD. A number of biologics are in development targeting key effectors (e.g., cytokines, cytokine receptors) of these biological responses. For targeting IL-17A, secukinumab, a human IgG1κ, anti-IL-17A monoclonal antibody, is developed and is FDA-approved for treatment of diseases like moderate to severe plaque psoriasis, active psoriatic arthritis, and ankylosing spondylitis, but result of AD trials are not yet reported (Toda et al. 2003).

Another pathway that is widely explored for AD treatment is phosphodiesterase-4 (PDE-4) enzyme inhibition, which is involved in chronic inflammation. Inhibition of PDE-4 increases levels of cyclic adenosine monophosphate (cAMP), which mediates downstream events culminating in the inhibition of inflammatory cytokine secretion (e.g., IL-12, IL-17, IL-23, IFN-γ). Though several PDE-4 inhibitors (e.g., apremilast, roflumilast) have not reported clinical success to date, topical crisaborole was FDA-approved in December 2016 for the treatment of mild to moderate AD in patients aged 2 years and older (Gisondi and Girolomoni 2016; Paller et al. 2016).

Table 9.6 Drawbacks of current treatment line of AD

Treatment	Drawbacks	Reference
Wet wrap treatment	Skill personnel requirement, increased cost of therapy, close medical monitoring required in case of steroidal drugs, adrenal suppression in children	Braham et al. (2010)
Topical corticosteroids	Skin atrophy, permanent telangiectasia, stretch marks, hypertrichosis, depigmentation, perioral dermatitis, acne rosacea, bacterial and/or fungal infections, steroidal phobia, inhibit the synthesis of collagen that causes epidermal thinning Local application of strong TCSs on large surfaces in children, especially infants, can cause undesirable systemic symptoms: inhibition of the hypothalamic-pituitary-adrenal axis, growth retardation, and osteoporosis	Green et al. (2005)
Topical calcineurin inhibitors	Burning and redness of the skin at the site of application disappear after termination of treatment	Schmitt et al. (2011)
Antimicrobial treatment	Chronic/long-term use of antimicrobial agent leads to development of bacterial resistance Depending upon class of antibiotic, hepatotoxicity, GI disturbances, or nephrotoxicity	Huang et al. (2009)
Antihistamines	Upper respiratory tract infections, gastrointestinal disorders, and exacerbation of allergic diseases	Simons (1999)
Cyclosporine A	Side effects usually resolved after discontinuation of treatment. Renal dysfunction, elevated blood pressure, and risk of nephrotoxicity are common side effects observed in case of high-dose regimen. Uncommon adverse effects are neurological symptoms like headaches, convulsions, paresthesia, as well as gastrointestinal disorders, infections, gingival hyperplasia, hirsutism, hyperlipidemia, electrolyte disturbances, increased risk of skin cancers, and lymph proliferative disorders	Schmitt et al. (2007)
Methotrexate	Hepatotoxicity, bone marrow suppression, pulmonary fibrosis, and renal failure. In addition, reduced resistance to infection, leukopenia, anorexia, dizziness, headache, abdominal pain, ulcerative stomatitis, inflammation, and ulceration of the bowel are frequently observed	Product summary (2007)
Azathioprine	Bone marrow failure and immune system disorders. Vascular disorders (vasculitis), gastrointestinal disorders (nausea, vomiting), and liver disorders	Roekevisch et al. (2014)
Phototherapy	Redness and tenderness after irradiation, itching, burns, and solar skin damage. Less common adverse events include skin cancers, melanoma (mainly with PUVA), lentigo, photosensitivity reactions (mainly polymorphic light eruptions), folliculitis, photo-onycholysis, reactivation of HSV, excessive facial hair, and cataract (also with PUVA). Patients who use psoralens complain of nausea, vomiting, and headaches	Sidbury et al. (2014)

9.2.8 Alternative Treatment Options for AD

Alternative treatment for AD generally involves treatment for its overall management and improving quality of life for the patients. This approach mainly involves remedies belonging to alternative systems of medicine, which mainly involve:

(a) Acupuncture.
(b) Relaxation therapy.
(c) Psychotherapy.
(d) Probiotics.

9.2.8.1 Acupuncture

This alternative system of treatment originated from Chinese system of medication. Basic philosophy of this system lies in hypothesis that many diseases are associated with skin as it communicates between inner and outer environment. Complete balance of energies is maintained in healthy body. Any disturbance in this equilibrium leads to a disease condition that immediately reflects on the skin. In case of AD, deficiency of energy is seen where tonification and moxibustion are preferred (mild stimulation of skin). Controlled clinical trials performed by Kim et al. (2018) which involve 30 patients over 19 years of age (SCORAD 10–40) showed significant betterment in SCORAD of verum acupuncture group to that of sham (placebo) acupuncture.

9.2.8.2 Relaxation Therapy

It mainly aims for controlling stress component in progression of AD.

It includes the following:

(a) *Biofeedback*—It is a combination of the words biology and feedback, meaning literally "biological return information." It shows good results with autonomic factor control in AD by controlling overrepresentation of sympathetic responses based on concepts like mind-body equilibrium inducing self-control in patient. It also includes individual's responsibility in curing of disease, etc. In this therapy, patients are trained cautiously to alter neurological responses. During training, special attention is required to pay for various responses generated in disease and controlling them. This is usually coupled with electromyography during training for monitoring of responses (Sarti 1998).
(b) *Hypnosis* —It refers to state of mind attained by patient under guidance of a therapist in which patient easily accepts suggestions and creates alternative reality. Heightened self-concentration may lead to improvements of symptoms of AD through patient's mind to body communications. Although exact mechanism for working of hypnosis is still unknown, it was hypothesized that it works on similar lines to that of biofeedback. It usually focuses on controlling of other parameters such as desensitization of affected area and aversion training. Autogenic training is similar to the self-guided hypnosis (Arndt et al. 2008).

(c) *Massage therapy*—It is a widely accepted form of relaxation therapy as it serves benefits such as stress relief action as well as topical emollient treatment. Study conducted by Eisenberg et al. (1998) included a set of children suffering from AD who underwent massage treatment daily for 15 min before their bedtime. Massaging material was medicated ointment. Similarly, in control group, the same ointment was applied without massage. Upon completion of 1 month, children who were receiving massage showed improvement with decreased redness and itchiness of skin compared to control group.

9.2.8.3 Psychotherapy

Itchiness and discomfort are important characteristics of AD. Continuous scratching induces skin rash and hampers skin integrity which further leads to progression of disease. Psychotherapy mainly focuses on reduction of feeling to itch, as well as betterment of patient's psychiatric conditions (Horne et al. 1989). Cognitive behavioral therapy (CBT) plays important role in modification of patient's response to anxiety and also shows betterment in dealing with frustrations, etc. It considers patients and their relatives' opinion in disease and tries to modulate and restructure their thinking pattern. In this treatment, therapist hypothesizes chain of thoughts which are percolated and accepted by patients, e.g., continuous motivation of patients using positive talk, decreasing intensity of scratching during itch, and solving any family disturbances by aid of counseling (Arndt et al. 2008). In a pilot open trial carried out by Erik Hedman-Lagerlöf et al., nine adults suffering with AD underwent psychotherapy for a period of 10 weeks. Patient showed statistically significant improvement with respect to SCORAD index compared to pretreatment and posttreatment data with $p = 0.020$ (Hedman-Lagerlöf et al. 2019).

9.2.8.4 Probiotics

This mode of treatment involves administration of bacteria to patients through various modes of delivery (oral, topical) in a calculated amount to confer health benefits. Colonized bacteria in human GI offer health benefits, and disturbance in their equilibrium usually leads to disease condition. Maintenance of this microbiota of the gut is very important. Detailed analysis of fecal bacteria of healthy children and adults compared to patients of AD gave rise to database for bacterial strain that might play a crucial role in progression of AD. Based on this database, commonly used bacterial strains for treatment of AD are *Lactobacilli*, *L .rhamnosus*, *Bifidobacterium lactis*, *L. fermentum, P. freudenreichii* subsp. *shermanii JS*, *L. reuteri*, etc. (Yeşilova et al. 2012).

9.3 Probiotics in AD

The science of using microbes in food fermentation as well as for conferring health benefits heralds back to some of the ancient civilizations of Asia and Europe (McGovern et al. 2004; Ozen and Dinleyici 2015). However, the link between human health and microbiome became more profound only in the early nineteenth

century, when beneficial effects of certain bacteria were systematically studied and reported by Ellie Metchnikoff—the father of natural immunity. He drew a direct correlation between yogurt consumption and postponed aging (Gordon 2008). Eventually, the term "probiotic" was coined by Werner Kollath and got associated with array of health ailments for its therapeutic, theranostic, and cosmeceutical benefits (Gueniche et al. 2009; Danino et al. 2015; Singh et al. 2017a). This circumvents cardiovascular, gastroenteric, pulmonary, and neurodegenerative disorders, cancers, bone health, reproductive system well-being, psychological conditions, lifestyle disorders, and skin diseases. The probiotics used for skin diseases and disorders with special emphasis on AD are discussed in the next section.

9.3.1 Probiotics in Skin Health

Microbes are omnipresent, and within the human body, their count is approximately 10^{14}, outnumbering the total number of human cells. Majority of them are habited in the GI track (particularly large intestine) followed by different locations like the oral cavity, conjunctiva, lungs, vagina, urethra, urinary bladder, and skin (Huttenhower et al. 2012). Being the largest organ of the human body with an average surface area of about 2 m^2, the skin houses a large amount of natural microbiome. They contribute to maintaining skin homeostasis and are often called as "good bugs." However, the number varies depending upon the anatomical positions, structural composition, epidermal thickness, skin secretions (sweat and sebum), and moisture content (Grice and Segre 2011). The microflora on the skin is subjected to change depending upon the thresholds of various environmental factors including hormonal, neuronal, and inflammatory signals. The physiological outcomes of these are often keratinocyte degeneration, excessive sebum production, degranulation of mast cells, and production of vasodilatory factors and proinflammatory cytokines like IL-1β, IL-6, and TNF-α (Arck et al. 2006; Choi and Di Nardo 2018). Such aberration manipulates normal functioning of the skin microbiome in maintaining good health and paves a way for skin ailments and diseases.

The fungal microbiome is thought to be progressively steady as compared to the bacterial microbiome, which is time and again prone to changes (Paulino et al. 2006; Gao et al. 2008). Bacteria's belonging to the phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* are generally found on the skin. At genus level, *Proteobacteria*, *Propionibacteria*, *Corynebacteria*, *Staphylococcus*, and *Streptococcus* are predominant and maintain healthy skin. However, the skin fungal microflora remains confined to only the yeast genus of *Malassezia* (Paulino et al. 2006; Gao et al. 2008; Grice et al. 2008).

The various skin diseases and disorders along with the probiotic treatment therapy are briefly described in the below section.

9.3.1.1 Probiotics and Acne

Overexpression of proinflammatory mediators like cytokines aggravates skin inflammation suppressing hair growth and keratinocyte proliferation, resulting in acne rosacea or acne Vulgaris. *Lactobacilli reuteri* BM 36301 and *Bifidobacterium longum* have demonstrated to stimulate hair follicle growth and reduce inflammation, respectively, in animal models and ex vivo skin explant studies (A. et al. 2010; J. et al. 2016).

Staphylococcus epidermidis and *L. brevis* DSM 17250 have shown inhibitory effect on the propagation of *Propionibacterium acnes*, the causative agent for acne, by releasing SCFAs and certain peptides which trigger *S. epidermidis* proliferation, respectively (Wang et al. 2016; Holz et al. 2017). Synergism along with reduction in adverse effect was seen when minocycline was administered along with a combination of three probiotics—*L. acidophilus*, *B. bifidum*, and *L. delbrueckii* (Jung et al. 2013). Oral administration of *Escherichia coli* alleviated inflammation due to the presence of immunoglobulin-A (IgA) and maintained the gut flora associated with skin dermatoses as well (Manzhalii et al. 2016). In vitro and topical studies for *Lactococcus* sp. HY 449 produced positive results against *P. acnes* by the release of bacteriocin (Oh et al. 2006).

9.3.1.2 Probiotics and Wound Healing

Innate process of skin tear or disruption healing generally involves four stages, namely, (a) hemostasis, blood flow cession to the damaged tissues, (b) inflammatory mediator release to prevent pathogenic infection progression and further maintain natural skin microbiome, (c) stimulation of growth factors for reepithelization, and (d) fibroblast proliferation and release of extracellular matrix proteins like collagen (Singh et al. 2017b). Probiotics work in stimulating and maintaining the above process at either of the stages.

An increase in collagen concentration and transforming growth factor beta 1 (TGF-β1) was observed when burn wounds were treated with *Saccharomyces cerevisiae* (Tsiouris and Tsiouri 2017). MRSA which are generally found in infectious wounds were inhibited by the use of *L. acidophilus* and *L. casei* (Sikorska and Smoragiewicz 2013). In another study, *L. reuteri* and *L. rhamnosus* inhibited the adhesion and conquest of *S. aureus* on the keratinocyte, thus preventing keratinocyte destruction and eventual death (Prince et al. 2012). Gaseous nitric oxide (gNO) production-mediated wound healing process was also studied against *S. aureus* using the probiotic *L. fermentum* (Isenberg et al. 2005). A few studies on the use of Kefir in topical applications have also shown significant microbicidal and healing effects (Huseini et al. 2012). Kefir has gained much scientific importance due to the presence of hydrogen peroxide, bacteriocin, lactic acid, and acetic acid suppressing bacterial growth, especially on burn wounds infected with *P. aeruginosa* infection (Rodrigues et al. 2005).

9.3.1.3 Probiotics and Skin Aging

There is an unavoidable link between increase in levels of stress, unhealthy lifestyles, and human skin getting prone to the appearance of fine lines, wrinkles,

surface roughness, and age spots. Internal (oxidative stress, pH change, etc.) and external (UV irradiation) factors further deteriorate natural skin microbiome. Plethora of preclinical and clinical data support maintenance of SC suppleness, protecting the skin from UV-induced photoaging using probiotics. These friendly organisms are now taking up a central stage in cosmetic applications as well, providing a more efficient and safe treatment for negative cosmesis.

Oral intake of *L. acidophilus* and *L. johnsonii* reduced UVB-induced skin wrinkles and alleviated photodamage on the skin (Bouilly-Gauthier et al. 2010; Im et al. 2016). Another study highlighted that fermented *Acanthopanax koreanum* root extract together with *L. plantarum* and *B. bifidum* delayed skin aging and reduced oxidative stress-induced damage on the human skin (Park and Bae 2016). Other endogenous approaches include the administration of *Vitreoscilla filiformis* that altered the activity of mitochondrial oxide dismutase, lowering cell apoptosis, and tissue damage induced by UV damage (Mahé et al. 2006).

In a study carried out by Baba et al., *Lactobacillus helveticus* showed enhanced moisture retention (Baba et al. 2006). Similar studies showed that *B. breve* B-3 supplementation maintained normal hydration levels in the skin along with a photoprotective action (Satoh et al. 2015).

9.3.1.4 Probiotic and Psoriasis

Immunomodulatory probiotic strains are of growing interest these days for autoimmune diseases like psoriasis and AD. Probiotics for psoriasis work by downregulation of proinflammatory mediators ILs and TNFs which otherwise cause epidermal hyperplasia leading to psoriatic lesions (Hawkes et al. 2017). A downfall of bacteria belonging to phyla *Actinobacteria* and *Proteobacteria* and the genus *Propionibacterium* is that they further aggravate the condition (Gao et al. 2007). Application of *L. pentosus* GMNL-77 and *B. infantis* 35,624 has shown reduction in inflammatory symptoms and relief from psoriatic plaques (Groeger et al. 2013; Chen et al. 2017).

L. sporogenes has also shown similar effects in pustular psoriasis (Vijayashankar and Raghunath 2012).

The section below draws a link between gut microbes and pathogenesis of AD along with the treatments using probiotics.

9.3.2 Microbiome in the Gut-Skin Axis in AD

A myriad of bacteria dwell in the human gut in symbiotic relation, monitoring and maintaining physiologic homeostasis. However, an alteration in this natural gut microbiome leads to dysbiosis, and the body's well-being is also imperiled. A potential link does exist between healthy skin and gut microbiota (Johnson and Ownby 2017). Taking advantage of this association, scientists have traced a way "from inside to outside" providing effective treatments for a complex skin condition as AD.

Table 9.7 Some probiotic stains used with their mode of action/function in AD

Probiotic strains	Mode of action/functions	References
Combination of *L. casei*, *L. plantarum*, *L. rhamnosus*, *B. lactis*, and sodium butyrate	Elevation in Th1 levels. Reduction in type 1 hypersensitivity	Kim et al. (2018)
L. plantarum IS-10506	Downregulation of inflammatory markers like IL-4, IL-17, and interferon- γ (IFN-γ). Increase in gene expression of Foxp3+ levels, increase IL-10 levels	Prakoeswa et al. (2017)
L. acidophilus	Decrease in Th2-mediated inflammatory responses	Inoue et al. (2014)
L. salivarius LA307 and *L. rhamnosus* LA305	Inhibition of skin inflammation, decrease in hyperplasia	Holowacz et al. (2018)
Lactococcus chungagensis CAU 28T	Anti-inflammatory and anti-allergic	Choi et al. (2016)
L. johnsonii NCC 533	Enhanced expression of antimicrobial peptides, inhibition of adhesion of *S. aureus*	Blanchet-Réthoré et al. (2017), Rosignoli et al. (2018)
Bifidobacterium lactis HN019	Alleviate allergic condition	Wickens et al. (2008)

The conventional therapies include regulating the degranulation of mast cells and alleviation of phenotypic symptoms (erythema, itching, and hemorrhage). Alongside, the research on the use of probiotics for AD is at an exponential increase (Table 9.7). Probiotics work by restoring microbial-gut flora, secretion of toxins (bacteriocin), and preventing the adherence of detrimental pathogens on the epithelial wall. They further modulate inflammatory and immunological signals by releasing SCFAs like butyrate, propionate, and acetate. The expression of tight junction proteins is also enhanced. Suppression of proinflammatory cytokines like IL-4, IFN-γ, and IL-17 and promotion of anti-inflammatory cytokines (IL-10, TGF-β) are regulated by probiotics. Upregulation and migration of regulatory T cells on the skin by probiotics inhibit Th2 and Th17 responses, ensuring therapeutic effect in AD (Kim et al. 2013; Chang et al. 2016; McCusker and Sidbury 2016; Rather et al. 2016).

The levels of *Clostridia*, *C. difficile*, *E. coli*, and *S. aureus* in the gut are elevated in patients with AD causing eosinophilic inflammation. On the other hand, the good bacteria like *A. muciniphila*, *Bifidobacteria*, *Bacteroidetes*, and *Bacteroides* are decreased (Penders et al. 2006; Abrahamsson et al. 2012; Nylund et al. 2015; Lee et al. 2016a, b). Song et al. reported a significant decrease in the levels of *Faecalibacterium prausnitzii* in infants affected with AD (Song et al. 2016). In order to compensate for the good bacterial loss, *Lactobacillus* and *Bifidobacterium* species are among the most explored for treatment of AD (Pité 2010; Enomoto et al. 2014).

