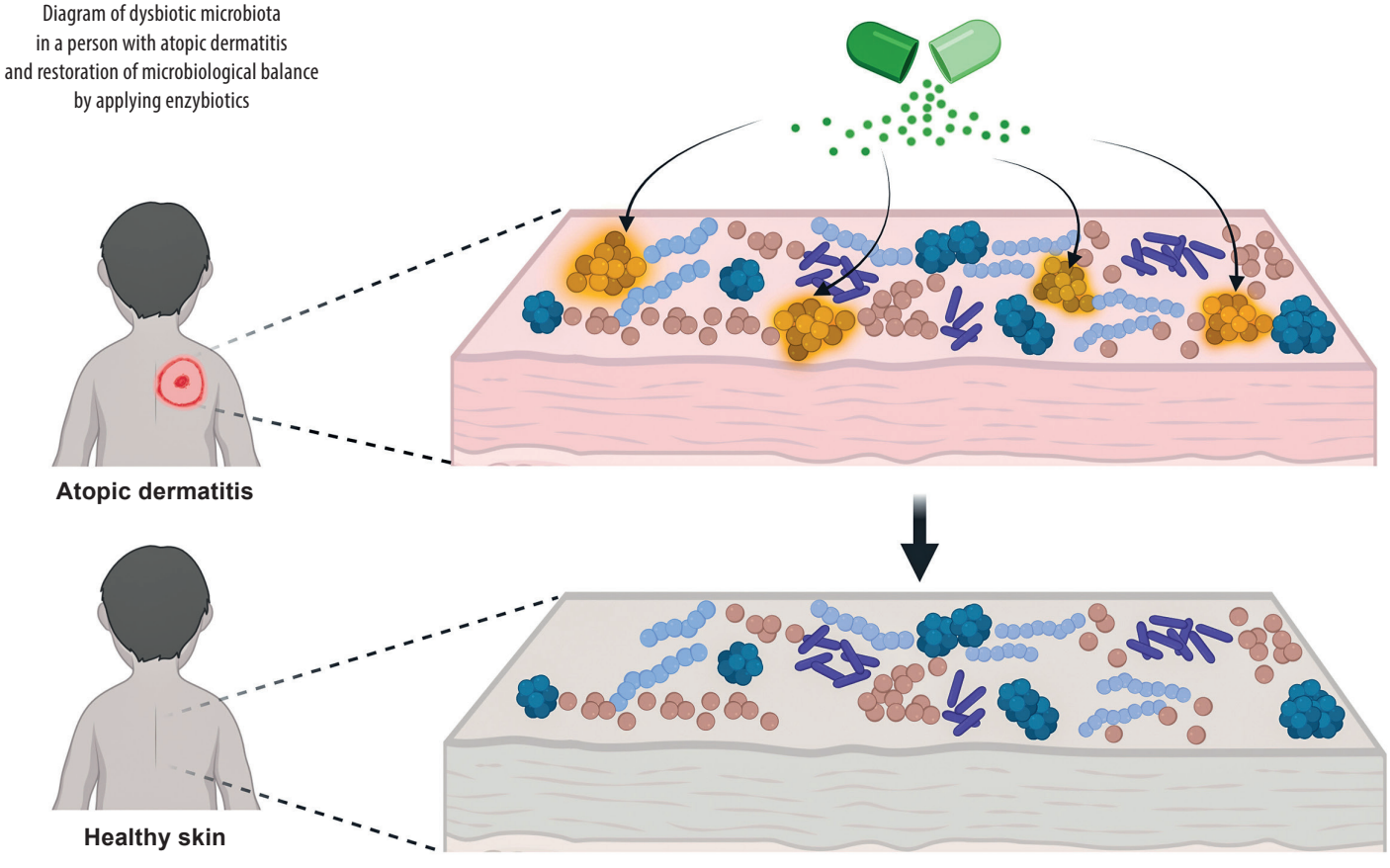
DOI: 10.24425/academiaPAS.2024.152926

Elżbieta Jagielska, PhD
conducts research on the properties of bacteriolytic enzymes for use as *enzybiotics* – modern antibacterial drugs.
ejagielska@imdik.pan.pl

The Microbiota: Freeloading Passengers?

The microorganisms on our skin create their own micro-worlds, living in symbiosis with us.

