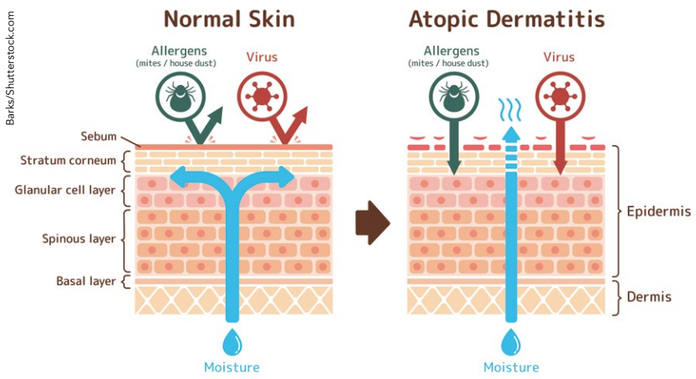
Taming *Staphylococcus aureus* in the eczema skin microbiome

Atopic dermatitis, commonly known as eczema, is a skin condition that affects millions of people globally. The bacteria Staphylococcus aureus *– a common and normally harmless member of the skin microbiome that can turn nasty in certain circumstances – has been linked to this condition. A team at the University of Michigan, USA, led by Drs Gabriel Núñez and Seitaro Nakagawa, in collaboration with Dr Greg Hillebrand of Amway Corporation, has conducted translational research into developing natural extracts of rosemary that could help disrupt the* S. aureus *virulence mechanisms involved in the itch, scaling, and redness of eczema and potentially other skin irritations.*

Atopic dermatitis – or atopic eczema – is a skin condition that affects around 5% of adults and 15–30% of children in industrialised countries. It is the most common type of eczema and is a chronic relapsing condition that affects many throughout their lifetime. The exact cause of eczema is not known but thought to involve multiple factors including compromised skin barrier function and skin microbiome imbalance. Eczema normally starts in childhood, with approximately 9.6 million children in the US experiencing it and 16.5 million adults experiencing atopic dermatitis that began after they were two years old (National Eczema Association, 2021).

Most often affecting the hands, insides of the elbows, neck, and legs, the condition can lead to sore, inflamed, itchy, and dry skin. Especially during eczema flares, the scratching of their itchy skin breaks down the skin's barrier function, leading to an increased risk of bacterial and viral infection. Many people with atopic dermatitis experience poor sleep due to the incessant itch and are embarrassed and self-conscious about the appearance of their skin, which can have a knock-on effect on mood and quality of life.



THE SKIN MICROBIOME AND ATOPIC DERMATITIS

The skin's primary role is to act as a physical and biochemical barrier to the outside world, protecting us from environmental stressors including invading pathogens. To that end, our skin plays host to a plethora of friendly microbes, including bacteria and yeast. This coexistence has no adverse effects and is often beneficial to us. However, these same friendly microbes can also turn on us. One particular species of bacteria that can be friend and foe alike, depending on the circumstances, is *Staphylococcus aureus*.

A round bacterium that is found in clusters, *S. aureus* can exist as a harmless part of the skin microbiome for a significant percentage of the human population. In this form, it acts as a commensal, colonising the skin without any harm to the host. However, *S. aureus* can switch personalities like Dr Jekyll into Mr Hyde and become harmful (virulent), earning the title of an 'opportunistic pathogen'. A well-known manifestation of this is methicillin-resistant *Staphylococcus aureus* (MRSA) infection, which was associated with 20,000 deaths in the US in 2017.

S. aureus is also thought to have a role in atopic dermatitis and other skin irritations. Previous data has suggested that 90% of atopic dermatitis patients have colonies of the bacteria present on the skin of their lesions, and biofilms of *S. aureus* have been directly associated with flare-ups of the condition.