It is reported that inflammatory signals in infants suffering from AD were reduced by an increased level of *Coprococcus eutactus*, a butyrate-producing bacteria (Nylund et al. 2015). Prenatal and postnatal probiotics have shown a positive effect in managing AD. In a clinical study involving pregnant women with AD as a family history and their infants for 2 months after birth, they were given a combination of *B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031. A reduction in AD score index was seen in probiotic-treated group as compared to the placebo group (Pité 2010).

Thus, the gut-skin axis is becoming a potential therapeutic approach for patients, involving relief from severe conditions of AD by harmonization of gut microbiota.

9.3.2.1 Mechanism of Action

Apart from the restoration of the gut microflora, there are three major mechanisms by which probiotics work in modulating the symptoms of AD, viz., immunomodulatory, metabolic, and neuroendocrine pathway.

Immunomodulatory Mechanism

The immunological imbalance involving the ratios of Th1/Th2 (types of helper T cells) leads to the overproduction of Th2 cytokines like IL-4, IL-5, and IL-13. This over-signaling triggers the production of IgE, causing hypersensitivity reactions leading to AD. This also aids the adherence of *S. aureus* to the skin causing epithelium disruption in AD (Huang et al. 2017b).

Probiotics work by regulating the immunogenic pathway by interacting with the gastric mucosa and gut-associated lymphoid tissue (GALT) (Lebeer et al. 2010). They further adhere to and interact with other immune cells like macrophages and dendritic cells in the gut mucosa triggering activation of anti-inflammatory cytokines. This includes elevation in IL-10 and TGF-β in the cytosol (Smits et al. 2005; Ueno et al. 2011). Due to this milieu, the induced regulatory T (Treg) cells, particularly the Foxp3+, increase, turning the immune response down (Werfel and Kapp 2002).

On the other hand, *S. aureus* with combination of superantigen and adhesion genes in the gut was negatively associated with progression of AD in infants irrespective of the fact that *S. aureus* on the skin had devastating effect in AD (Nowrouzian et al. 2017). To summarize, regulation of the immune environment by probiotics is linked with the association of the gut and skin in AD.

Metabolic Pathway

Modulation of AD via the metabolic pathway generally involves SCFA synthesis by probiotics having anti-inflammatory effect (Reichardt et al. 2014). Oral administration of linoleic acid and 10-hydroxy-*cis*-12-octadecenoic acid in preclinical studies maintained the gut microbiota and lessened the conditions of AD. Further, increased levels of kynurenic acid, a metabolite produced by oral administration of *B. animalis* subsp. *lactis* (LKM512), relieved the pruritic symptoms in mice model (Matsumoto et al. 2014).

The neonatal gut microbiome 3 (NGM3) state in infant fecal samples showed an increased level of 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME), a proinflammatory and immunomodulatory compound causing eczema or AD. Besides, it had protective function on human skin in vernix caseosa (Fujimura et al. 2016; S.R. et al. 2018). These data signify the presence of metabolite-mediated communication through the gut-skin axis in AD.

Neuroendocrine Pathway

A recent transition has also occurred in understanding the neuroendocrinal pathway in modulating the conditions of AD (Senra and Wollenberg 2014). The involvement of neurotransmitters and neuronal compounds released by the gut microbiome, either acting directly or indirectly, is being considered in AD treatment. They are further associated with skin barrier function and regulating the host immune system (Cryan and Dinan 2012; Jin et al. 2014).

Itchy sensations that occur due to the overproduction of tryptophan by the gut microbes are counteracted by the release of γ-aminobutyric acid (GABA). This is produced by *Lactobacilli* and *Bifidobacterium* species (Akiyama et al. 2011; Jin et al. 2014). Serotonin produced by *E. coli* and *Enterococcus* stains modulates the melanogenic pathway through serotonin receptors in skin pigmentation. It becomes an important parameter to be considered for overcoming negative cosmesis (hyper-pigmentation and hypopigmentation) that occurs in AD (Leung et al. 2004). These are examples of direct modulation of the symptoms.

Indirectly, neurosensory network disruption hampers brain functions, increasing anxiety and stress levels which are one of the reasons for aggravation of AD (Yokoyama et al. 2015). Under stress, cortisol release changes the gut microbiome, altering the gut permeability and barrier function (Cryan and Dinan 2012). Alongside, the levels of neuroendocrine compounds and neuropeptides like serotonin, tryptamine, and trimethylamine are reduced. This condition modifies the disrupted epithelial layers and reduces skin inflammation (Jin et al. 2014; O'Neill et al. 2016). Thus, these molecules or pathways can further be used as targets or therapeutic agents for combating the severity of AD.

9.4 Factors Affecting Viability of Probiotics

Viability concerns for probiotics in various applications have invited large number of studies as it greatly affects the therapeutic efficiency of the product. It is generally measured in number of active or viable cells per g or mL of the product (Karimi et al. 2011). As a general notation, a concentration of 10^6 to 10^8 cfu mL^{-1} of viable cells is considered satisfactory. Another study highlighted the intake of 100 g of probiotic product (10^9 viable cells) as a routine for maintaining good gut health (Vinderola et al. 2000; Karimi et al. 2011; Mohammadi et al. 2011).

However, various contributing factors affect cell viability. This generally includes many production attributes and exposure conditions during the storage of commercial products. Viability concerns are also associated with various GI

conditions after ingestion of the product. Brief summary on production parameters and storage conditions on cell viability are mentioned in Table 9.8.

Production parameters are the first check point in assuring probiotic sustainability and end up with product storage before consumption. It begins from selection of correct strain to circumventing fermentation and storage conditions like pH, molecular oxygen, and temperature, freeze-thawing, downsteaming processes like drying and centrifugation, compatibility with growth medium, toxic by-products, competition with other organisms, and packaging material and condition (Lacroix and Yildirim 2007; Tripathi and Giri 2014). Ambient production and storage criteria are a must for cell viability and therapeutic efficiency.

The effect of GI conditions on the viability of probiotics goes hand-in-hand with the development of dosage form. This is discussed in the further section involving probiotics and various dosage forms for AD.

9.5 Delivery Systems for Probiotics

9.5.1 Conventional Delivery Systems

Probiotic remedy as a treatment for atopic dermatitis has been rising in recent years. Undeniably, considering the increasing occurrence of eczema and the lack of a definitive cure, patients and parents often go to complementary and alternative medicine treatments when they are dissatisfied by the side effects of allopathic medicine (Astin 1998).

Various systems have been developed for the delivery of probiotics, which include both conventional and nonconventional probiotic systems. Conventional probiotics consist of liquid, capsule, sachet, and tablet formulation dosage form (Table 9.9), whereas milk, meat, curd, and cheeses are well-known nonconventional probiotics already available in public domain. A large number of studies have explored the potential efficacy of probiotics and proven the probiotics are very useful for the treatment of atopic dermatitis (Pessi et al. 2000; Matsumoto et al. 2007; Batchelor et al. 2010). Study report of Wickens et al. (2012) indicate that *Lactobacillus rhamnosus* GG (LGG) is the most frequently studied and very useful probiotic strain for the conventional treatment of AD. *Bifidobacterium breve* M-16V and *B. longum* BB536 administration over the time period 6–18 months to children with moderate to severe AD lowers the incidence of AD as concluded in a study performed by Enomoto et al. (2014). Also, mixture of prebiotics and probiotics supplement by conventional delivery showed good results against AD (Foolad and Armstrong 2014; Astin 1998; Govender et al. 2014).

9.5.2 Controlled Delivery Systems

Achieving optimum rate, amount, and site using controlled or targeted delivery systems is the most effective strategy rather than enhancing absorption/penetration

Table 9.8 Factors and their effect on probiotic strain viability

Parameter/ condition	Effect on cell viability	Examples	References
Selection of correct strain	Should ensure safety (clinical validation and health benefits data), in vivo viability, compatibility, appropriate sensory characteristics, withstand fermentation parameters, short fermentation time, and low cost	Acetic acid produced by *Bifidobacterium* spp. affects taste senses along with lowering pH and affecting viability of other cells	Tuorila and Cardello (2002)
pH and acidity	Low pH due to the formation of undissociated, lipophilic organic acid and their penetration in the cells, disrupt cell metabolism and affect their viability	Optimum pH for *L. acidophilus* is 5.5– 6.0, and for *Bifidobacterium*, it is 6.0–7.0. Acidic condition of fortified cranberry juice could not sustain both these species	De Vuyst (2000), Sheehan et al. (2007)
Molecular oxygen	Cells are affected by various concentrations of oxygen, can also cause peroxide and free radical formation from lipids leading to cell death	*Lactobacilli* have a little high tolerance for oxygen than *Bifidobacteria*	Tamime et al. (2005), Lee and Salminen (2008), Iravani et al. (2015)
Temperature	High fermentation temperature causes cell rupturing. Low temperatures preserve cell viability during storage	Optimum fermentation temperatures and storage condition from 37 to 43 °C and between 2 and 5 °C, respectively. *L. acidophilus* showed highest viability at 2 °C	Lee and Salminen (2008), Xiao et al. (2014)
Freeze-thawing operations	Mechanical stress of ice in freezing can cause cell wall disruption by condensation of solute or dehydration of cell. Melting causes chemical stress like change in osmotic levels, hydrogen ions, oxygen, and other poisoning components in media	*L. rhamnosus* GG freeze-thaw-tolerant strains have been developed using genome sequencing	Jay (1992), Kwon et al. (2018)
Drying process	Spray drying affects cell viability due to high working temperature, osmotic pressure, and mechanical stresses. Freeze-drying is the most preferred method	*Bifidobacteria* show altered viability depending on the type of atomization, air pressure, and the outlet temperature used in spray drying	Champagne and Møllgaard (2008)

(continued)

Table 9.8 (continued)

Parameter/ condition	Effect on cell viability	Examples	References
Packaging material and conditions	Cell viability gets affected if the product containing anaerobic cells is permeable to oxygen. Permeability depends on the material (glass or plastic) thickness and their manufacturing constituents	*Lactobacilli* and *Bifidobacteria* cells decrease in count due to high levels of oxygen in the product	Talwalkar and Kailasapathy (2004)

by conventional method. Some controlled delivery of probiotic approaches developed for the treatment of AD is explained below.

9.5.2.1 Encapsulation

This is the most common and well-known system for the controlled delivery of probiotics. Encapsulation is a process in which a layer of coating material is formed over a core material, preferably an active moiety entrapped in the carrier molecules. It is the most commonly used method in formulation containing microorganisms. Motive behind this method is to provide protection and perfect preservation of bacterial strains by inhibition of contact with external factors such as heat, oxygen, moisture, radiation, etc. Modification of release profile also can be achieved by encapsulation. Confining bacterial multiplication within system is achieved by encapsulation process (Maria Chavarri et al. 2012). There are various types of encapsulation such as:

(a) Reservoir type (active core surrounded by coating material, e.g., microencapsulation).
(b) Matrix type (active material embedded in matrix of coating materials, e.g., hydrogels).
(c) Coated matrix (amalgamation of abovementioned types).

There are many ways in which encapsulation can be achieved. Depending on active materials, processing condition and targeting site, type of encapsulation, and material for encapsulation, manufacturing method usually changes. Commonly used methods in case of probiotics are emulsion, extrusion, spray drying (matrix type of microcapsules), spray coating, and coextrusion (reservoir type of microcapsules) (Solanki et al. 2013).

Selection of encapsulating material plays a crucial role in preservation of bacterial strain during administration as well as during storage. Some commonly used biomaterials for encapsulation purpose are alginates, chitosan, gellan, and xanthan gum (Hood and Zottola 1988).

Various protein-based coating materials are also used in encapsulation such as gelatin, milk powder, and whey protein. Milk powder is the most commonly used

Table 9.9 Conventional forms for conventional delivery of prebiotic/probiotic

Formulation type	Probiotic strain	Marketed formulation	References
	Vitreoscilla Filiformis (*V.F.*)	La Roche-Posay Lipikar Balm AP®	
		La Roche-Posay - Moisturizing Cream®	Guéniche et al. (2006)
	Nitrosomonas eutropha	Mother Dirt AO + Mist lLiquid Spray	Lee et al. (2018), Paller et al. (2019)
Liquids	*Streptococcus thermophilus* + *Lactobacillus plantarum* + *Bifidobacterium bifidum*	Marie Veronique Pre + Probiotic Daily Mist®	Shin et al. (2016), Kim and Ji (2012)
	Vitreoscilla filiformis + *Staphylococcus epidermidis*	Columbia Skincare Probiotic Concentrate®	
		LaFlore Probiotic Concentrated® Serum®	Mottin and Suyenaga (2018)
Capsules	*Bifidobacterium bifidum* + *B. lactis* + *Lactobacillus acidophilus*	Complete probiotics Purayanti®	Rather et al. (2016), Kim and Ji (2012)
	Lactobacillus rhamnosus + *B. lactis* + *Bifidobacterium breve* + *B. longum*	Pro4-50®.	Pizano et al. (2017), Baldassarre et al. (2018), Kiousi et al. (2019), Kim and Ji (2012)
	Lactobacillus rhamnosus	Faulding	Pizano et al. (2017), Baldassarre et al. (2018)
		Probiotics Eczema Support	
	Lactobacillus fermentum	proTract®	Weston et al. (2005), Kim et al. (2019a, b)
	Lactobacillus acidophilus + *B. longum* + *B. lactis* + *Lactobacillus casei*	Hyperbiotics PRO-15 Probiotics®	Yang, Liu and Yang (2013), Gaucher et al. (2019)
	Lactobacillus acidophilus + *Lactobacillus rhamnosus* + *Lactobacillus plantarum* + *B. lactis* + *Bifidobacterium bifidum*	Custom Probiotics CP-1®	Żukiewicz-Sobczak et al. (2014), Huang et al. (2017a), Pizano et al. (2017)
Sachets	*Lactobacillus casei* + *Lactobacillus acidophilus*	Elizabeth Arden Superstart Probiotic Boost Skin Renewal	Blanchet-Réthoré et al. (2017)
		Biocellulose Mask®	

(continued)

Table 9.9 (continued)

Formulation type	Probiotic strain	Marketed formulation	References
	Lactobacillus rhamnosus GG	Culturelle Kids Probiotic®	Grüber et al. (2007), Rather et al. (2016)
	Saccharomyces boulardii	FlorastorKids	Rusu et al. (2019)
Tablets	*Lactobacillus acidophilus* + *Lactobacillus rhamnosus* + *B. lactis*	UltraBiotic Factors for Juniors®	Żukiewicz-Sobczak et al. (2014), Huang et al. (2017a, b), Pizano et al. (2017)
	Lactobacillus rhamnosus + *B. longum* + *Lactobacillus acidophilus*	Primadophilus	Özdemir (2010), Nakata et al. (2019)

coating material for infant formulations as it helps in ease of delivery and avoids any complications. Some synthetic encapsulating materials are also used such as cellulose acetate phthalate (CAP) which shows enteric release of probiotics, aiding their targeted activity.

9.5.2.2 Microencapsulation

Oral delivery of probiotics is typically a tedious job mainly due to the lack of sustainability of bacterial culture in gastric environment. Drastic change in pH, harsh enzymatic activities, and peristaltic movement adversely affect viability of microbes. Microencapsulation plays a major role in tackling these issues; along with that, it helps in achieving many other goals such as targeted delivery, delivery of multistrain combinations, and so on. Various studies have been carried out for microencapsulation of microbes and their incorporation in formulations, and Table 9.10 lists all such formulations.

9.5.2.3 Freeze-Drying

Freeze-drying, also known as lyophilization, is a dehydration process usually carried out under low pressure and temperature condition. It is the most preferred method for heat-labile substances such as proteins, cell, biological products, etc. It is a widely used process as loss of water preserves viability of actives and improves free-flowing nature of powders and stability of the product. These freeze-dried products can be delivered as conventional as well as modified release products. Many formulations considering different strains have been prepared and checked for their efficacy. Detailed list of such formulations is given in Table 9.11.

Lyophilized dosage forms are usually administered as powders for reconstitution as oral suspension. Due to poor viability of strains as well as due to inhospitable environment of GI tract, frequency of administration of these probiotics is twice daily. Many times, freeze-drying and microencapsulation techniques are coupled for better restoration of efficacy and viability of bacterial strains.

Table 9.10 Microencapsulated probiotics in treatment of atopic dermatitis

Bacterial strain	Method used	References
Bifidobacterium breve	Microencapsulation was carried out using chitosan and alginate. Further dried powder was obtained by fluidized bed dryer. Comparison for viability of microbes for non-FBD dried and FBD dried batches was conducted. Both the trial showed similar viability	Cook et al. (2011)
Lactobacillus acidophilus	Encapsulation was carried out using alginate and xanthan gum. Stability against pH change was observed at gastric pH	Kim et al. (2008)
L. rhamnosus, *B. longum*, *L. salivarius*, *L. plantarum*, *L. acidophilus*, *L. paracasei*, *B. lactis* type Bl-O4, and *B. lactis* type Bi-07	Microencapsulation was done using alginate by emulsion method in which palm oil and poly-L-lysine were used as coating material. Formed microencapsulated bacterial strains were found to have excellent stability in the gastric pH and in bile pH	Ding and Shah (2009)

Table 9.11 Freeze-drying of microbes for treatment of AD

Probiotic strain	Method used	References
Multiple bacterial strains including *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. infantis*	Freeze-dried along with fructo-oligosaccharide and dispensed in sachets	Farid et al. (2011)
L. fermentum VRI003 strain of bacteria was used as its predominance is vastly decreased in AD	Freeze-drying was carried out by using cryoprotectant like maltodextrin. Dispensed as oral suspension for reconstitution in 5–10 mL of water/milk	Weston et al. (2005)
L. rhamnosus 19070-2, L. reuteri DSM 12246	Bacterial strains were checked for its mucoadhesion to the gut wall prior to lyophilization. Further lyophilization done using cryoprotectant 10^{10} CFU/g	Rosenfeldt et al. (2004)
Four strains of microbe were combined based on dysbiosis study of microbes in AD. *L. rhamnosus* with *plantarus*, *lactis*, and *casei*	There strains were cultivated in enzymatically hydrolyzed casein, glucose, and lactose in certain fixed amount at 37 °C. Coated with protein and polysaccharide and freeze-dried	Yim et al. (2006)

9.6 Clinical Trials of Probiotics for AD

Several probiotics have been evaluated clinically to check their therapeutic efficacy and safety. The clinical trials pertaining to AD are depicted in Table 9.12.