Diagram of dysbiotic microbiota in a person with atopic dermatitis and restoration of microbiological balance by applying enzybiotics



Elżbieta Jagielska
Izabela Sabała

Protein Engineering Laboratory
Mossakowski Medical Research Institute,
Polish Academy of Sciences, Warsaw

The human skin, while forming a barrier that separates our body from harmful external agents, also allows for continuous, intense interaction with the environment through nerve endings. It has a complex, varied surface that covers up to two square meters. Although it may seem something like a barren desert at first glance, under a microscope the skin proves to be teeming with millions of microorganisms such as bacteria, viruses, and fungi. In fact, if we consider the skin areas around hair follicles and sweat glands, we will find as much as 25–30 m^2 of habitat ready for colonization. The skin's surface is generally cool, acidic, and dry, but its thickness and structure vary with depressions, folds, hair follicles, and sweat glands. This dive creates distinct niches on the skin's surface, each inhabited by different species of microorganisms that form a unique community known as the *microbiota*.

The qualitative and quantitative composition of this community depends not only on the local conditions of the specific skin area but also on individual characteristics of the host, including age, climate zone of residence, skin type, sex, condition of the immune system, diet, and body weight. The microbiota of each person will be distinct and unique, and its composition changes throughout human development – from newborns to adulthood, when it becomes stable and constant. The colonization of an infant's sterile skin first occurs during birth, and a child's microbiota develops while interacting with the immune system. During adolescence, changes in sebum production favor lipophilic bacteria, while aging skin, with reduced regenerative capacity and a visibly thinner epidermis, sees a shift in microbiota composition, increasing the risk of infection.

In samples taken from the skin of healthy adults, several million bacterial cells may be found on each square centimeter. The most common species belong to the genera *Corynebacterium*, *Cutibacterium*, *Micrococcus*, *Staphylococcus*, and *Streptococcus*. Skin areas that produce the most sebum, like the forehead, ear crease, the fold behind the ear, and the back, exhibit the lowest microbial diversity and are mainly inhabited by *Cutibacterium* bacteria. In contrast, moist areas such as the armpits, inner elbows, and groin folds are primarily colonized by *Staphylococcus* and *Corynebacterium*. The most microbiologically diverse regions are the dry areas, like the upper parts of the limbs (e.g., hands/forearms, thighs), where bacteria from the phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* are most commonly found.

The micro-world on our skin comprises a highly diverse group of bacteria coexisting within the same ecological niche and living in a fragile balance. The mechanisms that govern this world are varied: natural selection driven by the distinct conditions of different skin areas, competition for resources within each niche, and even specific tools actively used by some bacterial species to try to outcompete others. This antimicrobial "arsenal" includes bacteriocins – proteins or peptides with bactericidal properties that can *lyse* (break down) competitors' cell walls or disrupt their membranes, which lead to cell death. This weapon is often designed to selectively target specific bacterial species, opening up the possibility to precisely control the microbiota's composition.

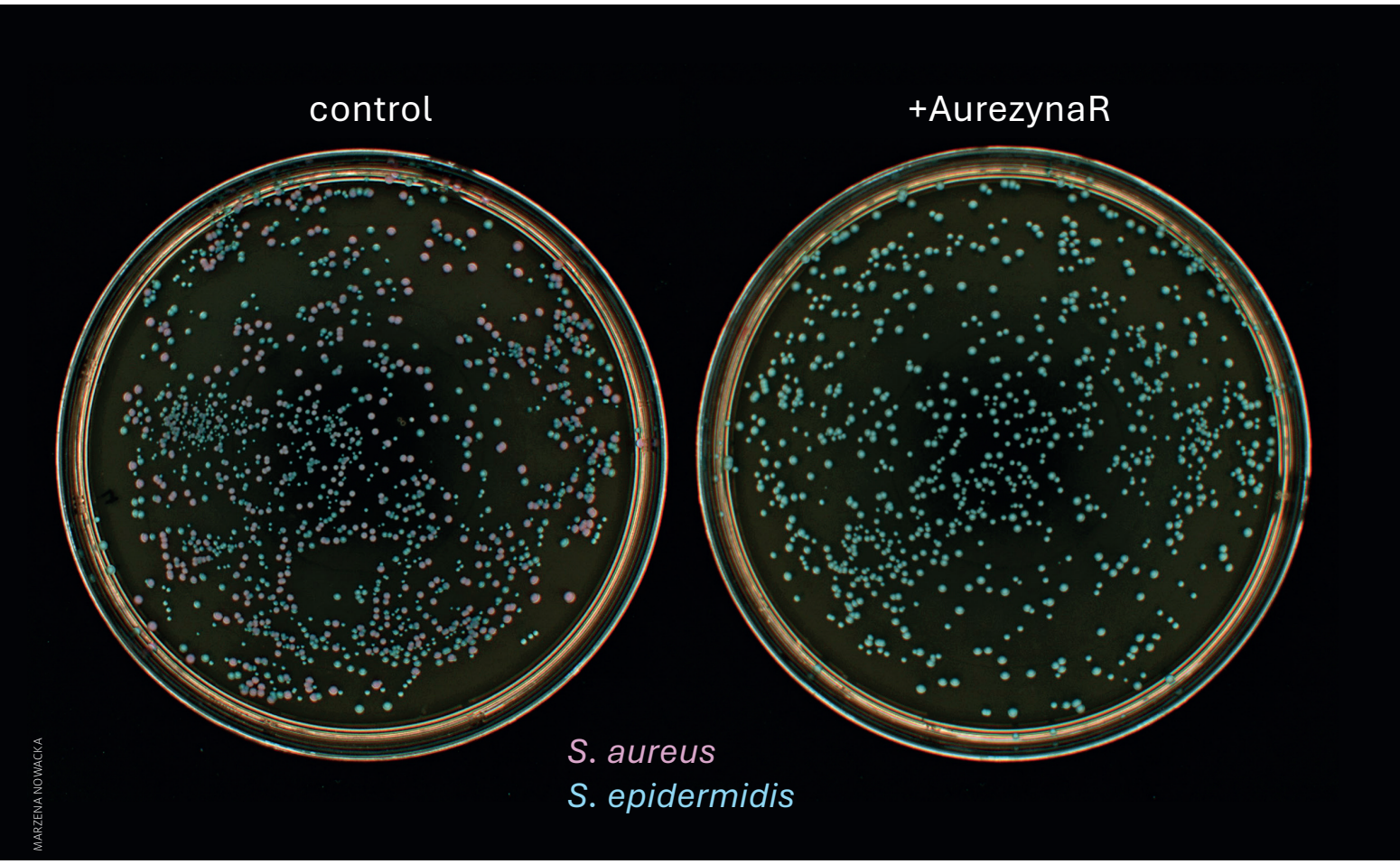
Izabela Sabała, PhD, DSc
develops new, non-antibiotic antibacterial therapies using bacteriolytic enzymes.
isabala@imdik.pan.pl