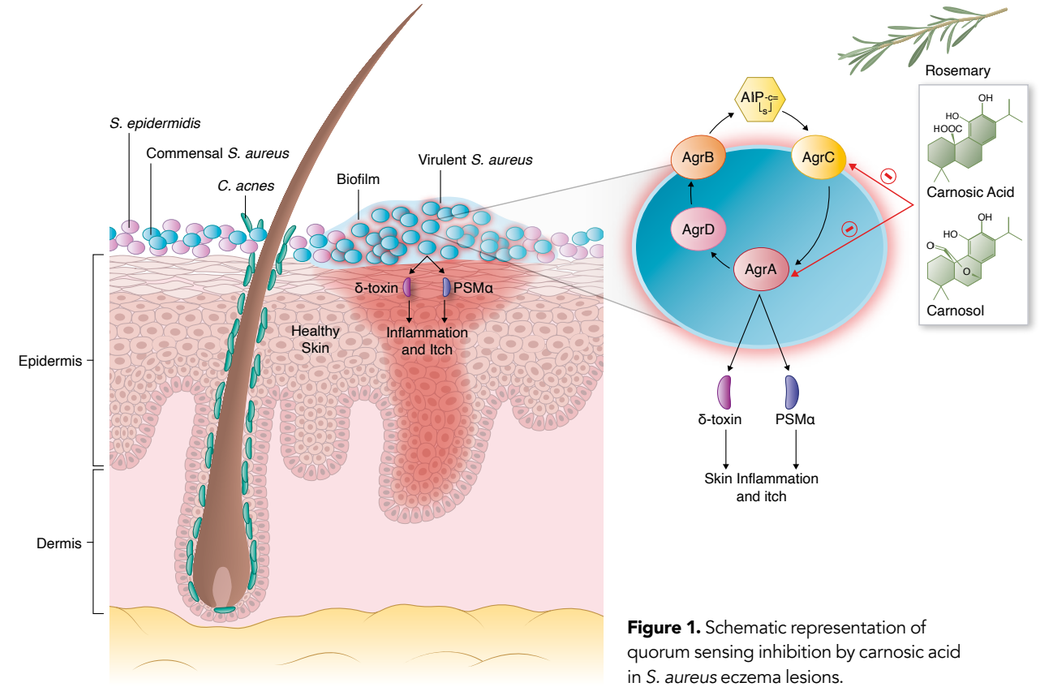


Figure 1. Schematic representation of quorum sensing inhibition by carnosic acid in *S. aureus* eczema lesions.

HOW DOES *S. AUREUS* SWITCH FROM HARMLESS TO HARMFUL?

The transformation of *S. aureus* from a harmless commensal to virulent pathogen involves bacterial cell-to-cell communication called quorum sensing. Once the density of *S. aureus* reaches a certain level, or quorum, the entire colony will change its behaviour from that of a friendly commensal to that of a pathogen. The biochemical mechanisms involved in quorum sensing are well understood and so targeted inhibition of quorum sensing is proving to be an attractive approach for preventing *S. aureus* virulence.

In *S. aureus*, quorum sensing is regulated by a section of the bacterial genome known as *agr*, which stands for accessory gene regulator. Two transcriptional units, RNAII and RNAIII, are coded for by agr. After RNAII and RNAIII are produced, they go on to trigger the production of virulence factors. These include δ-toxin and PSMα, which cause mast cell degranulation and release of histamine and keratinocyte alarmins, which produce skin inflammation and itch. Thus, compounds that inhibit *S. aureus agr* activation might be useful in treating and mitigating skin irritation and the symptoms of eczema.

HOW ROSEMARY CAN HELP INHIBIT QUORUM SENSING IN *S. AUREUS*

Humans are not the only ones who can come under attack by unruly bacteria – plants also face the threat of bacterial infection by common virulence mechanisms and, therefore, are our allies. Importantly, on an evolutionary scale, plants have been in this war a lot longer than we have and have evolved sophisticated strategies and compounds to combat bacterial infection, which we can study to use for our own benefit. One such plant is rosemary.

***S. aureus* can switch personalities like Dr Jekyll into Mr Hyde and become harmful, earning the title of an 'opportunistic pathogen'.**

Professor Gabriel Núñez, a molecular biologist and immunologist at the University of Michigan Medical School, screened over 4,000 compounds for *agr*-inhibiting activity. Of these, carnosic acid was found to be one of the most potent. Found in rosemary leaves at levels between 3 and 10%, carnosic acid is a diterpene that has been shown to have antioxidant and antimicrobial properties and is naturally present in several additives used in the food industry.

This discovery of carnosic acid as a potent *agr* inhibitor spurred Núñez and his colleague Dr Seitaro Nakagawa from the University of Michigan, and Dr Greg Hillebrand from Amway Corporation, to conduct further studies on this compound, as well as natural extracts of rosemary leaves containing carnosic acid. The findings from their research were published in the journal *Antibiotics* in 2020.

The team tested three different compounds found in rosemary leaves at high concentration for their effectiveness at inhibiting *agr*-stimulated virulence in *S. aureus*, the first being carnosic acid. The second compound they tested was carnosol, another diterpene that is a derivative of carnosic acid. The third

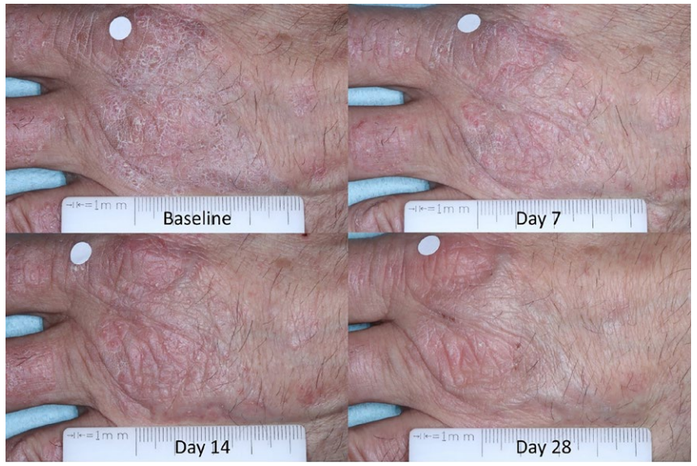


Figure 2. Good response of hand eczema to topical treatment with a formulation containing rosemary extract.

compound was rosmarinic acid, which likely is where rosemary got its name.