Table 9.12 Clinical trial database of probiotics for AD

Title	Subject	Method	Results	References
Lactobacillus fermentum VRI-033 PCC supplemented for 8 weeks	Infants (6–8 months old), $n = 56$ provided with 16109 *Lactobacillus fermentum* VRI-033 PCC supplement	Randomized double-blind placebo-controlled trial	Reduced the scoring of AD (SCORAD) and the severity of AD	Weston (2005)
Gut microbiota profile in children impacted with AD	AD patients ($n = 19$) in the age range of 1–6 years wherein probiotic consumed was *B. breve* BR03 and *L. salivarius* LS01	Prospective study	Significant reduction in the SCORAD index in patients administering probiotics	Reddel et al. (2019)
Impact of gastrointestinal symptoms and small intestine permeability in children with AD	Moderate and severe AD children ($n = 41$) in the age range of 1–13 year administered with probiotic lactobacilli (*Lactobacillus rhamnosus* 19070-2 and *L. reuteri* DSM 12246)	Double-blind, placebo-controlled crossover study	Prominent reduction in severity in AD	Rosenfeldt et al. (2004)
Evaluation of the significance of probiotics to treat atopic eczema in children	Infants ($n = 27$) with an early onset of AD treated with *Lactobacillus GG* and *B. lactis Bb-12*	Randomized double-blind study	A significant improvement in skin conditions of patients given probiotic-supplemented formulae as compared to control group	Isolauri et al. (2000)
Evaluation of the clinical and anti-inflammatory effect of probiotic supplementation in children with AD	1–13-year-old children with severe AD where probiotic consumed was lyophilized *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460	Double blind, placebo-controlled, crossover study	56% patients experienced improvement of eczema, whereas 15% believed improvement after placebo treatment. The extent of eczema decreased from 18.2% to 13.7%	Rosenfeldt et al. (2003)
Evaluation of the effect of a mixture	Children aged 4–17 years old	Randomized, double-blind,	Mean reduction in the SCORAD	

(continued)

Table 9.12 (continued)

Title	Subject	Method	Results	References
of oral probiotics and its subsequent evaluation on the use of topical steroids in young patients	($n = 50$) with moderate AD administered with probiotic strains *Bifidobacterium lactis* CECT 8145, *B. longum* CECT 7347, and *Lactobacillus casei* CECT 9104	placebo-controlled intervention trial	index in the probiotic group was 19.2 points greater than the control group	Navarro-López et al. (2018)
Influence of probiotic intervention on the immune system of healthy adults and patients with AD	15 healthy volunteers and 15 AD patients administered with a combination of *Lactobacillus paracasei* Lpc-37, *Lactobacillus acidophilus* 74-2, and *Bifidobacterium animalis* subsp. *lactis* DGCC 420	Double blind, placebo-controlled, randomized, crossover study	*Lactobacillus paracasei* Lpc-37 and *B. lactis* 420 colonized the intestine transiently, thus proving that probiotics have a potential to modulate peripheral immune parameters	Roessler et al. (2007)
Clinical effects of probiotic *Bifidobacterium breve* supplementation in adult patients with AD	24 adult patients (8 men, 16 women) consuming probiotic *Bifidobacterium breve* supplementation	Randomized study design	SCORAD was found to be decreased	Yoshida et al. (2010)
Clinical trial concerning *Lactobacillus salivarius* LSOI (DSM 22775) on adult AD	Patients aged 18–46 years old ($n = 38$) with moderate and severe AD treated with *Lactobacillus salivarius*	Double-blind, placebo-controlled, randomized trial	Significant decrease of T-helper cell type 1 (Th1) cytokines [(IL-12), interferon (INF)-γ] and Th1/Th2 (IL-12, IFN-γ/IL-4, IL-5) ratio	Drago et al. (2011)
Reduction in gut microbial translocation and cure of AD on incorporation of probiotics	Adult patients ($n = 48$) suffering from moderate to severe AD treated with *L. salivarius* LS01/*B. breve* BR03 combination for 12 weeks	Randomized study	Marked improvement in SCORAD and immunological profile [Th1/Th2 ratio, T-helper cell 17/regulatory T-cell (Treg) ratio]. Also, reduction in	Raone et al. (2014)

(continued)

Table 9.12 (continued)

Title	Subject	Method	Results	References
			microbial translocation	
Faecalibacterium prausnitzii subspecies- level dysbiosis in the human gut microbiome underlying AD	132 subjects including 90 AD patients	16S rRNA gene and metagenome sequence analyses	Enrichment of a subspecies of the major gut species *F. prausnitzii* associated with AD	Song et al. (2016)

9.7 Constraints of Probiotic Delivery

In spite of having numerous health benefits of probiotics, there are certain inherent limitations in their applications which are attributed to their unstable nature, certain safety concerns, and lack of regulatory norms (Verna and Lucak 2010). Probiotics are highly sensitive to the environmental factors like temperature, humidity, oxygen, etc. Also, their viability may vary with type of strains, molecular oxygen, pH change, additives, drying, and freeze-thaw operations and may need special attention during processing and packaging and till shelf life (Cheng et al. 2019).

Probiotics, being sensitive to gastric pH, face a primary hurdle of getting degraded by the harsh gastric environment before it reaches the intestine to elicit the therapeutic effect. Hence, formulations were developed using gastro-resistant, enteric-coated polymers wherein the compatibility poses a significant issue. Ideally, viable microbes should survive, adhere, and then colonize for producing therapeutic activities. Yet, several digestive enzymes like proteases, lipases, and amylases can affect their viability. Along with acidic pH and digestive enzymes, probiotics also have to survive with lysozymes, bile acids, and other microorganisms in the body which challenge the number of viable cells reaching to the small intestine (Sotoudegan et al. 2019).

Lyophilized probiotic formulations are helpful in improving the stability, but adequate care must be taken during the processing of lyophilized products so that bacterial viability remains unaltered. Although cryoprotectants can be used to avoid the problem arising from harsh conditions of freeze-drying, the type of cryoprotectants decides the viability, storage conditions, and shelf life of the product. Traditionally lactose has been widely used as a cryoprotectant; however, the effect of lactose is short-lived with bacterial viability which decreases after few months of its manufacture. Similar excipients such as ascorbic acid have been more effective to protect viability of bacteria; thus the combination of lactose and ascorbic acid can be used as amalgamation (Govender et al. 2014).

The safety of probiotics has to be evaluated systematically with respect to their pathogenicity, virulence, metabolic actions, toxicity profile, etc. The risk associated with probiotic therapy is related to the safety concerns in immune-compromised individuals (pregnant women, elderly) and critically ill, hospitalized patients (Ayichew et al. 2017). Probiotics have a potential to interact with commensal bacteria and exert positive or negative impact. Thus, understanding the mechanism of probiotics is a major challenge. Moreover, cocktail formulations of probiotics are available commercially wherein elucidation of health benefit associated with each microbial strain is not very clear and its intake level is also a limiting factor.

Probiotics are classified under various categories in different countries such as dietary supplements, functional food, nutraceuticals, live biotherapeutic agent, and food supplement and in some countries as drugs. Therefore, common categorization of probiotics is an essential requirement globally. Being a supplementary product, there are no strict regulatory norms for probiotics unlike therapeutic drugs, and the regulatory requirements of probiotics vary with country. Thus, a well-defined regulatory framework is very much essential to govern this emerging sector and achieve better regulatory fit for their safety, efficacy, and labeling issues (Kothari et al. 2019).

9.8 Future Trends and Conclusion

The practice of supplements for intestinal flora and usage of probiotics for AD can be traced back from the early twentieth century. Research in the field of probiotic supplements has greatly enhanced in the last decade with over 5000 publications, stating the applications and their subsequent delivery systems to facilitate health-promoting effects.

Probiotics are not only actively recommended for health-promoting benefits by normalizing microbial flora, but they are also widely used to cure several clinical manifestations including AD. There exist numerous clinical trials and its corresponding data confirming the capability of probiotic bacteria to modify allergic inflammation and establish a balance in the gut microbial flora. In addition to this, there is emerging evidence that probiotics are major pioneers to modulate and upgrade immunological system.

To address the major constraints of probiotics, viz., stability and their viability, several advances are required with respect to formulation and technology development. Technological improvement should result into stable probiotic product which can deliver adequate amount of microbes at targeted site. Apart from formulation aspect, sophisticated analytical techniques are required to be developed which can analyze the viable strains of probiotics clinically to establish their safety and efficiency. Also, the packaging material can be studied and improvised which gives sufficient protection to microbes for lasting viability and activity throughout shelf life.

In the future, a detailed and systematic elucidation of mechanism of actions of probiotics not only helps in more specific delivery of probiotics but also facilitates to

explore new routes of administration. The molecular-level mechanisms of probiotics have recently been studied which may lead to genetically modified probiotic products in the future.

Also, synbiotics is an upcoming approach in this area in which combination of prebiotic and probiotic has been recommended. Prebiotics are categorized into different dietary fibers which feed gut microbiota and help to nourish their growth. The combination of prebiotic and probiotic works synergistically to improve the gut microflora. Although few products of synbiotics are commercialized, there are no evidences available for clinical studies and needs detailed investigation. Thus, synbiotics can be a very good option for treatment of AD.

The entire focus of probiotic delivery is held on administering probiotics in the form of most efficient formulation wherein the bacteria remain viable during its course in the body and capable of exerting therapeutic actions without getting degraded.

Therefore, reported studies and their outcomes are very encouraging for use of probiotics in AD. Probiotics are helpful in improving skin condition and reducing the SCORAD index of AD. Although clinical trials for probiotics are conducted in AD population, extension of clinical study is still insufficient to demonstrate their therapeutic efficacy and safety profile comprehensively. Thus, after getting substantial clinical evidences, probiotic therapy may be used alone or in combination with first-line therapies for AD in the future.

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Safety Concerns, Regulatory Guidelines, Current Market Trends, and Future Directions toward the Use of Probiotics in Gut-Brain-Skin Axis

10

Swati Misra and Shailendra Raghuwanshi

Abstract

The concept of "gut-brain skin axis" is a newly emerging and important avenue of investigation for researchers to treat emotional state (stress, anxiety) and various skin diseases influenced by alteration in normal intestinal microflora. The role of probiotics and correlation between the gut, emotional state, and skin microflora was explored. Before using probiotics as a drug, the parametric assessment in terms of risk versus benefits must carefully be made. Depending on the intended use of a probiotic (drug vs. dietary supplement or food), regulatory requirements differ. However, the position of the regulatory system for probiotics is quite vague and unclear even within the existing categories in the international market which leads to legal uncertainty and confusion. The current market trend like R&D in innovative product development, product launches, brand mergers, or acquisitions for probiotics related to gut-skin and gut-brain axis is studied. It requires more in-depth research for existing and exploring newer potential probiotic strains for varied applications and provides a future direction for probiotic research for routine healthcare practice. This article focuses on safety assessment parameters of probiotic strain, and specific requirements for manufacturing, labeling, and safe delivery, proposed regulatory guidelines by the FDA (for marketing probiotics in a global context) and FSSAI for manufacturing and selling probiotics in an Indian market, approved list of probiotic cultures and licensing of brands, current market trends, and future directions are mentioned.

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Keywords

Gut microbiome · Disease · Probiotic supplements · Regulatory guidelines · Market trends · Future developments

10.1 Introduction

In the past decade, the importance of gut microbiota in terms of human health has been a topic of scientific study for researchers and clinicians. Reports suggest that the interaction between host and microbiota is mainly influenced by the host immune system through protection against exogenous pathogens and priming immunoprotective responses. However, any alteration in the intestinal microbiota could lead to autoimmune and inflammatory diseases in the gut-brain-skin axis (Kosiewicz et al. 2011) (Fig. 10.1). In the early 1930s, the two eminent dermatologists, John H. Stokes and Donald M. Pillsbury, proposed that there is an interrelationship between emotional states, the intestinal flora, and systemic and skin inflammation (Stokes and Pillsbury 1930) which was later unified as gut-brain-skin axis models (Arck et al. 2010).

10.1.1 Strategies Used to Validate the Relationship Between Gut Microbiota and Diseases

The validation of this concept of a causative relationship between gut microbiota and diseases is clarified through certain strategies. The strategies could be either the use of oral administration of antibiotics or prebiotics or probiotics or a combination of prebiotics and probiotics or fecal transplantation (Mikó et al. 2016). The use of antibiotics is mainly involved in the management of cutaneous inflammation. However, their use should be limited due to the development of resistance. On the other hand, the strategy of fecal transplantation has been practiced in China for treating

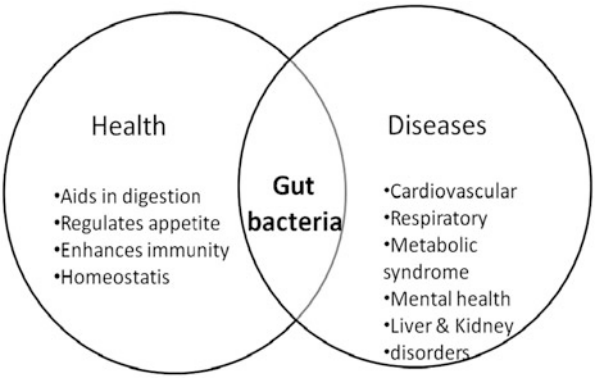

Fig. 10.1 The Venn diagram clearly indicates the positive influence of gut bacteria on a wide range of health conditions and in the prevention of diseases

abdominal diseases from the medieval period, but globally its medical acceptance and importance have been realized in recent years as an effective strategy in *Clostridium difficile* infection. This promising strategy aids in the treatment of gastrointestinal disorders associated with the disturbed gut flora which thereby leads to skin disorders (Salem et al. 2018). Being a prominent strategy still, there are very few chances of it entering the cosmetology or dermatology clinical practice at a faster pace. To modulate the gut microbiome with potential benefits on the skin, prebiotics have proven to be an appealing strategy. Herein, the dietary components are fermented through gut microbiota, and the high nutritional value products released supports the growth of bacteria or helps to maintain the gut microbiota. Among the prebiotics are fructooligosaccharides, galactooligosaccharides, inulin, polydextrose, lactulose, sorbitol, or xylitol (Collins and Reid 2016). Besides this, the product which has gained momentum in the past few years is the use of probiotics; it is live microbes which when consumed in adequate amount confer health benefits on the host (Food and Agriculture Organization/World Health Organization (FAO/WHO) 2001). Probiotics are categorized into probiotic drugs, probiotic food (e.g., foods, food ingredients, and dietary supplements), direct-fed microbials (probiotics for animal use), and designer probiotics (genetically modified probiotics) (Sanders 2009).

10.1.2 Role of Gut Microbiota in Metabolic Disorders

Researchers observed that in cultures (traditional groups) wherein diet rich in fiber is emphasized (Conlon and Bird 2015; Hills et al. 2019), the population had good health in terms of having diverse gut microflora in contrast to modern westernized populations colonized by far who have less diverse microbiota due to high carbohydrate diet along with less fiber. Lower diversity in microbiota is associated with metabolic disorders such as obesity, Crohn's disease, IBS, and colorectal cancer (Mosca et al. 2016). On the other hand, sometimes, a high diversity is also not an indicator of good health or healthy microbiome, as has been established in the case of the vagina, wherein high diversity could cause vaginitis (Charbonneau et al. 2016). Therefore, it is very difficult to explain the concept of dysbiosis, an abnormal microbiome since, eubiosis, a healthy or homeostatic microbiome itself occurs in a heterogeneous state (Lloyd-Price et al. 2016).

The specific strains carrying health benefits are the following genera: anaerobic microbes, *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Escherichia coli*, and aerobic *Bacillus* strains (Fijan 2014).

Among these aforesaid microbes, the studied microbes having beneficial effects on skin health after oral administration are *Bifidobacterium* and *Lactobacillus* (Roudsari et al. 2013). Researchers though noted in their outcomes that probiotic administration leads to an increased level of IL-10 that induced peripheral regulatory T (Treg) lymphocytes and the secretion of health-stimulating hypothalamic hormones which improve epithelial integrity and immune tolerance (Levkovich

et al. 2013). In this case, a detailed study on probiotic administration and its effect on the composition of gut microbiota has not been carried out. The link between the gut and skin offers targetable pathways with therapeutic potential in dermatological practice. The molecular mechanism has not yet been explored to a great extent. The scientific evidence determines that the gut epithelial innate immune response is stimulated locally on probiotic administration and improvises intestinal inflammation. The improvement of epithelial barrier function, on increased production of TNF-alpha by epithelial cells and activation of the NF-kappa B pathway as has been observed on probiotic administration (Pagnini et al. 2010).

Reports suggest that the difference between two probiotic bacteria could be larger and similarly it could be between the two strains; the function of one probiotic strain could not be extrapolated for another strain of the same species. It is referred to as strain specificity. It has been explained by Douillard et al. (2013) wherein it was observed that the use of 100 strains of *Lactobacillus rhamnosus*, obtained from human and dairy sources, showed different characteristics such as bile acid resistance, carbohydrate transport and metabolism, and production of mucus-binding pili. This study helps to understand that taxonomic profiling is an inadequate measure of functional capacity and that probiotic strains should be selected for products based on evidence of their phenotype rather than simply relying on the popularity of their species name.

Metabolomics, an emerging technology, is a comprehensive analytical profiling technique used for measuring and comparing large numbers of metabolites present in biological samples. To understand the relationship dynamics of gut microbiota and metabolic disorders, patients suffering from metabolic diseases were checked for fecal and serum metabolomics. These analyses could help to understand the probiotic action and accordingly design appropriate probiotic application. It is evident from the scientific research reports and ongoing studies that to understand probiotics, it is necessary to better characterize their gut microbiota composition and metabolome. Better designing of clinical investigations is essential, as studies conducted by large have shown that probiotic administration is heterogeneous (Yelin et al. 2019). Therefore, comprehensive studies are needed to assess clinical applicability, since there is a variation in the probiotic strains used, in the formulation of the probiotics, and the timing and duration of the probiotic intervention between the studies.

10.1.3 Probiotic Supplementation

To date, there is no scientific evidence of any potential side effects with the application of oral probiotics (Mahasneh and Mahasneh 2017; Hadj-Hamou et al. 2020; Olaimat et al. 2020). Probiotic supplementation is a cheap and simple method in the treatment of skin and brain diseases. The main problem with probiotic supplementation is that bacterial colonization of the gut is transient and could sustain for less than 2 weeks after cessation of intake (Firmesse et al. 2008; Frese et al. 2012). The successful colonization depends on the adaptation of probiotics to the gut

ecosystem which is determined by intestinal phylogenetic limiting and resource availability in the individual (Maldonado Gómez et al. 2016). A study was conducted by Maldonado and coworkers demonstrating that a strain of *Bifidobacterium longum* was able to persist for more than 6 months in those individuals wherein it was originally absent. Due to persistence of *B. longum*, the fecal microbiome was enriched with functional genes associated with *B. longum* (Maldonado Gómez et al. 2016).