> *Enzybiotics* – enzymes that recognize specific cell wall components – are promising candidates for selective treatment to remove a single pathogen.

Healthy Skin

In fact, we should not treat the microorganisms that inhabit our skin as freeloaders, stowaway passengers, or intruders, but more like friendly neighbors, forming a community with us. These microbes play an active role in maintaining skin homeostasis and its function as an immune barrier. They secrete proteases, lipases, ureases, and fatty acids that contribute to the renewal of the skin's outer layer and help maintain a stable pH, while also protecting us from colonization by harmful bacteria.

The cooperation between our microbiota, skin cells, and immune system is essential to keeping our skin healthy. But how does our body tell the difference between "good" and "bad" bacteria? The immune system learns to recognize them early in infancy through T cells – for instance, tolerating beneficial bacteria



MARZENA NOWACKA

Selective removal of *Staphylococcus aureus* by AuresineR. Bacterial cultures before and after incubation with an enzybiotic on agar plates

like *Staphylococcus epidermidis* (commonly present on the skin) while targeting *Staphylococcus aureus*, a pathogenic species. *S. epidermidis* is recognized by the immune system via specific molecules on its cell wall, like teichoic acids, or through substances it secretes, which then trigger signal cascades in skin cells. These signal cascades allow *S. epidermidis* to colonize the skin without causing inflammation while also preventing colonization by other species, such as *S. aureus*. Furthermore, *S. epidermidis* cells alert the host's innate immune system to the presence of *S. aureus*, initiating a bactericidal response, including the production of antibacterial peptides. *S. epidermidis* also produces bacteriocins that eliminate pathogens on the skin, including *Streptococci*. Other commensal staphylococcal species, like *Staphylococcus capitis*, *S. hominis*, and *S. lugdunensis*, can also produce bactericidal peptides.

Besides protecting the host from infection, *S. epidermidis* supports the skin's protective barrier by breaking down sphingomyelin – a component of skin cell membranes – into ceramide, helping to prevent water loss.

In recent years, advancements in molecular techniques have made bacterial identification in skin swabs faster and more accurate. Amplifying and sequencing the conserved *16S rRNA* gene enables identification of microorganisms down to the order level, while the rapid development of whole-genome sequencing now allows identification down to the species, even strain level, and sequencing of the entire skin microbiome. With a growing body of microbiome data, we can now make detailed comparisons of skin ecosystems across individuals, observing how they vary over time and through different life stages. These analyses are helping us understand the mechanisms behind certain skin conditions and guiding the development of new, targeted, and effective treatments for skin diseases where microbiota imbalance is a contributing factor or symptom.