10.1.4 Interaction Between Gut Microbiota and Skin Microflora

To maintain the homeostasis between host and organism, the different microbial communities (gut, skin, oral, vaginal, brain) should not be considered as separate but as a complex, interacting ecosystem of commensals residing in distant body parts. It is also a fascinating field of microbial research that is yet to be explored whether the interaction between gut microbiota and skin microflora is unidirectional or bidirectional. A large diversification and variation in skin microflora allow only a certain set of microbes to colonize in certain skin regions. In general, the healthy skin microbiome is composed of *Staphylococcus* spp. (*S. epidermis*), *Propionibacterium* spp. (*P. acnes*, *P. granulosum*, and *P. avidum*) and *Corynebacterium* spp. (*C. simulans*, *C. tuberculostearium*, *C. tenuis*, *C. jeikeium*, and *C. xerosis*) (Byrd et al. 2018), while *Staphylococcus aureus*, a skin pathogen, is responsible for several skin infections, including impetigo, furuncles, subcutaneous abscesses, skin ulcers, and several systemic infections, when they get into the bloodstream (Olaniyi et al. 2016). The variation in the cutaneous microflora is noted which could be due to individual-specific factors, such as sex, age, occupation, clothes, and use of hygienic products (Grice and Segre 2011; Szabó et al. 2017). Very few studies are conducted on how probiotic supplementation could influence inflammatory skin disorders. The results thus obtained are promising, but due to the heterogeneity of the applied supplemental regimen in different studies, it is difficult to implement in clinical practice. The existing research has shown that inflammatory skin diseases are linked to the imbalanced gut microbiome. Therefore, the modulation of the gut microbiota to improve skin condition seems to be a feasible approach. In this respect, oral probiotics could be a simple, safe, and cheap modality in the therapeutic management of skin inflammation.

Therefore, due to these individual-specific factors, a specific microenvironment is created in the skin, and it requires personalized solutions in order to manage any skin conditions with oral probiotics. The microbial communities could be manipulated through the usage of topical probiotics, oral and topical probiotics, or the combination of prebiotics and probiotics, called synbiotics. Topical has proven to have special significance in the improvement of skin defense in cosmetology. Topical applications could sometimes create a challenge in the formulation, or environmental conditions of the skin prevent colonization by probiotic (Tester and Al-Ghazzewi 2012). On the other hand, the use of the most suitable oral probiotic strain in combination with topical probiotics and/or prebiotics might help in the

individualized design of treatment of skin disorders. To explore the potential of oral probiotics, clinical trials need to be conducted and determine the optimal formulation of the most effective probiotic strain or the combination of certain strains, the duration of the supplementation or treatment, and also the inclusion criteria of the subjects in the study. Oral probiotic supplementation is relatively cheap, and if proven to be effective, it could serve as a useful, supportive therapy for the management of microbiome-associated cutaneous disorders.

10.1.5 Relationship Between Gut Microbiota and Brain Disorders

In recent times, the interrelationship of gut microbiota and neuropsychiatric conditions has evolved rapidly and is termed as the gut-brain axis. It is a bidirectional communication that exists between the gastrointestinal tract and central nervous system utilizing neurological, immunological, and hormonal signaling pathways (van Hemert et al. 2014). During preclinical trials, it has been noted that probiotic supplementation could lead to amelioration of anxiety and depression in mice (Sun et al. 2018). Several other studies on probiotic consumption in rodents were carried out, and data reveal that consumption of probiotics could prevent an increase in the level of stress hormones including ACTH, corticosterone, and epinephrine via the hypothalamic-pituitary-adrenal axis (Wallace and Milev 2017). Though, at present, very few literature reports are available to study the effect of probiotic supplementation on neuropsychiatric conditions in terms of human clinical trials. Akkasheh and coworkers noted a sharp reduction in the depressive symptoms when supplemented with *Lactobacillus acidophilus*, *L. casei*, and *Bifidobacterium bifidum* for a period of 8 weeks (Akkasheh et al. 2016). In similar lines, the term "psychobiotics" was coined by Romijn and Rucklidge for probiotics producing health benefits on patients suffering from a psychiatric illness (Romijn and Rucklidge 2015). It could be concluded that a substantial amount of research work is still required to provide healthcare providers with the confidence to embrace probiotics into regular practice.

10.1.6 Role of Probiotics During COVID-19

Several clinical evidences have shown that lung diseases influence the gut microbiome. The alteration of gut microbiota leads to dysbiosis, thereby causing the translocation of endotoxins and microbial metabolites from the gut, which in turn affects the lung through hematological communication. There were some hurdles during COVID-19 which cause major impact such as lack of preparedness for such unprecedented transmission, intrinsic virulence of the pathogen and its contagiousness, asymptomatic spreaders, unavailability of a vaccine, and lack of effective antiviral agents.

Prof. N K Ganguly, President, Gut Microbiota and Probiotic Science Foundation (India), and Former Director General, Indian Council of Medical Research (ICMR), and Dr. Neerja Hajela, Secretary, Gut Microbiota and Probiotic Science Foundation

(India), shared the insights that there is a strong relationship between probiotics and immunity during COVID-19 pandemic. Though the dreaded COVID-19 disease continues to threaten us, but still it has not affected everyone, and some of them are symptomatic, while others recover quickly. The variation in terms of response toward virus lies in our natural immunity as noted by researchers at the Peter Doherty Institute in Australia, and this study was published in *Nature Medicine*. A strong immune system can fight the virus and divert the course of the disease.

However, realizing the severity of the disease and the absence of proven effective therapy for COVID-19, there was a need to search for alternative strategies. Recently, scientific supporting documents have proven that the effective way of building strong immunity lies in improving intestinal health, since 70% of the body's immunity resides in the intestine which in turn depends on the gut microflora. Therefore, the natural immunity could be restored using probiotics to fight against COVID-19. Some strains of *Lactobacillus gasseri*, *B. longum*, and *Lactobacillus casei* strain Shirota have shown to boost immunity and reduce the incidence of upper respiratory tract infections or common cold and flu (https://www.business-standard.com/content/press-releases-ani/building-immunity-with-probiotics-in-the-times-of-covid-19-new-insights-from-gut-micro). Probiotics exert certain beneficial immune-modulatory effects and helps in microbial restoration of the gut and respiratory tract which might be relevant to prevent lung injury, acute respiratory distress syndrome (ARDS), and multiple organ failures which are complication endpoints of COVID-19 (Kohli 2021).

This chapter addresses the current knowledge of probiotic research, provides a brief overview on safety concerns and regulatory guidelines for the use of probiotics and market trends of oral probiotics in existing applications in different sectors, and discusses the potential future directions of probiotic investigations and subsequent translation to the global industry and market.

10.2 Safety Concerns

Probiotics are similar to beneficial microorganisms found in the human gut and, when consumed, have the potential to confer health benefits to consumers by maintaining or improving their intestinal microbial flora. Probiotics are available to consumers mainly in the form of dietary supplements and foods.

10.2.1 Role of the Food and Drug Administration (FDA) in the Marketing of Probiotics

The safety of dietary supplements is carried out post-marketing. Since, unlike drugs, these do not require FDA approval or any evidence that substantiates the safety, benefits of their product before marketing to the FDA and only notification to the authority will suffice. It is the responsibility of the manufacturers to authenticate the product claims with adequate research evidence to avoid any falsification. In case

the dietary supplement contains any new ingredient not included in the list before October 15, 1994, then it has to be notified to the FDA before marketing and to demonstrate that the product is safe to use as a supplement. In 2007, the FDA introduced the rule of current Good Manufacturing Practices to ensure that the identity, purity, quality, strength, and composition of dietary supplements are strictly met by all those who are involved in manufacturing, packaging, or holding dietary supplements [Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements. 21 C.F.R. 111 (200 (Bowe and Logan 2011). If any report received having serious implications/ adverse effects on health associated with the use of the product, it is the responsibility of the manufacturers and distributors of dietary supplements to inform FDA through records as per the Dietary Supplement and Nonprescription Drug Consumer Protection Act in 2006.

The FDA encourages voluntary reporting of adverse events by healthcare professionals, consumers, or patients on MedWatch Form 3500 (The Dietary Supplement and Nonprescription Drug Consumer Protection Act. Pub. L. No. 109-62, Stat. 3469 (Dec 22, 2006]. The FDA gives a nod to dietary supplement manufacturers to make structure/function or health claims for their products in addition to nutrient content claims. The health claim must be authenticated by field experts that the claim is truthful and not misleading. The data does not need to be disclosed to the general public and no need to take the approval from the FDA before marketing. It is only the evidence that could substantiate that the claim is less than the health claim. When a structure/function claim is made, manufacturers need to put a disclaimer that FDA has not evaluated the claim and the product does not intend to "diagnose, treat, cure, or prevent any disease" (Regist. 2000; Heimbach 2008). This claim can only be made if it fits as a health claim, reducing the risk of disease or health-related condition, and FDA reviews through supporting scientific evidence and to define the relationship between probiotic and disease. The data supporting a health claim must be published and therefore apply to any product meeting the criteria for the claim (Saldanha 2008).

10.2.2 Guidelines to Use Probiotics as Drugs

An attempt was made in 2001, wherein probiotics were attempted to make a health claim, and the FAO and WHO expert consultants developed guidelines for evaluating probiotics in food having health benefits. The proposed recommended guidelines are (1) genus and species identification of probiotic strain through a combination of phenotypic and genotypic tests as clinical evidence have suggested that the health benefits may be strain-specific, (2) in vitro tests to know the mechanism of probiotic effect, and (3) substantial clinical health benefits of probiotics with human trials and safety assessment of probiotic strain.

The safety assessment parameters of probiotic strain are (a) determination of antimicrobial drug resistance; (b) metabolic activities; (c) adverse side effects, if any, observed in humans during or after clinical trials; (d) any type of toxin production or

hemolytic potential if the strain possesses any sort of such properties; and (e) lack of infectivity in animal studies.

10.2.3 Complexity and Requirements for Probiotic Formulation

In pharmacology, probiotic formulations are more complex compared to inert drugs and requires detailed pharmacokinetic studies. Maintenance of these products requires special requirements for manufacturing, labeling, and safe delivery. In the case of manufacturing of probiotics, the quality assurance program such as Good Manufacturing Practices (GMP), Codex General Principles of Food Hygiene, and Guidelines for Application of Hazard Analysis Critical Control Point (HACCP) should be followed, while the following information should also be mentioned on the label beside the labeling requirements under food laws: (a) genus, species, and strain, (b) minimum viable numbers of probiotics at the level at which efficacy is claimed and at the end of shelf life, (c) health claim(s), (d) serving size for efficacy, and (e) storage conditions (Saldanha 2008).

The manufacturers are responsible for guiding consumers or presenting available scientific evidence to clinicians about the type and extent of safety assessments conducted or the therapeutic potential of the probiotic products until more stringent regulations are in place. However, very few manufacturers have conducted small, randomized controlled studies in humans in order to prove the safety and efficacy of their products. The labeled health claim on probiotic food items is allowed with the sole responsibility of the manufacturers and with sufficient scientific evidence available to clinicians to authenticate it to be considered as a health claim. However, constant reports on novel probiotic strains associated with novel health benefits should be authenticated with scientific evidence and clinical studies on humans. Any improper use of the term probiotic should be avoided since the lack of awareness and credibility of health claims associated with the probiotic products are posing a major threat to consumers and the probiotic industry. Taking this into consideration, the safety issues with probiotics, the use, selection, and design of probiotics remain an important challenge for the scientific community. Therefore, careful risk assessment for patients and proper handling of the probiotic during administration need to be conducted before using probiotics as drugs.

10.2.4 Cross-Contamination During Probiotic Administration

At present much of the safety data on the use of *microbe* as a probiotic "drug" are derived from case reports. In several reports, it has been noted and reported that *Saccharomyces fungemia* is the most severe complication secondary to administration of the probiotics *S. cerevisiae* and *S. boulardii*. The factors which could lead to fungemia during probiotic administration are immunocompromised state during critical illness, live yeast spore contamination of healthcare workers via hands during blend preparation (formulation) or during filling in capsules/sachets, and

contamination through hands by healthcare workers to catheter sites. The data procured through several case studies showed that the organism retains for 30 min in the air after opening the packet and for 2 h on table surface (Hennequin et al. 2000). Therefore, *S. boulardii* administration should be avoided by immunocompromised individuals or have a central venous catheters (Cohen et al. 2010).

In terms of safety guidelines, the institutional guidelines must be followed: (a) all healthcare providers should wear gloves during probiotic administration and then discard gloves and wash hands with soap and water and (b) probiotic capsule/sachet should not be open in front of patients suffering from central venous catheters since aerosolized spores could cross-contaminate sterile sites and environmental contamination too. It has to be taken into account that any probiotic administration as a dietary supplement in the form of a drug to immunocompromised patients should be evaluated for its safety in these individuals.

10.3 Global Regulations for Probiotic Products

In the global scenario, probiotics became a commercial commodity due to widespread healthcare settings and are growing at a fast pace. Though, the large number of probiotic products has been introduced in the international market under different categories in different countries such as natural health products in Canada; food supplements in Sweden, Denmark, and Finland; dietary supplements, drugs, medical food, and live biotherapeutic agent in the USA; functional foods in Japan; and biotherapeutic/pharmaceuticals in European countries like Belgium and Germany. Due to ongoing developments in probiotics in different countries, the views of the regulatory authorities are too changing, so there is a need to characterize it with a certain degree of harmonization. At present, within existing categories for probiotics, the regulatory system becomes vague and quite unclear. The need of an hour is to have internationally accepted consistent terminology, appropriate use of different terms related to probiotics on an international basis, clear demarcation of food-based probiotics with nutritive claim and drug-based probiotic products with health claims to achieve adequate regulatory control for discussion of probiotic-related issues, and development of methods and techniques (in vitro and in vivo) to evaluate the efficacy and safety on a common basis and to remove legal uncertainty and confusion in the path for the development of a mature market.

A common regulatory framework is required which will allow the free exchange of products with harmonized guidelines for safe and efficacious use of probiotics and to minimize the confusion of different regulations.

10.3.1 Regulatory Guidelines

In this direction, the International Scientific Association for Probiotics and Prebiotics (ISAPP) (2013) organized a panel meeting to define clearer guidelines and standards for using probiotics and for the determination of what products can be included in the

scope of probiotics (Hill et al. 2014). The new definition of probiotics as mentioned aforesaid in this chapter was postulated in 2001. It is important to know that this definition could differentiate between commensal microbes and probiotics. Generally, probiotics are derived from gut commensals, and until these strains have shown proper characterization and health effects on the host, they cannot be called probiotics (Hill et al. 2014). A need of an hour is to adopt the criteria defining probiotics so that the misuse of the term, probiotics, in the absence of proven health effects and other misleading information could be stopped from dissemination among the consumers and researchers.

The importance of probiotic strains in terms of health benefits has been studied and reported in the literature with time immemorial. However, the European Food Safety Authority (EFSA) has listed three main regulatory issues to be addressed when making a health claim. Due to these regulatory issues, many health claims reported have now been dismissed.

These regulatory issues include the requirement for the demonstrated evidence of:

- *Characterization of the probiotic product*.
- *Substantiation of the health benefit* (i.e., demonstration that the biomarker in question contributes to the claimed health benefit).
- *Extrapolation to the general, healthy population*.

According to the EFSA, the health claims must be specific, and the claim such as the strengthening of the immune system is considered to be vague and could not be considered (Binnendijk and Rijkers 2013). To date, researchers could not establish evidence that probiotics have an impact on approved biomarkers correlated with clinical endpoints (Rijkers et al. 2011). As per the regulatory authority, the claimed health benefits must be demonstrated in at least two randomized, placebo-controlled clinical trials, with a demonstrated cause and effect relationship. The lack in the demonstration of health claims of probiotics could be due to poor study design, a lack of investment in research and development, and a lack of fundamental knowledge about the biological mechanisms of action. Therefore, due to these aforesaid factors, a sufficient evidence base could not be created to obtain the regulatory approval for the claimed health benefits of probiotics (van den Nieuwboer et al. 2016).

10.3.2 Specific Guidelines from the Food Safety and Standards Authority of India (FSSAI) for Probiotic Products to be Sold in India

Due to exceptional health benefits along with health awareness amongst consumers over a while has led to the mushrooming of many nutraceutical as well as health supplement industries in order to launch their brands in India.

But at the same time, in order to keep track of rightful companies, the Food Safety and Standards (health supplements, nutraceuticals, food for special dietary use, food

Table 10.1 List of strains approved by the FSSAI as probiotics (live microorganisms) to be sold in India (as specified in Schedule VII)

1. *Lactobacillus acidophilus*	10. *Bacillus coagulans*	19. *Bifidobacterium lactis*	28. *Lactobacillus gasseri*
2. *Lactobacillus plantarum*	11. *Lactobacillus fermentum*	20. *Bifidobacterium breve*	29. *Bacillus clausii*
3. *Lactobacillus reuteri*	12. *Lactobacillus caucasicus*	21. *B. longum*	30. Established Probiotic strains of *Bacillus subtilis*
4. *Lactobacillus rhamnosus*	13. *Lactobacillus helveticus*	22. *Bifidobacterium animalis*	
5. *Lactobacillus salivarius*	14. *Lactobacillus lactis*	23. *Bifidobacterium infantis*	
6. *Lactobacillus casei*	15. *Lactobacillus amylovorus*	24. *Streptococcus thermophilus*	
7. *Lactobacillus brevis*	16. *Lactobacillus gallinarum*	25. *Saccharomyces boulardii*	
8. *Lactobacillus johnsonii*	17. *Lactobacillus delbrueckii*	26. *Saccharomyces cerevisiae*	
9. *Lactobacillus Delbrueckii* (subsp. *bulgaricus*)	18. *Bifidobacterium bifidum*	27. *Lactobacillus paracasei*	

for special medical purpose, functional food, and novel food) Regulations 2016 has been notified by the FSSAI on December 23, 2016. As per the notification, the following guidelines/regulations for food business operators (FBOs) were laid down to ensure compliances of their existing and new probiotic products with all the provisions of these regulations to be implemented by January 1, 2018, in order to sold their products in the Indian market (https://www.foodsafetymantra.com/2018/11/x-pro-and-prebiotics-products-that-can-be-sold-in-india; FSSAI Gazette Notification related to the standards of Food or Health Supplements, Nutraceuticals, Foods for Special Dietary Uses, Foods for Special Medical Purpose, Functional Foods and Novel Food. (Uploaded on: 06.01.2017)—fssai.gov.in/dam/jcr:c0b36c0c-ccc9-446d-ada6-5b164de27460/Gazette_Notification_Functional_Foods_06_01_2017.pdf):

- The microorganisms should be declared on the label with full information and have to be non-GMO.
- Only approved strains of microorganisms as specified in *Schedule VII* can be added to probiotic product (list as mentioned in Table 10.1).

- The viable number of microorganisms in food with added probiotic ingredients shall be $\geq$10 CFU in the recommended serving size per day.
- The probiotic food shall not claim or refer to have any property of preventing, curing, or treating a human disease.
- The Food Authority will allow a statement related to structure or function or the general well-being of the body only if it is supported by scientific evidence.
- The packaging of the probiotic food should have the following information:
  - "PROBIOTIC FOOD" to be mentioned clearly on the label
  - Genus and species including strain designation or culture collection number, if applicable, in the list of ingredients.
  - Viable numbers at the end of the shelf life of the probiotic strain.
  - Recommended serving size, duration of use, storage conditions, and "best by" date after the container is opened.
  - "NOT FOR MEDICINAL USE" written prominently
  - Any other warning or precaution to be taken while consuming, known side effects, contraindications, and product-drug interactions, as applicable.
- Only additives specified in *Schedule VA* to *Schedule VF* can be used in probiotic preparation.

The list mentioned below can be updated by the Food Safety and Standards Authority of India to add any new strain of microorganism which possesses probiotic properties supported with scientific evidence. The FSSAI has permitted under aforesaid regulations that any probiotic preparation may contain added prebiotics.

A lot of probiotic supplements are available in the Indian market; below (Table 10.2) is the list of some of the certified probiotic brands which are safe for consumption and on which the consumers can completely rely on. In this manner, the consumers have the option to select as per their needs from the mentioned probiotic supplements.

The mechanism of action of probiotics still needs to be deciphered though there are many pathways wherein probiotics modulate host immune and metabolic processes in the gut-skin-brain axis.

10.3.3 Mechanistic Approach of Probiotics

The heterogeneous nature of gut flora due to different microbial species leads to variation in the mechanisms of action. The biological effects due to probiotics are divided into the following categories:

- *Pathogen resistance*: Probiotics reduce pathogenic invasion and colonization by occupying all the functional niches or through the alteration of the local environment by the secretion of short-chain fatty acids (SCFAs), lactic acid, bacteriocins, and reactive oxygen species which inhibit the growth of pathogenic organisms. It helps to maintain host-microbial homeostasis (Harper et al. 2018). Several *lactic acid bacteria* (LAB) probiotics produce ribosomally synthesized substances that

Table 10.2 List of some of the certified probiotic supplement brands available in India (http://bestsupplementsforlife.in/probiotic-supplements/)

Probiotic supplement	Quantity and cfu	Certifications	Other remarks
Carbamide forte (gluten-free and vegetarian supplement tablet)	100 tab, 30 billion	FSSAI	Uses a total of 16 approved strains
Boldfit probiotics	120 tab, 30 billion	FSSAI, HACCP	Includes prebiotics as well as probiotics, uses a total of 16 strains, works well for both men and women, great for detox, and cleanses your body
Neuherbs daily probiotics	60 tab, 20 billion	FSSAI, GMP, ISO	Includes vitamin C, vitamin E, and selenium
Pure nutrition Progut plus probiotics	60 tab, 25 billion	FSSAI, GMP, ISO, HACCP	Made with seven powerful strains, improves gut health, boosts immunity and energy for the whole day, increases hormone production
Inlife	60 tab, 25 billion	FSSAI, GMP	Uses five strains, affordable price for budget buyers, increases nutritional absorption in the body
Now foods (gluten-free and vegetarian supplement tablet)	50 tab, 25 billion	GMP	Uses a total of ten acid-resistant probiotic strains, improves gut health
Nature's velvet	300 g	FSSAI, GMP	Includes decent amount of dietary fibers, boosts immunity, affordable supplement
Cipla ActivKids Unobiotics junior	10 sachet, 30 billion	FSSAI	Great for kids, sugar-free, boosts number of good bacteria in gut, boosts immunity levels

are protein in nature and exhibit bactericidal activity. These substances are collectively termed bacteriocins. Some of these bacteriocins have also been demonstrated to be active against viruses.

- *Nutritional functionality*: Certain gut microbes contribute to vitamin availability and SCFA production. The vitamins involved are vitamin K, vitamin B12, pyridoxine, biotin, folate, nicotinic acid, and thiamine which can be produced by gut microorganisms (Vandenplas et al. 2015). In terms of SCFAs, butyrate produced is a major energy source in enterocytes and is involved in the maintenance of the enteric mucosa (Ríos-Covían et al. 2016).
- *Immune functionality*: Probiotics prove to have diverse effects on the immune system. Certain probiotics are classed as immunostimulatory with characterization to induce IL-12 and natural killer (NK) cell immunity (Aziz and Bonavida 2016), while other species are classed as immunoregulatory due to their ability to induce IL-10 and regulatory T-cell pathways (Azad et al. 2018).
- *Amelioration of contaminants*: Reduces the risk from ingested hazardous compounds; it has been explained through the example of *Pediococcus*

pentosaceus wherein it breaks down fumonisins, a group of mycotoxins produced from fungi, which are found on a wide variety of crops (Vandenplas et al. 2015).
- *Xenobiotics*: An evidence to show that dietary and environmental chemical pollutants can interfere with gut bacterial function (transcription, metabolism, etc.)
- *Bile acid metabolism*: Certain species of the gut microbiota—such as *Bacteroides intestinalis*—have been shown to deconjugate and dehydrate primary bile acids to convert them into secondary bile acids (Jandhyala 2015). Secondary bile acids can inhibit *Clostridium difficile* spore germination and, therefore, suppress the vegetative growth of *C. difficile* (Ridlon et al. 2006).

The global realization of the importance of probiotics in terms of health benefits led to the exploration of market analysis trend, like evaluating changes in the probiotic market (trend), how it is expected to grow, the need/requirements, and the demand of the customers to be evaluated and studied under market trends.

10.4 Market Trends

It is predicted that the probiotic market will grow at a CAGR of 6.7% from 2020 to 2027 and reach $76.7 billion by 2027 (https://www.meticulousresearch.com/download-sample-report/cp_id=5113). The growth of the market is driven by factors such as health benefits associated with probiotic-fortified foods and technological advancements in probiotic products. It is an ever-growing entity with the continual expansion of products being taken into the market.

10.4.1 Major Impacting Factors in the World Probiotic Market

The major impact on the probiotic market could be due to the growing health concerns which are going to increase in the near future and low awareness among consumers which could be created through publications, functional food fact sheets, and conferences by the International Food Information Council (IFIC). Besides this, a rise in the consumption of functional foods due to increasing concerns on preventive healthcare in conjunction with the development of efficient probiotic strains and stringent government regulations for probiotic products may also impact the probiotic market. In the growth of the probiotic market, a large amount of investment in research and development (R&D) activities, research equipment, labs, and hiring of trained professionals is required which may create a barrier (Fig. 10.2).

10.4.2 Current Market Trend of Probiotics

The occurrence of the COVID-19 crisis has greatly impacted the entire value chain of the probiotic industry due to strict measures implemented by the government such

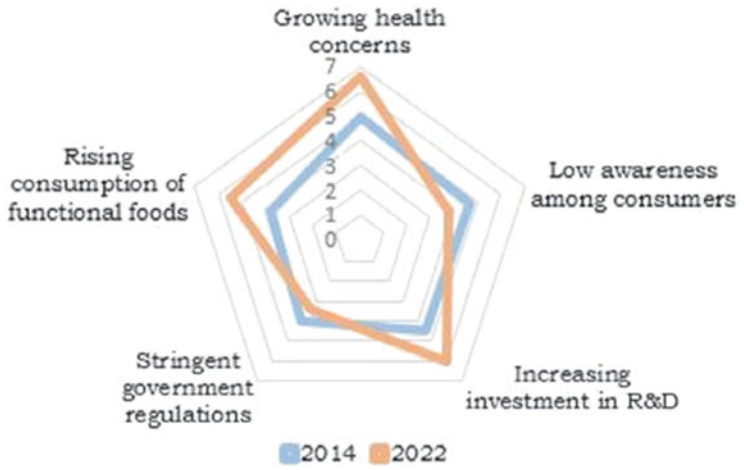

Fig. 10.2 The impacting factors in the world probiotic market (www.alliedmarketresearch.com/probiotics-market)

as complete lockdown in certain regions which mainly affected the logistics, truncated air freight capacity, port congestion, and roadblocks. Also, there were truck shortages, reduced deliveries, and employees contracting COVID-19. Therefore, the aforesaid factors restricted the demand for probiotics, and several well-known probiotic companies noted a drop in sales in the first quarter of 2020.

However, with recent scientific evidence, it is proven that the coronavirus directly affects the immunity of humans. This thereby led to the gradual increase in the usage of immunity products among the health-conscious consumers around the world. The preference for probiotic bacteria-fortified food has become popular among consumers who are seeking natural solutions over pharmaceutical products in order to treat numerous health ailments mainly gastrointestinal conditions, boosting immunity, maintaining gut health, and weight management. In recent years, there is an emerging trend of veganism that focuses on plant-based diets, and in this manner, the demand for plant-based products will increase. As a result, several key players are embarking on the launch of plant-based probiotics to increase their consumer base. In August 2020, Chobani an American food company introduced a new probiotic drink. Similarly, Danone launched new plant-based variants for its Danio and Activia yogurt brands in the Netherland market (https://www.fortunebusinessinsights.com/industry-reports/probiotics-market-100083).

The increasing evidence of health benefits linked with product consumption along with the growing popularity of probiotic strains being natural, cost-effective, and safe substitute for pharmacological solutions is expected to boost the demand for bacterial strain probiotics and will positively impact the probiotic market growth. In the second quarter, from April 2020 onwards, several large as well as small manufacturers and retailers noted a surge in the demand for immunity products from the consumers.

10.4.3 Major Breakthroughs in the Global Probiotic Market

The increase in investment by some of the prominent players on *research and development (R&D) of innovative products* will positively impact the probiotic industry growth. The Amorepacific group opened its novel green tea probiotic research center in February 2020 to study *Lactobacillus* found in Jeju organic green tea garden. Chr. Hansen Inc. launched the science-based online probiotic platform in the US market. It is intended to educate consumers and healthcare professionals about the benefits of probiotics. The available *scientific evidence* for the use of probiotics in specific applications, thereby correlating with health benefits, will become a breakthrough in the market and is anticipated to improve the quality of the probiotic products.

The *product launch* is the most widely adopted strategy by leading companies' in order to expand their product portfolio and to enhance their market share. In this direction, Danone North America announced the launch of its new kid's yogurt brand, Danimals. This newly launched low-fat yogurt contains vitamin C and vitamin D along with probiotic products that claim to be beneficial for kid's immunity. Both Danone and Nestle S.A. announced the launch of their probiotic-infused refrigerated yogurt bars.

In one of the articles, Nikki Hancocks mentioned that due to the stress of the COVID-19 pandemic, mental well-being has been in the spotlight elevating it to the same level of importance as other aspects of physical health. It can be predicted that the key consumer trend for 2021 would be an increased global interest in products offering cognitive health benefits. As per the market analyst's report, 42% of the global consumers would like to improve mental well-being, 58% are interested in products that could alleviate stress levels, and 63% of the global consumers will opt for products which are offering natural energy boost. This gave rise to the health phenomenon which is the gut-brain axis, thereby opening newer avenues for innovation in the coming years. One of the leading players in the nutraceuticals sector, FrieslandCampina Ingredients has developed two new consumer product applications as part of its Biotis Brain Health Benefits Solutions range—the D-Stress sachet and Relax health shot. A lot of new and innovative products along with solutions are developing and yet to come in this sector (https://www.nutraingredients.com/Article/2020/12/14/Microbiome-modulating-innovations-tap-into-mental-health-concerns).

It is postulated that the reason for skin diseases is due to emotional stress which alters the intestinal microflora and releases endotoxins increasing the intestinal permeability, thus leading to systemic inflammation and aggravating the skin conditions (Bindurani 2019). Studies have shown that using probiotics in both pill and topical forms may help in the prevention and treatment of skin conditions including eczema, acne, dry skin, and UV-induced skin damage. The most common probiotics claimed on the label are *Lactobacillus* spp. and *Bifidobacterium* spp. (Cinque et al. 2017) which when administered orally aids in managing skin inflammation. *Streptococcus salivarium* spp. *thermophilus* S244, an ingredient in cosmetic formulations, has been reported to produce enzymes that reduce skin dryness, loss of

tone, and water, hence slowing the process of skin aging (Cinque et al. 2017). Dr. Jart+ Vital Hydra Solution Biome Night therapy mask, an exogenous probiotic formulation containing *Streptococcus thermophilus* ferment, produces a skin protection enzyme, sphingomyelinase (SMase), which improves the skin health, moistures, and is highly efficient in the treatment of dermatitis and other skin ailments (Lew and Liong 2013). The well-known probiotic supplement available is Culturelle Daily Probiotic (10 billion cfu/g), and it prevents eczema through ceramide production. In this upcoming field, further investigations and deep research are required to improve the understanding of the complex mechanisms underlying the gut-skin axis and potentially expand the therapeutic manipulation to include the commensal gut fungi and viruses too in order to fully harness the gut microbiome's influence in the treatment of skin disease and to investigate the therapeutic potential of long-term modulation of the gut microbiome (Salem et al. 2018).

At present, several leading companies are trying to diversify their product portfolio and consumer offerings through *mergers and acquisitions*. In this direction, Chr. Hansen acquired HSO Healthcare, an Austrian-based company that specializes in women's health, and this will strengthen Chr. Hansen probiotic offerings (https://www.fortunebusinessinsights.com/industry-reports/probiotics-market-100083).

10.4.4 Segmentation of World Probiotic Market

The world probiotic market is segmented based on ingredient (bacteria, yeast), application (food and beverages, dietary supplements, animal feed), function (regular, preventative healthcare, therapeutic), end use (human probiotics, animal probiotics), and geography (North America, Europe, Asia-Pacific, and LAMEA) (Fig. 10.3).

Among the probiotic genus, the market trend is that *Lactobacillus* holds a major share of the market owing to their diversified applications and benefits. The *Lactobacillus* strains have the inhibitory potential to minimize the growth of microbes and pathogens, thereby extending shelf life and hygienic superiority of foods, having potential benefits in reducing enteric diseases in humans. Among yeast, *Saccharomyces boulardii* is one of the widely utilized yeasts for the production of probiotic drinks. It regulates the intestine and protects the intestinal lining from harmful pathogens. Rising awareness about the benefits associated with this product consumption is going to increase the product sales (https://www.fortunebusinessinsights.com/industry-reports/probiotics-market-100083). The emergence of *Bacillus coagulans* strains like Ganeden BC30, spore-forming bacteria, highly resistant to extreme conditions of pH, heat, cold, and pressure compared to vegetative cells, makes it a good option for everyday food fortification ranging from teas and coffees to muffins, pizza, and peanut butter. There are certain probiotic strains that require prebiotic to enhance beneficial effects, while certain strains can perform without them like Ganeden BC30 (https://www.nutritioninsight.com/news/trending-in-gut-health-branded-probiotics-build-consumer-trust-says-kerry-expert.html).

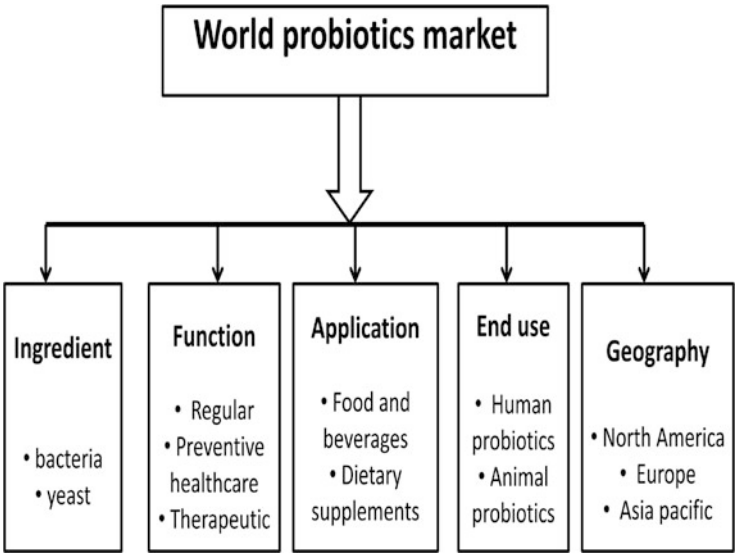

Fig. 10.3 A schematic representation of the segmentation of the world probiotic market. (Source: www.alliedmarketresearch.com)

On the basis of application, the probiotic functional food and beverage segment accounts for the largest share of the overall probiotic market; products under this category offer distinct health benefits due to specific ingredients.

While observing the sales channel, it is only the offline sales segment that could to a certain extent contribute to the largest share in the probiotic market due to heavy impact on the entire value chain, which thereby gave an edge to the 24 × 7 pharmacies and supermarkets to operate during the entire lockdown with the available stocks. The supermarkets and hypermarkets remain to be a major distributor due to various cashback or discounts offered by them. Since lockdown is raised and there is certain relaxation in rules, the growth in online sales is expected with players like Amazon, online pharmacies, and local online stores. Besides this, offices resumed which led to busy lifestyles and hectic schedules, thereby pressing the consumers to go for online purchases which in turn will positively impact the product sales through online channels.

Among the regions in the world, it is expected that Asia-Pacific commands the largest share of the global probiotic market due to a rise in disposable income, increased demand for nutritional food products, and a growing technology base which could create a niche for an increased demand for probiotics. An increasing trend across the Asia-Pacific region for non-dairy probiotic food and the presence of a number of companies in the digestive health product category offers lucrative growth opportunities for the key players operating in this market. India has surged in the highest number of new food, drinks, and supplements that bear immune system-

boosting claims, followed by Australia and Indonesia (https://www.mordorintelligence.com/industry-reports/probiotics-market).

It is expected that the liquid form of probiotics holds the largest share of the overall probiotic market with an increased usage of liquid supplements and probiotic-enriched drinks such as kefir water, probiotic juices, and yogurt-based drinks which are healthy options for daily supplementation and are convenient to use (https://www.marketsandmarkets.com/Market-Reports/probiotic-market-advanced-technologies-and-global-market-69.html). On the other hand, the major drawback is that it requires refrigeration and has a short shelf life which could restrict the demand for liquid probiotics, while the dry form segment is expected to grow owing to better handling, ease of use, high shelf life, lower expenses, and chances of avoiding expensive formulation mistakes.

The key players in the global probiotic market are Kerry Group Plc. (Ireland), DowDuPont Inc. (USA), Chr. Hansen A/S (Denmark), BioGaia AB (Sweden), Probi AB (Sweden), glac Biotech Co., Ltd. (Taiwan), Bifodan A/S (Denmark), Lallemand Inc. (Canada), UAS Laboratories (USA), and Biena Snacks (USA).

The main challenge on industry players is to get satisfactory returns on investments due to intensive competition among pharmaceutical, biotechnological, and food and beverage industries to establish their footprint in the market (https://www.alliedmarketresearch.com/probiotics-market).

10.4.5 Probiotic Research and Technological Advancements in Host-Bacteria Interactions

The web search on MEDLINE/PubMed clearly shows 19,000 results for probiotics with almost 10,000 results associated with terms "probiotics," "health," or "disease." Reports indicate a rapid increase in research papers with approximately three-fold in the previous decade and 34-fold increase since 1998 (Day et al. 2019). The focus areas for publications are related to clinical trials, reviews, and meta-analyses in the gut-brain-skin axis. Through research papers and articles, researchers claimed a large number of probiotic strains having health benefits, but on a commercial scale, very few strains are available for the clinical purposes to maintain healthy gut flora and to overcome diseases associated with a gut-brain-skin axis (van den Nieuwboer et al. 2016). Till date, several clinical studies conducted could not be translated into health or mediclaims of probiotics. This is because there is a wide interpersonal variation in commensal bacteria as well as fundamental differences between probiotic strains.