Out of balance

A disruption in the composition of the skin microbiota, called *dysbiosis*, is linked to skin conditions such as atopic dermatitis (AD), psoriasis, impetigo, and

even challenges with wound healing. In patients with AD, the skin microbiota can shift at various stages of the disease, but generally, significant reduction in microbial diversity is observed, with an overgrowth of *Staphylococcus aureus*. Interestingly, the presence of commensal species (like *S. epidermidis* and *S. cohnii*) in children under two months of age has been found to reduce the likelihood of developing AD later, highlighting their protective role. During AD flare-ups, *S. aureus* levels rise on the skin of 90% of patients. *S. aureus* produces alpha toxins that lyse epidermal cells and proteases that break down connections between these cells and activate T cells, intensifying inflammation. These effects further damage the skin's protective barrier, causing water loss and greater exposure to environmental factors.

Current antibacterial treatments typically rely on broad-spectrum agents like chlorhexidine. However, because these are nonspecific, they remove not only *S. aureus* but also other beneficial bacteria. Antibiotic treatments, such as mupirocin or fusidic acid, can disrupt the entire microbiota and increase the risk of *S. aureus* developing antibiotic resistance, which can spread across entire bacterial populations. Notably, 94% of *S. aureus* strains isolated from AD patients who had used fusidic acid for three months were found to have become resistant to it. As a result, modern AD treatments focus primarily on: (i) reducing inflammation with steroids and cyclosporine, (ii) blocking the interleukin-13 signaling pathway, (iii) moisturizing and softening the skin to restore its natural barrier, and, more recently, (iv) restoring a healthy microbiota balance.

Modern therapies

Among the many innovative methods – still in the research phase – for combating the excessive colonization of *Staphylococcus aureus* on the skin of patients with atopic dermatitis (AD) is the use of its natural competitors: commensal bacteria that form part of the normal microbiota. Staphylococcal species like *S. epidermidis*, *S. hominis*, *S. warneri*, and *S. capitis*, taken from healthy skin areas of AD patients, were cultured and then applied to irritated skin areas on the same individuals (a procedure known as *autologous skin microbiota transplantation*), resulting in a 99% reduction in the number of in *S. aureus* cells. Similar effects were achieved by transplanting the Gram-negative bacterium *Roseomonas mucosa* from healthy donors onto AD skin or by modulating the microbiota composition through the application of prebiotics, probiotics, and postbiotics.

Targeted antibacterial therapies that selectively eliminate *S. aureus* strains have been under investigation for some time now. *Enzybiotics* – enzymes that recognize specific cell wall components, such as those unique to *S. aureus*, lysing only this species without harming other microbiota members – are promising candidates for such selective treatments. This selective removal of a single pathogen allows the microbiota to recover its balance, boosting the growth of commensal bacteria and restoring the skin's natural protective barrier, thus promoting gradual healing. Importantly, these enzymes are safe for humans, animals, and the environment. Furthermore, as the medical community contends with the rising issue of antibiotic resistance, enzybiotics are seen as next-generation antibacterial agents. They pose a lower risk of inducing resistance as compared to antibiotics, offering hope for avoiding a crisis in the treatment of many infections.

Despite extensive research worldwide on bacteriolytic enzymes, most of these technologies have yet to demonstrate effectiveness beyond *in vivo* studies on animal models. The only protein-based product currently on the market that can modulate the skin microbiota is Staphefekt, registered as a dermocosmetic in the Gladskin line. It has been shown to effectively eliminate *S. aureus* cells without affecting *S. epidermidis*, and further studies on this enzybiotic have reached the clinical trial stage.

> We should not treat the microorganisms that inhabit our skin as freeloaders, stowaway passengers, or intruders, but more like friendly neighbors, forming a community with us.

Our team has been working for years on enzybiotics that selectively eliminate pathogenic bacteria, including *S. aureus*, while sparing bacteria essential to the natural skin flora. Our patented AuresineR* removes *S. aureus* without harming commensal bacteria like *S. epidermidis*. Furthermore, this enzybiotic offers competitive advantages over Staphefekt, thanks to its unique efficacy and speed of action. We are continuing research to confirm AuresineR's effectiveness in patients with atopic dermatitis, psoriasis, impetigo, and other conditions, with the hope that our enzybiotic will soon bring relief to patients. ■

*Patent PL243304 – Peptidoglycan hydrolase, compositions containing it, its applications, and method of hydrolysis utilizing it.

Further reading:

Byrd A., Belkaid Y., Segre J., The human skin microbiome, *Nature Reviews Microbiology* 16/2018, doi.org/10.1038/nrmicro.2017.157

Zhu Y., Yu X., Cheng G., Human skin bacterial microbiota homeostasis: A delicate balance between health and disease, *mLife* 2023, vol. 2(2), doi.org/10.1002/mlf2.12064

Swaney M.H., Kalan L.R., Living in Your Skin: Microbes, Molecules, and Mechanisms, *Infection and Immunity* 2021, vol. 89(10), doi.org/10.1128/iai.00695-20