The use of multi-omics technologies will accelerate microbiome science, and advanced analytical technologies will revolutionize the microbiome study. Each technology will be able to provide insightful data and improved connectivity of these technologies and will provide an increased ability to delineate the physiological and biochemical processes of host-bacteria interactions. This will give a better understanding of complex host-bacteria interactions and will aid in translating laboratory research into clinical treatments using probiotic supplements at a fast pace to influence conditions related to both health and disease.

10.5 Futuristic Approach

It has been observed that there is a vast literature available on probiotics with a majority available on the analysis of gut microbiota which provides a picture of microbiome diversification, but still very few literature reports are available with little research conducted in terms of understanding the mechanics of the complex microbiome. The link between the gut, skin diseases, and mental health is complex and could not be deciphered through a single avenue of pathogenesis, and therefore, scientists and clinicians should be open-minded to expect the unexpected pathways. In the current scenario, we could not rely on anecdotes, inferences, and uncontrolled observations carried out by scientists and must approach this hypothetical landscape of gut-brain-skin triangle with scientific temperament and with the will to find the solution (Bowe and Logan 2011).

In the next few years, it is predicted that generation and data combination from multiple *omics* platforms will be focused. Through this study, the detailed characterization of the microbiome in terms of genetic makeup and transcription products (metagenomics/metatranscriptomics) to its proteins (metaproteomics) and metabolic products (metabolomics/metabonomics) could help in unlocking the workings of this complex ecosystem and to understand which microbial components are functionally relevant.

Though, the biggest challenge in microbiome research would be to integrate these meta-*omics* datasets to create a systems-level framework that links the data through a comprehensive mechanistic model of the microbiome (Waldor et al. 2015). Once this complex interaction of microbiome in a host is defined, then the health benefits of individual strains could be deciphered for creating a probiotic treatment for specific diagnosis and scaled up the process for clinical trials in industries to reap health benefits. At present, the research is at a very nascent stage, and a need of an hour is to better understand the mechanisms of action, in conjunction with high-quality phase III clinical studies, if probiotics are to be integrated into widespread mainstream clinical practice through the generation of confidence in product efficacy to clinicians and health authorities.

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Probiotics as Edible Vaccines

11

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Abstract

The expansion in the knowledge and applications of probiotics is on a constant rise over decades. The history of vaccine development, on the other hand, ranges back to the early nineteenth century and has been advancing ever since. Design of probiotic-based vaccine has been a boon and can be an important contributor in the field of immunization. Building up the human immunological tolerance has been the aim in order to achieve a superior level of human longevity. Orally deliverable vaccines have been preferred over the conventional parenteral formulations owing to their high patient compliance and ease of delivery. Bacteria and yeast are among the most prevalent microbiota utilized as probiotics, and owing to their significant immunomodulatory effects, they have garnered attention as promising candidates for developing edible vaccine. A legion of organisms has been regarded as safe for human consumption, thereby encouraging their adoption into probiotic-based vaccine development. A variety of bacteria- and yeast-based edible probiotic vaccines are highlighted in this chapter catering to a plethora of physiological conditions in humans. The chapter is majorly devoted to representation of the most frequently applied strategies involving bacteria and yeast in the conception of oral probiotic vaccines, both preclinically and clinically. Despite the presence of conglomerate vaccine-based strategies, a quest for newer probiotic oral vaccine platforms still persists.

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Keywords

Edible vaccines · Bacteria · Yeast · Recombinant microbes · Immunoprotection · Immunomodulation

11.1 Introduction

Despite many advances in the field of medical science for public health services, infectious diseases remain to be a formidable challenge for global health. A vaccine is a smarter and safer way of providing immunization and protection against infectious diseases for years and works on the principle "prevention is better than cure." Vaccines are biological preparations that contain microorganisms similar to disease-causing agents in the killed or weakened form or its toxins or surface proteins, which provide immunity against a particular disease. The goal of vaccination is to encourage a defensive immunity to the targeted pathogen and prevent future encounters with diseases without the risk of promoting them. They are mostly administered intramuscularly; few vaccines are given subcutaneously, intradermally, or orally. Injectable vaccine formulations and their manufacturing systems have biosafety and sterility concerns and often require a trained person for administration. They also require cold chain systems for their storage, transport, and stability (Rosales-Mendoza et al. 2016; Vetter et al. 2018; Kumar and Kumar 2019). In contrast to prophylactic vaccines that are meant to prevent infectious diseases, therapeutic vaccines are required to remove foreign cells or abnormal cells via T-cell-mediated immunity. Therapeutic vaccines are expected to induce cell-mediated cytotoxicity executed by CD81 CTLs and CD41 T-helper responses. Although injectable vaccines elicit robust systemic humoral responses, they confer weak T-cell-mediated immunity and mucosal protection. This necessitates the need for novel vaccines especially in developing countries, where vaccines are essential. It is thus imperative to develop vaccines that are stable and can be stored at room temperature, cost-efficient, transport-convenient, and self-administered. Most importantly, they must produce the required amount of antigens to confer protective immunity, and additionally, in case of oral/edible vaccines, they must escape from enzymatic digestion in the gastrointestinal tract (Levine 2006).

Vaccines that can be ingested, known as edible vaccines, are emerging as one of the most interesting approaches for administering novel vaccines. Oral vaccines are self-administrable and relatively inexpensive, avoid the need for purification, and can be manufactured on a large-scale basis. They are relatively stable in freeze-dried formulations and can attain the required bioavailability to induce immune responses. Oral immunization offers mucosal protection and often induces both local and systemic immune responses, leading to an effective eradication of foreign organisms. Many infectious microorganisms such as viruses, bacteria, and pathogens enter the body via the mucosal surfaces of the body. The intestine, one of the immunological organs of the human body, acts as the first line of protection, with majority of the antibodies produced being secreted into the gastrointestinal

tract. Oral vaccines elicit immunity via the gut-associated lymphoid tissue, which includes Peyer's patches, lymph nodes, and lymphoid follicles in the gastrointestinal tract. The administered antigens are transported into the Peyer's patches by the M cells and presented to T cells by the antigen-presenting cells. This further activates B lymphocytes to grow, proliferate, and differentiate to plasma cells. Another important criterion while developing oral vaccines is to achieve a balance between immunogenicity and mucosal tolerance, which prevents unnecessary immune responses in the gut mucosa (Rosales-Mendoza et al. 2016; Criscuolo et al. 2019). Figure 11.1 depicts the schematic description of rationale and immune responses elicited in the case of oral vaccines.

The food-grade organisms have emerged as an attractive alternative for the development of oral/edible vaccines. Research in this direction has investigated plants, bacteria, yeasts, algae, and insects that can be developed as biofactories or oral delivery vehicles for subunit vaccines. Moreover, this approach is postulated to offer better vaccine efficacies for extended duration at relatively lower costs and ease of administration. The edible vaccines developed so far are based on attenuated disease-causing pathogens and hence pose the risk of reversal to pathogenic form. Further, majority of the edible vaccines nearing commercialization are plant-based that may not be appropriate for oral delivery and require purification for the final formulation. Few products manufactured with insects and animal cell lines necessitate high-priced culture media incurring high production costs and extended time than those for microbial system (Pouwels et al. 1998; Mishra et al. 2008; Rosales-Mendoza et al. 2016). In this context, probiotics having vaccine prime-boost potentials and adjuvant effects are excellent candidates to develop oral vaccines requiring minimum processing for production. Microorganisms have been a major source of innovative therapeutic agents in vaccination, which has led to a shift of focus toward probiotics as a potential source of novel vaccination. Probiotics are defined as live microorganisms which, when administered orally in adequate amounts, are beneficial to the host health. Until recently, very few researchers have investigated probiotics as vaccines. They have been found to stimulate immune responses when used as adjuvants to vaccination and have immunomodulating effects reducing the risk/severity of diseases (Link-Amster et al. 1994; Lei et al. 2017; Yousefi et al. 2019). The compelling evidence that the gut microbes influence immune responses led to the hypothesis of employing probiotics as edible vaccines or vaccine adjuvants. The probiotics may affect the gut microbiota by colonization resistance, competing for adhesion sites, normalizing perturbed microbiota, and changing the pathogen-associated molecular patterns presented to the gut-associated lymphoid tissue. They may further influence immune responses by short-chain fatty acid production, regulation of intestinal transit, gut barrier reinforcement, increased turnover of enterocytes, acid and vitamin synthesis, and bile salt metabolism (Strukelj et al. 2012; Maidens et al. 2013; Hill et al. 2014).

The most often used microorganisms for vaccine development are *Lactobacillus* spp., *Bifidobacterium* spp., and *Saccharomyces cerevisiae*. *Bacillus subtilis* and whole-cell yeast-based vaccine manufacture and delivery are very attractive owing to their "generally recognized as safe" (GRAS) status, easy genetic manipulation,

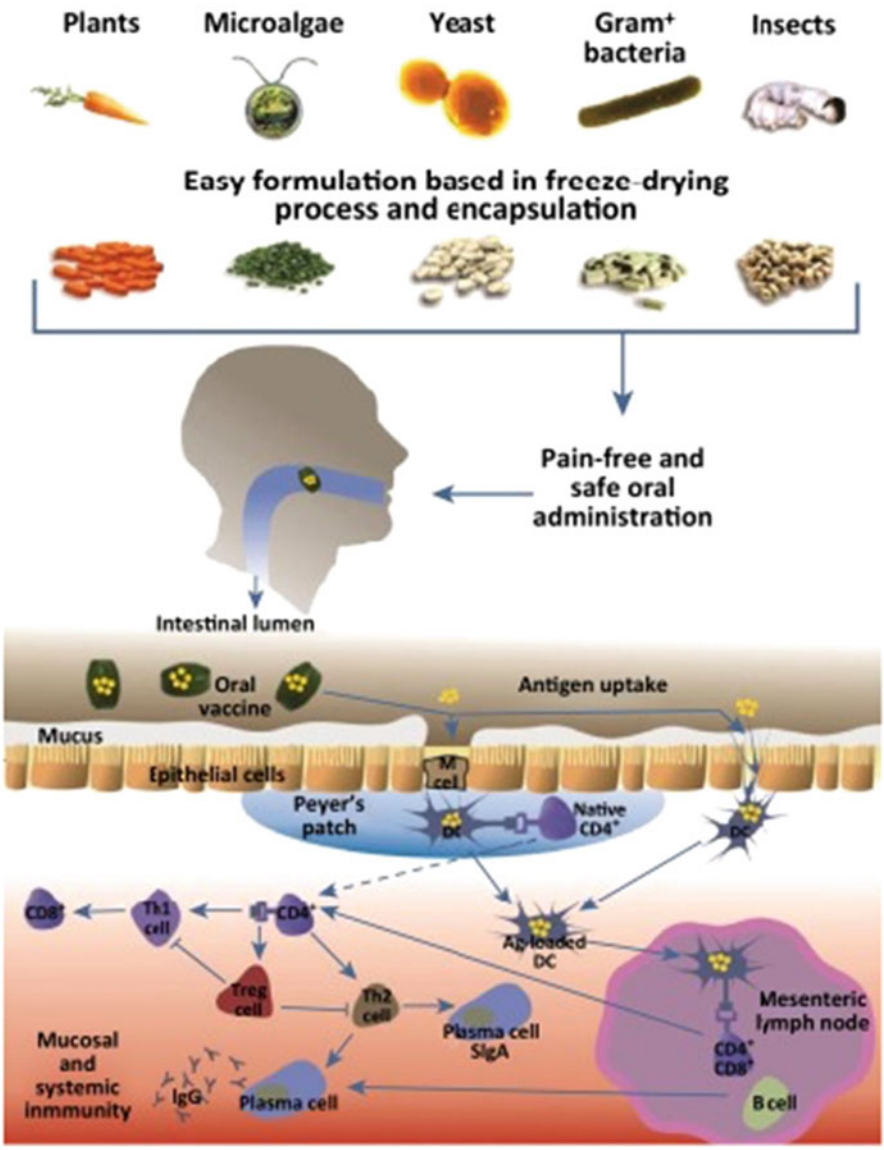

Fig. 11.1 Schematic description of rationale and immune responses elicited in the case of oral vaccines. (Reprinted with permission from Rosales-Mendoza et al. 2016)

adjuvant properties, mucoadhesivity, capability to provoke an immune response, and industrial scalability (van Baarlen et al. 2013; Bermúdez-Humarán et al. 2013; Zimmermann and Curtis 2018). The best candidate for an oral vaccine, known as *Lactobacillus acidophilus* bacterium, is present in fermented milk products. Several preclinical and clinical studies involving *Lactobacillus* spp., *Bacillus subtilis*, and

whole-cell yeast-based vaccines against various infectious diseases have been reported (Miraglia del Giudice 2014). For instance, oral administration of *Bacillus subtilis* spores causes system-specific humoral and mucosal immunity and provides protection against infectious diseases such as *Helicobacter* infection by producing high levels of specific IgA and systemic IgG. Further, *Bacillus subtilis*, being heat-stable, can survive indefinitely at temperatures as high as 70 °C and requires no cold chain for storage and transportation (Zhou et al. 2015). In some other findings, colostrum/milk components affect initial probiotic colonization, and together, they modulate neonatal antibody responses to oral attenuated human rotavirus vaccine in complex (Chattha et al. 2013). Oral immunization with yeast *Saccharomyces cerevisiae* against an infectious agent *Actinobacillus pleuropneumoniae* revealed that this delivery system could induce a protective mucosal and systemic immune response. Further, whole recombinant yeast provokes antigen delivery for humoral immune response by oral administration (Shin et al. 2005; Kim et al. 2014).

The fact that many probiotic compounds have already entered into the market proves that probiotics have their throttlehold in the area of vaccination. Several probiotic-derived vaccines have been approved for clinical use or are in clinical development phases. In the global biopharmaceutical pipeline, several companies are investing in the execution of food-grade vaccine platforms. SporeGen® is producing *Bacillus subtilis*-based vaccines. SporeVax® exploits bacterial spores as a vaccine delivery agent, for enhanced natural immune responses. GlobeImmune has implemented the production of whole-cell recombinant yeast-based vaccine Tarmogens (a yeast-based immunotherapy) to offer prevention or therapy of range of human cancers and novel therapies in the short term. Similarly, ActoGenix and Bioneer have developed lactic acid-based technology platform for biopharmaceutical production and delivery (Rosales-Mendoza et al. 2016).

The detailed discussion on the use of probiotic bacteria and yeasts for the development of oral vaccines, including the status of clinical evaluations, has been given in the following sections.

11.2 Bacteria-Based Vaccine Probiotics

Vaccines have been introduced to the human kind since the Asian influenza pandemic, way back in the year 1957, by American microbiologist Maurice Hilleman ("Viruses and Evolution | History of Vaccines"). Since then, vaccines are considered to be one of the highly regarded achievements in the healthcare industry. Preventive vaccines, in particular, have garnered immense interest of microbiologists as well as the pharmaceutical industry. With the point of view of route of administration, oral route has always been considered as the most convenient of all. In order to achieve this, numerous formulation strategies have been put into effect along with constantly evolving microbiological aspects. Probiotics, on the other hand, are live bacteria and yeasts, often referred to as "good microbes" for maintaining and restoring health. The bacteria majorly belong to the *Lactobacillus* or *Bifidobacterium* species which may or may not be a part of the normal commensal microbiota of the body (Forsythe

and Bienenstock 2010). Microbes causing either no damage or damage ineffective to show any clinical outcomes are referred to as commensal organisms. These probiotics have been found to serve as therapeutics and nutritional supplements since over a decade now. The role of probiotics, however, has been found to be versatile with its origin as gastrointestinal (GI) system developer and modulator to its ability to regulate immunomodulation outside of the GI system. The mechanism of action of the immunomodulatory effect of probiotics can be either a suppression of undesired immune response or an immunostimulatory effect related to immunoglobulin secretions (Forsythe and Bienenstock 2010). The ability of the bacteria to bestow desired immunomodulatory properties is established based upon the strain, underlying properties, and the capability to interact with native immune system of the host. An in-depth understanding of these factors is essential to develop strategies for bacteria-based edible probiotic vaccines.

11.2.1 Bacteria as Vaccines

The bacterial biogeography of the humans is a widely studied subject due to the diverse bacterial species, which exist in a healthy human at various sites. The gastrointestinal tract in particular is housed by majority of these. Human intestines are the hosts for an extensive range of microscopic organisms including bacteria, viruses, archaea, and microbial eukaryotes, which can be grouped under the umbrella of human microbiome (Ursell et al. 2012). Numerous bacteria-based vaccines composed of "killed" whole bacterial cells for typhoid, shigella, and cholera were developed for parenteral delivery but had the disadvantage of immunogenicity due to bacterial components (Hilleman 2000). A whole-cell vaccine for pertussis based on the bacteria *Bordetella pertussis* is one of the only few, still in use (Ada 1998). Oral vaccines against enteric infections including enterotoxigenic *E. coli* (ETEC) and cholera also utilize killed bacterial cells. Vaccine composed of killed whole-cell *Vibrio cholerae* serogroup O1 with a recombinant B-subunit of the cholera toxin (WC/rBS) is in markets since the 1990s for primary immunization, and similar vaccines based on the serogroups O1 and O139, devoid of the B-subunit, are available in India and Vietnam for protection against traveller's cholera. Dukoral® (SBL Vaccines) is the brand name for the vaccine against serogroup O1, whereas ShanChol® (Shantha Biotec) and Euvichol-Plus® (Eubiologics) against serogroups O1 and O139 and, recently, Vaxchora®, which is a lyophilized single-dose live oral vaccine against serogroup O1, are all approved by the WHO and FDA for cholera management ("Vaccines | Prevention and Control | Cholera | CDC,"). The attenuated variants of pathogenic bacteria have been an attractive strategy for vaccine development, the earliest approach being the oral BCG vaccine based upon *Mycobacterium bovis* (Maclennan and Mutreja 2016). The live attenuated vaccines enabled the design and development of vaccines by monitoring and controlling the virulence by an in-depth knowledge of genetics and DNA sequences. An example of this approach is deletion of mutagenic genes in *S. typhi* bacteria to make the desired vaccine, but the licensing for human use is still pending (O'Callaghan et al. 1988).

Vaccines based on the membrane complexes of bacteria are another type wherein the bacterial membrane is extracted and delivered which helps in immune recognition of the bacteria (Acevedo et al. 2014). The membrane components like proteins and antigens make the immune system capable for production of specific antibodies, thereby rendering protection. A detergent-extracted outer membrane vesicle (dOMV)-based vaccine called MeNZB was developed in New Zealand following the meningococcal serogroup B epidemic (Holst et al. 2013). The approach was good but also led to removal of useful lipoproteins which hampered the immunoprotective ability which was overcome by the use of gram-negative bacteria which shed the native outer membrane vesicles (NOMV), spontaneously, hence avoiding loss of functional components due to detergent treatment (Maclennan and Mutreja 2016). A research conducted on the prevention of meningococcal disease caused by serogroup A and W-135 in the African meningitis belt showed that the bivalent A + W-135 outer membrane vesicle or OMV vaccine has the potential of providing a broader protection against the disease (Norheim et al. 2012). The research also suggested that the OMV-based vaccines could be used as alternatives to the conjugate type of vaccines available for dealing with meningococcal disease in epidemic situations. Similarly, strategy utilizing OMV, an interface between the conventional and the newer methods of vaccine production, for vaccine development from *N. meningitides* serogroup B, serogroup A, serogroup W, and serogroup X was designed at the Finlay Institute in Cuba in association with the Norwegian Institute of Public Health, Norway, following a GMP process (Tunheim et al. 2013; Acevedo et al. 2014). The relative immunogenicity of the killed whole-cell bacterial vaccines led to the advancement into the toxoid-based vaccines, which comprised of well-defined antigen components devoid of bacterial cells.

The most prevailing bacterial species in the human gut belong to the division (phyla) of *Bacteroidetes* and *Firmicutes* followed by *Proteobacteria*, *Fusobacteria*, *Actinobacteria*, and *Verrucomicrobia* (Vitetta et al. 2017). The intestinal lining has a humongous surface area which is native to secretions like immunoglobulins, antibacterial peptides, mucous, etc. which function as the human immunomodulators, serving the human body with their protective actions. The gut immune system and the bacteria within have their own interrelated mechanism to render and regulate immunity. An imbalance in the microbiome is, therefore, a cause of many physiological conditions. Probiotics come into picture in this scenario because of their ability to restore and replenish this microbiome balance in humans. Probiotics act as the microflora modifiers and use mechanisms like competitive inhibition of pathogen, production of toxins against the pathogens, and protection and maintenance of epithelial lining, rendering immunomodulatory support to the host immune system (Linares et al. 2016). Bacteria majorly belonging to the species of lactic acid bacteria, namely, *Lactobacillus*, *Bifidobacterium*, and *Bacillus subtilis*, have been majorly studied for their probiotic potential via vaccine delivery. Figure 11.2 lists salient features of most commonly used bacteria for fabrication of edible oral vaccine.

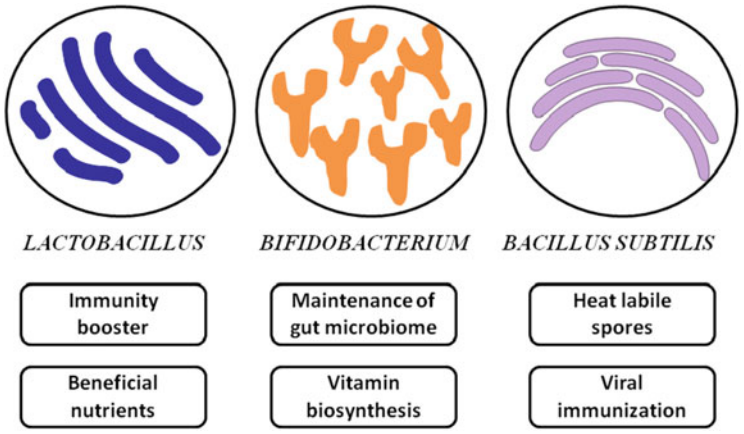

Fig. 11.2 Salient features of most commonly used bacteria for fabrication of edible oral vaccine

11.2.1.1 Lactic Acid Bacteria

Lactic acid bacteria (LAB) fall under the gram-positive bacteria category and also possess features like lack of sporulation and nonpathogenicity. Lactic acid bacteria have been used by the food industry since decades as a food preservative as well as a component for numerous food productions. There are numerous health claims pertaining to LAB owing to their ability of immunostimulation via functional foods as well as pharmaceuticals. Along with this, certain therapeutic roles exhibited by this bacteria include its use in production of anti-HIV antibodies by *Lactobacillus jensenii* and the functional expression of bacteriocin EntA, an antilisterial, by *Lactobacillus casei* (Criscuolo et al. 2019). Citing these functions, the lactic acid bacteria serve as potential candidates for development of bacteria-based probiotic vaccines. The bacteria can elicit favorable characteristics with respect to protection against pathogen, immunomodulation, biodegradation, and biocompatibility. Lactobacilli have innate features, which make them ideal candidates as immunomoduators as well as vaccine adjuvants. *Lactobacillus* and *Bacillus subtilis* species have also been used as therapeutics for infectious diseases. For example, vaccine with *Lactococcus lactis* expressing HEV antigen ORF2 gave rise to cell-mediated immune response giving rise to production of specific immunoglobulins, whereas spores of *Bacillus subtilis* expressing urease B of *Helicobacter pylori* provided protection against *Helicobacter* infection upon oral administration (Criscuolo et al. 2019). To add a feather in the cap, both *Lactobacillus* and *Bifidobacterium* have the coveted "generally recognized as safe" (GRAS) status making them highly attractive carriers and therapeutics for vaccine delivery of probiotics. *Lactococcus lactis* is another species used for development of bacteria-based vaccines. Immunization against influenza virus has been shown upon repeated administration of *Lactobacillus* strains by causing neutralization of viral antibodies. A daily intake of *Lactobacilli* has been suggested before the annual influenza vaccine may therefore result in an enhanced immunity and protection thereof

(Yasui et al. 1999). *Lactobacilli casei*, a major component of dairy products, has also been found to elicit immune response against specific influenza vaccine in elderly population (Boge et al. 2009). A patient study was conducted during a rotavirus epidemic to study the effects of different strains of *Lactobacilli* on their efficiency to help in clinical recovery of rotavirus gastroenteritis in children (Majamaa et al. 1995). *Lactobacillus casei* with a subspecies *casei* strain GG, *Lactobacillus casei* with subspecies *rhamnosus*, and a combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* with subspecies *bulgaricus* were the strains which were studied. Of these, *Lactobacillus casei* with a subspecies *casei* strain GG was found to promote serum and intestinal immunoreactivity toward rotavirus and further inferred to have an important role in establishing immunity in cases of rotavirus reinfections (Majamaa et al. 1995). Physiological messengers like cytokines are on the forefront while eliciting an immune response as a part of the host defense mechanism. It has been postulated that lactic acid bacteria possess the ability to elevate the levels of Bcl2 protein, alter cytokine production, and influence the immune balance of Th1 and Th2, i.e., T-helper cells 1 and 2, respectively (Perdigón et al. 2002). The aim of the study, herein, was to examine the role of the interaction between LAB and the gut immune system to put forward their correct application as probiotic and further oral adjuvants. A mucosal immunostimulatory role of orally administered LAB was found to influence the balance of Th1/Th2 because of the underlying effect on cytokine release patterns. The researchers reported a dose-dependent immune response on the cytokine profiles elicited by the oral administration of LAB (Perdigón et al. 2002). Studies, to find if the anti-allergy effect of LAB can also be strain-dependent, were also conducted in order to establish the numerous factors influencing the Th1 and Th2 balance. The immunostimulatory effects of more than 100 LAB strains on the Th1/Th2 balance were tested in vitro and in vivo, wherein it was found out that the effect was influenced by the LAB strain rather than the species. Oral administration of *Lactobacillus paracasei* KW3110 showed alteration of expression of splenocytes, cytokines, as well as co-stimulatory molecules on the cell surface, suggesting LAB impact on Th1/Th2 balance and its anti-allergy effects (Fujiwara et al. 2004).

Inflammatory bowel disease (IBD) can be broadly classified into two sub-diseases, namely, ulcerative colitis or proctitis and Crohn's disease, along with some nonspecific IBD like collagenous colitis, eosinophilic enteritis, Behcet's disease, transient colitis, microscopic colitis, prestomal ileitis, pouchitis, and solitary rectal ulcer (Lennard-Jones 1989). A lyophilized oral probiotic containing four lactobacilli strains, three bifidobacteria strains, and one streptococcus strain was formulated to cater to cases of chronic pouchitis. An observation was made earlier that *Lactobacillus* sp. is capable of reducing the colitis in interleukin-10 gene-deficient murine model by restoring the native mucosal adhesion and bacterial pattern in the colon (Madsen et al. 1999). Following this, many studies were conducted to examine the role of lactobacilli in IBD, one of which included development of a spontaneous colitis murine model with interleukin-10 deficiency. *Lactobacillus plantarum* 229 V strain was orally administered in this model, and its effect on the mucosal immunostimulation was observed with respect to histology,

secreted colonic immunoglobulin isotypes, and interferon gamma production. The results suggested that germ-free mice with a *Lactobacillus plantarum* colonization under specific pathogen-free conditions showed desired response to the therapy and can be a potential therapeutic in clinical IBD (Schultz et al. 2002). Further, *Lactobacillus rhamnosus* GG strain was compared to the *Lactobacillus plantarum* 229 V strain to find the effective one among the two in treatment of cases of colitis recurrence. An animal model comprising *Bacteroides vulgates*-induced colitis in HLA-B27 transgenic rats was established wherein vancomycin and imipenem were administered to the animals for 2 weeks followed by bacilli. *Lactobacillus rhamnosus* GG strain was found to show superior effects compared to *Lactobacillus plantarum* 229 V strain in combination with the antibiotic pretreatment suggesting a synergistic therapeutic effect toward immunomodulation (Dieleman et al. 2003). Another research conducted in direction implicated the efficacy of *Lactobacillus rhamnosus* GG strain in a randomized clinical trial in ulcerative colitis patients, who were given the strain alone and in combination with mesalazine. The results were surprising as the group with oral bacteriotherapy with *Lactobacillus rhamnosus* GG strain showed a better clinical efficacy in ulcerative colitis remission maintenance treatment as compared to the combination group proving that a natural physiological probiotic approach (as preferred by the patients) is a promising one (Zocco et al. 2006). Clinical trials with patients of symptomatic uncomplicated diverticular colon disease have been conducted to check the efficacy of *Lactobacillus casei* in combination with mesalazine, wherein the combination therapy was found to prevent recurrence of the disease, for long-term maintenance, and also proved the efficacy in comparison to placebo treatment (Tursi et al. 2006, 2008, 2013).

The presence of LAB in the gut ecosystem of humans has led to their selection as a probiotic strain and made them huge contributors in development of immunomodulatory vaccine systems. The knowledge of these species has been prevalent since decades, and hence their exploration for numerous vaccine applications is extensive. Numerous strains of LAB have been reported along with their specific applications, and hence strain selection is an important task in order to achieve desired immunomodulation in full capacity. With the basic knowledge of their properties and immunostimulatory characteristics, LAB have been studied for applications ranging from microbial and viral infections, maintenance of cytokine balance, anti-allergy effects, colon-specific therapy, use of the bacilli in synergy with therapeutics, as well as remission prevention approaches.

11.2.1.2 Bifidobacteria

Bacteria belonging to the genus *Bifidobacterium* are among the initial microbes to have been found in the human gastrointestinal tract and were first isolated in the early 1900s and termed as *Bacillus bifidus*. The bacteria are gram-positive, catalase-negative, nonspore-forming, and nonmotile bacteria with a variety of shapes and around 30 species. The prevalence of bifidobacteria is highest in infants, and a reduction in their numbers is observed as the age increases, the least being present in geriatrics. The nomenclature of some of the species is therefore according to their profile, viz., *Bifidobacterium infantis* and *Bifidobacterium breve* in infants,

Bifidobacterium adolescentis in adults, and *Bifidobacterium longum* being present later throughout a person's lifespan (Gomes and Malcata 1999). Bifidobacteria along with LAB have been the most highlighted bacteria for use as probiotics due to their potential applications in fermented products in the dairy industry. Some strains of bifidobacteria are already in markets as probiotic-based food supplements including *Bifidobacterium breve* M16-V, *Bifidobacterium longum* BB536, and *Bifidobacterium bifidum* BGN4 (Mauras et al. 2018). They have been termed as health-promoting microbes and are highly researched over the past decade for their therapeutic applications. One major reason is that even bifidobacteria have obtained the GRAS status, and hence there has been a multifold increase in their commercial viability. The use of probiotics in IBD has been a well-sought strategy because of their high prevalence in the GIT. As is the case with lactobacilli, even bifidobacteria have been explored in different modalities of IBD. It has been reported that the prevalence of bifidobacteria is around 10%, whereas that of lactobacilli is 0.01% in human gut indicating the suitability of bifidobacteria for designing probiotics catering to conditions of the gut (Benno and Mitsuoka 1992; Shiba et al. 2003). *Bifidobacterium infantis* 1222 and *Bifidobacterium adolescentis* 1275 were utilized as probiotics against *Bifidobacterium vulgates*-induced IBD, wherein their oral administration in *Bifidobacterium vulgates*-implanted mice showed a reduction in serum antibody titer and ratio of antibody-producing cells, thereby suppressing *Bacteroides* in the gut and ameliorating IBD (Shiba et al. 2003). The expected beneficial effects of bifidobacteria have been tested on the repression of inflammation in gnotobiotic mice associated with *Bifidobacterium vulgates* strains, and the results suggest that *Bifidobacterium* strains control the inflammation by inhibiting the growth of *Bifidobacterium vulgates* strain (Setoyama et al. 2003). The potential of bifidobacteria in prevention and treatment of colorectal cancer has been studied by numerous researchers. Major studies have been preliminary murine model-based wherein a combination of prebiotics and bifidobacteria has been hypothesized to help in reduction of carcinogen-induced cancer cells in the animal models (Callaghan and Van Sinderen 2016). Being among the important gut microbiota, bifidobacteria have been studied for numerous conditions of the colon. Anti-inflammatory potentials of eight strains of bifidobacteria, viz., *Bifidobacterium adolescentis* NCC251, *Bifidobacterium lactis* NCC362, *Bifidobacterium longum* NCC2705, *Bifidobacterium bifidum* NCC189, S16, S17, *Bifidobacterium longum/infantis* E18, and *Bifidobacterium breve* MB226, have been studied (Riedel et al. 2006). The properties of these strains were tested in vitro on the intestinal epithelial cell lines including Caco-2, T84, and HT29 and later in murine models of colitis. *Bifidobacterium bifidum* S17 (highly adherent strain) and *Bifidobacterium longum/infantis* E18 (nonadherant strain) were found to show higher anti-inflammatory potential in the in vitro testing and were then delivered orally in the colitis-induced murine models. The authors report that *Bifidobacterium bifidum* S17 strain exhibited highest anti-inflammatory effect in the murine models of colitis, confirming its future potential as an oral probiotic vaccine (Preising et al. 2010). Live oral vaccine of *Bifidobacterium infantis* expressing ETEC antigens has been constructed as a vehicle for expressing heterologous antigens. ETEC is a major cause of bacterial

diarrheal illness along with traveller's diarrhea and other diarrheal conditions in low-income countries because of its easy mode of transmission via food, water, and feces, necessitating an effective prophylaxis and treatment thereof. Expressions of antigen protein subunits of ETEC in *Bifidobacterium infantis*, namely, CfaB, which is a fimbrial subunit, and LTB, a B-subunit of heat-labile enterotoxin, were studied in murine models as a probiotic oral vaccine with immunogenic function. Both antigen proteins elicited systemic and mucosal immune responses in the form of an oral probiotic vaccine, and the *Bifidobacterium infantis*-based system was proposed to be a versatile platform for developing probiotic vaccines against numerous pathogens (Ma et al. 2012). Enterovirus 71 (EV71), a single-stranded virus, is responsible for hand, foot, and mouth disease, paralysis, neurological complications like encephalitis, as well as cardiac complications (Yi et al. 2017). A recombinant *Bifidobacterium longum* to be delivered as an oral vaccine against EV71 infection was designed wherein VP1 protein from EV71 was amplified by creating an expression vector pBBADs-VP1 followed by its insertion into *E. coli-Bifidobacterium longum* as a shuttle expression vector. Oral immunization of BALB/c mice with the resultant pBBADs-VP1-transformed bacteria resulted in potent immune responses against EV71 infection, and immunization of mother mice was found to confer protection to the neonatal mice, strengthening the immunity in the next generation as well (Yu et al. 2013).

Demonstration of the role of commensal bifidobacteria in spontaneous antitumor immunity enhancement in multiple tumor setting was given in comparison to the programmed cell death protein 1 ligand 1 (PD-L1)-specific antibody therapy (Sivan et al. 2015). Sequencing the 16S ribosomal RNA indicated the association of *Bifidobacterium* with antitumor effect as compared to other commensal microbiota, and the effect was to the same degree as PD-L1 suggesting a possibility of cancer immunotherapy modulation incorporating commensal *Bifidobacterium* (Sivan et al. 2015). A new Bifidobacteria Expression SysTem (BEST) was developed to enable production of heterologous proteins in *Bifidobacterium bifidum*; the system was validated by cloning murine interleukin-10 or IL-10 and using resulting plasmids in BS42 strain of *Bifidobacterium bifidum* (Mauras et al. 2018). The fact that bifidobacteria confer numerous advantages toward development of probiotics is an indicator of its importance. The applications are subject to the particular strains of bifidobacteria and their allied applications. The studies, nevertheless, are majorly restricted to animal models, and their translation into clinical practice is still to be achieved on a larger scale.

11.2.1.3 *Bacillus subtilis*

Bacillus subtilis is a gram-positive bacilli capable of growing in diverse physiological environments including the human GIT (Earl et al. 2008). The benefits of *Bacillus* subtilis in the form of probiotics are enormous because of the inherent characteristics of its endospore to survive the extreme physiological conditions. The bio-resistant properties of the spore make *Bacillus subtilis* an attractive alternative for development of oral vaccine with a bonus of heat stability. The utility of these organisms has been noted in animal models against viral and bacterial pathogens in

diseases including human clonorchiasis, foot and mouth disease virus in veterinary medicine, and *Helicobacter pylori* infection using only the bacterial spores (Rosales-Mendoza et al. 2016). An oral vaccine of spore-based *Bacillus subtilis* expressing murine VP6 rotavirus has been studied for its ability to provide allied immunization. A potential approach pertaining to viral immunization using a bacterial species has been established in a first of its kind experiment (Lee et al. 2010).

Oral administration of *Bacillus subtilis* spores, expressing *Clonorchis sinensis* TP22.3 protein, to cater to *Clonorchis sinensis* infection in humans was found to confer protection against the infection (Zhou et al. 2008). *Bacillus subtilis* harboring an envelope protein VP28 was used as delivery vehicle in an attempt to confer protection against white spot syndrome virus in shrimp or crayfish which showed significant resistance to the infection, further indicating the potential of this system in development of vaccines with different target viral antigens (Fu et al. 2008). The use of *Bacillus subtilis* has been ranging from an oral vaccine carrier to a host for antigen production, applications which present a wide scope for future research in this direction (Ferreira et al. 2005).

The bacterial strains used for the development of probiotics are generally picked up from the ones which are already present in the host biological system. Major reason behind this selection criterion is the fact that the native organisms have an inherent capability to withstand the harsh environment in the human body, especially the gastrointestinal tract. Newer species of bacteria belonging to the genera of *Bacteroides*, *Clostridium*, *Faecalibacterium*, and *Akkermansia* are referred to as next-generation probiotics, but the lack of studies confirming to their benefit versus risk ratio is yet to be derived (Novik and Savich 2019). Need for evidence-based results with respect to these bacteria is a prerequisite to ensure an absence of pathogenicity along with the presence of efficacy with their use. *Lactobacillus* and *Bifidobacterium* are most prevalent microbiota in humans due to their proven safety and efficacy and are, therefore, preferred over other strains in majority of cases.

11.3 Yeast-Based Probiotic Vaccines

The GRAS status, possibility of posttranslational modifications, simple, fast, as well as scalable growing methods, high biomass yields, and safe pathogen-free production are some of the salient attributes that have led to the widespread utilization of yeast cells for the expression of heterologous proteins for biopharmaceutical applications. Yeasts have stable haploid and diploid state that enables genetic analysis and efficient homologous recombination, hereby simplifying gene replacement/mutation. Further, cellular wall components of yeast cells aid in protecting the antigens in the gastrointestinal tract and act as natural adjuvants that can incite or modulate immune responses. Thus, engineered yeast cells pose as excellent candidates for the development of edible vaccines. However, hyperglycosylation of the recombinant proteins has been reported as a major limitation of these systems, which can be addressed by using yeast strains that are defective for N-glycosylation (Nielsen 2013; Wang et al. 2016; Kumar and Kumar 2019).

Although yeast cells are nonpathogenic, dendritic cells and macrophages take them up and generate danger signals related to microbial infection. The polysaccharides beta-1, 3-D-glucan, and mannan in the yeast cells possess strong adjuvant nature and cause efficient phagocytosis by antigen-presenting cells. The foreign antigens expressed in yeast cells are displayed on the cell surface through major histocompatibility complex (MHC)-I and MHC-II after degradation of the yeast cells in proteasomes and endosomes, respectively. These antigens are then recognized by CD81 cytotoxic T lymphocytes and CD41 T-helper cells, which mediate T-cell immunity. The yeast cells also enhanced the interleukin secretion. Another important observation was that dead or alive yeast cells generated the same magnitude of antigen-specific responses (Stubbs et al. 2001; Franzusoff et al. 2005; Munson et al. 2007; Saegusa et al. 2009).

The advancements in the molecular biology techniques have facilitated efficient expression of heterologous proteins that can interact effectively with immune cells and stimulate an immune response. Integration of a gene into non-transcriptional spaces of rRNA gene or at chromosomal centromere or telomere regions of the yeast is easily possible. Non-integrating plasmid-based expression vectors, like episomal-based expression vectors, allow sustained gene expression but need continuous selection pressure to maintain clones. Further, auxotrophic selection is preferred for yeast strains compared to antibiotic selection owing to the development of resistant mutants and the insensitivity to certain antibiotics. The auxotrophic strains of yeast can be grown in media containing the missing nutrient; however, poor and slow growth in these media can pose an issue. The use of integrating vectors permits long-term maintenance of the clones, but the number of antigen copies is less (Stearns et al. 1990). Thus, it is important that appropriate selection of host (yeast species) and expression vectors enable stable expression of target genes and permits rapid and economical protein purification.

Unicellular yeasts like *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Pichia pastoris*, and *Hansenula polymorpha* possess extremely efficient heterologous gene expression systems and are commonly employed for the production of therapeutic proteins. Among these, the most studied eukaryote is *Saccharomyces cerevisiae* and has been employed for several purposes owing to its cheap and easy cultivation, fermentation, and feasibility of large-scale production. It was also used for developing the first commercialized recombinant vaccine, the hepatitis B vaccine, which is administered intramuscularly. *Saccharomyces cerevisiae* was employed for commercial production of hepatitis B antigens, as initial attempts to use *E. coli*-based expression systems did not succeed in providing immunoprotection (Fujisawa et al. 1983; Keating and Noble 2003) (Keating and Noble 2003). Further studies also investigated the possibility of developing oral live vaccines using *Saccharomyces cerevisiae* expressing hydrophilic regions of the hepatitis B virus surface antigen (Schreuder et al. 1996). Another study reported that oral administration of two recombinant *Saccharomyces cerevisiae* strains led to secretion of peptides in mice gut, thereby paving the way for the development of live oral vaccines (Blanquet et al. 2004).

The use of whole yeast cells expressing antigens has the potential to emerge as an innovative, low-cost immunization approach with very less purification requirements. Most of the yeast-based vaccines have been tested in parenteral administration modes with very few being developed for oral administration. In one of the earlier studies involving whole recombinant yeast, heat-killed *Saccharomyces cerevisiae* was used for the expression of recombinant myostatin that is induced by a copper-inducible promoter. Oral vaccination of mice caused a similar growth and immune response as that of subcutaneous injection, which was measured in terms of myostatin-specific antibodies found in mouse serum. The enhanced growth performance of the vaccinated mice indicated that these engineered whole yeast strains were excellent candidates for oral vaccination against muscular atrophy (Zhang et al. 2011). Further, they engineered a yeast strain that stably expresses mammalian myostatin for 2 years by integrating the corresponding gene into the ribosomal DNA of *Saccharomyces cerevisiae* and employed the Cre-LoxP system to remove the antibiotic selection marker. Establishment of such engineered yeast strains can act as stepping stones for developing yeast-based edible vaccines (Zhang et al. 2012). The same recombinant *Saccharomyces cerevisiae* vaccine was orally administered in rabbits also to investigate if similar immune responses could be generated. Myostatin-specific antibodies could be detected in the serum of the treated rabbits, which grew faster and had heavier muscles as compared to the control group (Liu et al. 2016). In another study of antibody involving *Saccharomyces cerevisiae* as the host, immune responses after oral immunization with whole recombinant yeast expressing the antigen intracellularly were compared with that of conventional purified recombinant antigen. The model antigen used was the recombinant capsid protein of red-spotted grouper nervous necrosis virus (RGNV), which causes viral nervous necrosis in marine fish cultivation. Whole recombinant yeast producing the antigen induced 9–27-fold higher IgG antibody titers than purified antigen. In addition, sera from the recombinant yeast-immunized mice had neutralizing activity against RGNV. This study thus posed the possibility of using mouse-derived neutralizing antibodies for passive immunization or oral immunization using recombinant yeast producing RGNV capsid protein to generate neutralizing antibodies against RGNV infection in fish (Kim et al. 2014). In a similar study, freeze-dried recombinant *Saccharomyces cerevisiae* producing RGNV capsid protein was used for oral vaccination of convict grouper fish. The serum of the orally vaccinated group showed higher RGNV neutralizing antibody titers, which sustained for at least 95 days after immunization. In addition, when challenged with RGNV, the vaccinated groups suffered significantly low mortality and RGNV titers. Virus-like particles (VLPs) are protein complexes consisting of recombinant virus capsid proteins and are considered a novel vaccine platform that is noninfectious and can induce neutralizing antibodies efficiently. RGNV VLPs produced in *Saccharomyces cerevisiae* were also employed to confer protective immune response in convict grouper fish via intraperitoneal injection and oral immunization (Wi et al. 2015). *Saccharomyces cerevisiae* expressing the ApxIIA protein was also used to induce both mucosal and systemic immune responses against *A. pleuropneumoniae* serotype 2 Korean isolate via oral immunization.

The vaccinated mice showed dose-dependent increase in ApxIIA-specific IgA antibody titers in the intestine, lung, and sera. Upon challenging the efficacy of the oral immunization with a minimal lethal dose of *A. pleuropneumoniae* serotype 2 isolate, half of the 30 mg dose group and 30% of the 15 mg dose group survived, while none of the control mice survived after 36 h (Shin et al. 2005). Oral immunization with *Saccharomyces cerevisiae* expressing ApxIA antigen was also studied and compared with that of ApxIIA antigen. Mice immunized with both ApxIA and ApxIIA antigens had significantly low infectious burden and survival rate following the *A. pleuropneumoniae* challenge, thereby confirming that immunization against ApxIA antigen is inevitable for optimal protection (Shin et al. 2007). Surface-displayed ApxIIA expressed on *Saccharomyces cerevisiae* was also found to stimulate dendritic cells and upregulate the production of tumor necrosis factor-α and interleukins (IL-1β, IL-12p70, and IL-10), thereby eliciting specific T-cell proliferation. Further, there was an increase in the number of cells secreting antigen-specific IgG and IgA antibodies in the spleens, Peyer's patches, and lamina propria of the orally immunized mice. This study is an excellent example suggesting that genetically engineered *Saccharomyces cerevisiae* could elicit both systemic and mucosal immunity postoral administration (Shin et al. 2013). In another study, *Saccharomyces cerevisiae* was engineered to express the neutralizing epitope of the spike protein from porcine epidemic diarrhea virus on its outer surface layer. The neutralizing epitope on the surface of yeast cells were visualized using confocal microscopy and further verified using a Western blot analysis. These yeast cells were then proposed to be administered orally along with diet to combat porcine epidemic diarrhea virus (Park et al. 2007).

Yeast-based vaccines against infectious disease-causing organisms like hepatitis B virus and *Plasmodium* were developed using other species of yeast also. In addition to *Saccharomyces cerevisiae*, methylotrophic yeasts *Hansenula polymorpha* and *Pichia pastoris* were also employed to express hepatitis B antigens. Currently, three commercially available hepatitis B vaccines, namely, HepavaxGene® (Johnson & Johnson), Gen Vax B® (Serum Institute of India), and Biovac-B® (Wockhardt), are produced using antigens expressed using *Hansenula polymorpha* (Li et al. 2013; Manfrão-Netto et al. 2019). Another most commonly used yeast species for heterologous protein expression is *Pichia pastoris*. As compared to *Saccharomyces cerevisiae*, it produces properly folded and secreted proteins and performs posttranslational modifications much closer to human protein modifications in exceptionally high-density cultures. Further, the amount of endogenous proteins secreted by *Pichia pastoris* is low, thereby simplifying the purification process. However, it requires stringent culture conditions that are possible in continuously stirred tank bioreactors and requires a lot of investment in terms of time and equipment (Darby et al. 2012; Wang et al. 2016). *Pichia pastoris*-derived hepatitis B antigens are the main active pharmaceutical ingredient for the commercial vaccine, Heberbiovac HB® vaccine (Heber Biotec, S.A., Havana, Cuba), which has shown enhanced efficacy in terms of antibody titers and immune responses as compared to other available yeast-derived hepatitis B vaccines. It has been proved to be safe, effective, and immunogenic even at lower doses in both adults and infants

(Hernández-Bernal et al. 2011; Gurramkonda et al. 2013). *Pichia pastoris* has also been used for developing subunit vaccines against human papillomaviruses (Bolhassani et al. 2014), *Plasmodium berghei* (Jacob et al. 2014), and dengue virus type 2 (Arora et al. 2013). However, all of the abovementioned systems were developed for the parenteral route of administration. Recently, heat-inactivated recombinant *Kluyveromyces lactis* were used to express virus capsid forming protein VP2 against infectious bursal disease virus and used for vaccination in mice and chickens via subcutaneous and oral administration. While subcutaneous administration provided full protection against a subsequent infection, oral vaccination had a protection rate of 10% only. Thus, *Kluyveromyces lactis* was established as a powerful tool for vaccination, but further studies are warranted to improve the efficiency of the oral immune response (Arnold et al. 2012).

Thus, it is clear that most of the yeast-based vaccine candidates have exhibited remarkable immunoprotection in preclinical studies. The enhanced immunogenicity of these systems could be attributed to the adjuvant properties of the yeast cell wall components including β-glucans that can bind with the innate pathogen receptors on immune cells like macrophages, dendritic cells, and neutrophils (Huang et al. 2013). There are currently two clinical trials involving parenteral administration of yeast-based vaccines GS-4774 and GI-5005 against hepatitis B and hepatitis C virus, respectively. In phase I clinical trials, GS-4774 was administered as two immunization regimens (five subcutaneous injections per week with one monthly booster and three subcutaneous injections per month) in healthy subjects and was found to generate immune responses at all evaluated doses (Gaggar et al. 2014). In the phase II study, it was evaluated in patients having chronic hepatitis B infection and receiving oral antiviral therapy. It was found to be safe and well tolerated in patients but could not reduce the serum antigen levels (Lok et al. 2016). In another study, recombinant *Saccharomyces cerevisiae* cells (GI-5005) were found to induce potent antigen-specific T-cell responses and Th1-type cytokine secretion upon subcutaneous administration in mice (Haller et al. 2007). Currently, phase I clinical trials are being carried out in patients having chronic hepatitis C virus infection. These studies are indicative of the advantages of yeast-based vaccines, and the different commercial peptide vaccines against hepatitis B are proof of this hypothesis. With the identification of novel protein antigens and the development of recombinant yeast species, it would be feasible to develop low cost-efficient vaccine preparations in the near future. Table 11.1 lists few important examples of bacterial and yeast-based oral vaccines.

11.4 Way Forward in Formulation Development

Pursuit for safe and efficacious edible vaccines has been constantly evolving since decades. Along with vaccine development, another evolving allied area of research has been development of formulations for the delivery of these vaccines. Considering the harsh physiological environment, a formulation has to surpass upon oral delivery; formulations like enteric-coated tablets, capsules, and granules have been

Table 11.1 Representative examples of bacterial and yeast-based oral vaccines

Target disease/ causative agent	Antigen description	Immunogenic properties
Bacterial vaccines		
Leishmania major	*Lactococcus lactis* expressing *Leishmania* antigen, LACK, in the cytoplasm, secreted or anchored to the bacterial cell wall or co-expressing mouse IL-12	Oral immunization induced a LACK-specific mucosal immune response and Th1 immune response in splenocytes and mesenteric lymph node cells, partially protecting BALB/c mice (Strukelj et al. 2012)
Clostridium difficile	*Bacillus subtilis* expressing carboxy-terminal repeat domains of toxins A and B	Induced humoral responses at systemic and mucosal levels in murine models. Protected hamster from *C. difficile* spores (Permpoonpattana et al. 2011)
Foot and mouth disease virus	*Bacillus subtilis* expressing epitopes of foot and mouth disease virus and cholera toxin B-subunit	Induced humoral responses in mice at systemic and mucosal levels to protect against foot and mouth disease virus (Hu et al. 2011)
Hepatitis E virus	*Lactococcus lactis* expressing ORF2 antigen	Induced specific mucosal IgA and serum IgG and cellular immunity in mice (Gao et al. 2015)
Testicular cancer	*Lactobacillus plantarum* expressing testicular cancer antigen NY-ESO-1	Induced NY-ESO-1-specific antibodies and T-cell responses in mice (Mobergslien et al. 2015)
Peanut allergy	*Lactococcus lactis* expressing peanut allergen Ara h 2	Induced secretory IgA and regulatory T cells at the local level in orally immunized mice (Ren et al. 2014)
Staphylococcus aureus	*Lactococcus lactis* expressing staphylococcal enterotoxin B	Resulted in cellular or systemic immune responses in mice (Asensi et al. 2013)
Helicobacter pylori	*Bacillus subtilis* expressing urease B	Induced responses in terms of fecal IgA and serum IgG in mice models (Zhou et al. 2015)
Yeast-based vaccines		
Muscular atrophy	*Saccharomyces cerevisiae* expressing myostatin	Induced humoral responses in immunized mice with enhanced growth performance (Zhang et al. 2012)
Infectious bursal disease virus (IBDV)	*Kluyveromyces lactis* expressing the VP2 protein of IBDV	Induced IBDV-neutralizing antibodies in mice and chicken; immunized chicken were protected against pathogen challenge (Arnold et al. 2012)
Red-spotted grouper nervous necrosis virus (RGNV)	*Saccharomyces cerevisiae* expressing the capsid protein of RGNV	Induced strong humoral responses with neutralizing activity against RGNV in mice (Kim et al. 2014)

(continued)

Table 11.1 (continued)

Target disease/ causative agent	Antigen description	Immunogenic properties
A. pleuropneumoniae	*Saccharomyces cerevisiae* expressing ApxIIA#5 antigen	Induced humoral responses at systemic and local levels and systemic cellular responses in mice (Shin et al. 2013)

Adopted with permission from Rosales-Mendoza et al. (2016)

under use for delivery of live bacterial cells (De Barros et al. 2014). An edible recombinant *Lactococcus lactis*-based vaccine has been developed in the form of enteric-coated mini-capsules, wherein *Lactococcus lactis* vectors based on influenza virus H5N1 HA antigen were fabricated which enabled enhanced IgG and fecal IgA antibody production postoral administration (Lei et al. 2010). Another study investigating the efficiency of dry alginate chitosan microcapsules as enteric delivery system for probiotic bacteria was carried out with *Bifidobacterium breve* as the model organism. The role of chitosan in increasing the pH stability of alginate microcapsules has been highlighted which was responsible for enhancing the survival rate of *Bifidobacterium breve* in simulated gastric fluid (Cook et al. 2011). A novel approach exploiting the film drying technology, using the enteric coating polymer Eudragit L100–55 alone and in combination with ethylcellulose (50:50), has been tried. The concept involved examining if the live bacterial cells withstand direct drying onto the polymer film and stay unharmed in the gastric environment. Organisms like *Bifidobacterium breve* and *Salmonella typhimurium* were tried in the initial trials, which showed desired outcome. The preliminary studies conducted demonstrated feasibility of the enteric-coated film formulation, but further studies are warranted to prove the efficacy in preclinical models (De Barros et al. 2014). Studies majorly pertaining to use of excipients rendering protective action against digestive environments have been explored with a focus on controlled release and mucoadhesive polymers, path breaking research on this front is eagerly awaited.

11.5 Conclusion

Bacteria and yeast have demonstrated a strong potential for development into probiotic-based edible vaccines over a brief timespan. Majority of these organisms enjoy the benefit of being bio-resistant due to their inherent properties. Organisms like *Lactobacilli*, *Bifidobacterium*, and *Saccharomyces cerevisiae* have achieved the coveted GRAS status enabling their extensive exploration toward fabrication of edible vaccines. There has also been a great financial interest in the field of edible vaccines due to the enhanced knowledge about probiotics in the consumers. A recent report (2019) by Fortune business insights states that global market for probiotics has been valued at USD 42.55 billion in 2017 and it is expected to jump up to USD 74.69 billion by the end of 2025 with a CAGR of a whopping 7.3% ("Probiotics Market Size, Growth | Industry Report by 2025," n.d.). According to the same report,

Lactobacillus and *Bifidobacterium* are the most widely utilized organisms in the probiotic industry. Other candidates falling in the category of bacteria and yeast are being studied majorly at the preclinical stages, and their transformation into clinical practice would mandate substantial efforts. Studies to investigate the relationship and underlying mechanism between the probiotic candidates and the host immune system would be required to establish solid conceptual rationale for a desired vaccine development. A need for efficient molecular-level evaluation techniques also arises in this regard so as to understand the exact mechanism.

In spite of the major outcomes in this field, a lot of area remains unexplored and needs in-depth research to be carried out to develop a proof of concept followed by the actual clinical application. Owing to the highly diverse and equally complex immune system, building up of the existing knowledge base is a prerequisite for us to enter into the era of clinically effective next-generation vaccines. The progress of edible vaccine technology in the coming years would definitely be an area to look out for.

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