First, the team assessed the three pure compounds individually for how effective they were at inhibiting agr RNAIII gene expression in a laboratory strain of *S. aureus*. They found that both carnosic acid and carnosol significantly inhibited *agr* gene expression at a concentration as low as five micromolar (μM), indicating that these compounds were very potent. These two compounds were also found to significantly inhibit the expression of the *psmα* gene, which codes for the virulence factor *PSMα*. When rosmarinic acid was tested, it showed no significant inhibition of agr RNAIII or *psmα* gene expression, even up to 100μM. So, of the three most prominent compounds in rosemary, only carnosic acid and carnosol were quorum sensing inhibitors. None of the compounds showed synergy when combined with each other, which meant that they did not act together to produce more effective inhibition than if they were used alone. When the team tested nine different rosemary extracts containing all three compounds, all significantly inhibited RNAIII gene expression at concentrations as low as 5μg per ml.

The researchers then tested the effectiveness of rosemary extracts, carnosic acid, and carnosol in non-laboratory strains of *S. aureus* isolated from patients with atopic dermatitis. They stimulated these 'real world' bacteria to activate *agr* expression in the presence and absence of various concentrations of the test compounds. Three hours later, they collected the bacteria and isolated the mRNA. Quantitative real-time PCR (qPCR) was used to measure the expression of RNAIII and *psmα*. They found that all three test compounds inhibited these virulence genes. The researchers also measured the growth of the bacteria in the presence of each compound. The results showed that the extracts and compounds were *specifically* inhibiting agr expression and quorum sensing and not acting through an antimicrobial mechanism of action by blocking the growth of the organism. This is important because compounds that specifically target virulence rather than killing off the bacteria are less likely to induce bacterial resistance, a serious problem associated with antibiotic use.

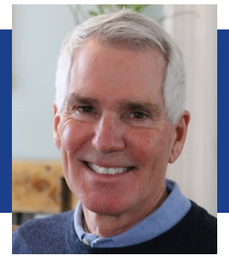
Using these plant-derived molecules to inhibit quorum sensing in S. aureus holds great potential.

The exact mechanism by which carnosic acid and carnosol blocks *agr* activation is not known. Carnosic acid could inhibit one or more of the several steps in the quorum-sensing signalling pathway from synthesis (AgrD) and export (AgrB) of the auto-inducing peptide (AIP) to the kinase receptor for AIP (AgrC) or the gene regulator (AgrA). However, since carnosic acid inhibits AIP-induced expression of both RNAIII and *psmα*, it likely inhibits AgrC or AgrA that act downstream from AgrD and AgrB (AIP synthesis and export) but upstream from both RNAIII and *psmα* gene expression (as shown in Figure 1).

TREATING PEOPLE WITH ATOPIC DERMATITIS AND OTHER SKIN IRRITATIONS

Most of the current treatments for atopic dermatitis have limited effectiveness, negative side effects, or are expensive. For example, first-line treatments with topical corticosteroids can be used to reduce inflammation during an eczema flare, but they can also cause thinning of the skin, leaving it more susceptible to damage. Antihistamines can also be used to relieve itching but have side effects including sleepiness and headaches. Injectables (eg, dupilumab) can be very effective but also very expensive. A promising new pharmaceutical approach using beneficial bacteria from healthy human skin is in early development but likely several years from approval.

Using natural plant-derived molecules with well-established safety profiles to inhibit quorum sensing in *S. aureus* holds great potential for treating and preventing eczema in a practical and cost-effective manner. In the future, compounds such as carnosic acid and carnosol (or rosemary extracts) could be incorporated into skincare formulations as active ingredients to specifically target flare-ups and occasional skin irritations where this bacterium has got a little out of hand. In fact, the first vehicle-controlled randomised clinical trial of a topical rosemary formulation for the treatment of eczema lesions was just completed by the Amway–University of Michigan team. The results look very promising and have been presented at a skin microbiome conference in Boston in November 2021, with a peer-reviewed paper describing the results of that study to be published soon. An example of the effectiveness of the new formulation containing rosemary extract in treating hand eczema is shown in Figure 2.



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Research Objectives

Drs Greg Hillebrand, Gabriel Núñez, and Seitaro Nakagawa research compounds found in rosemary which might prevent *Staphylococcus aureus*-related skin problems.

Detail

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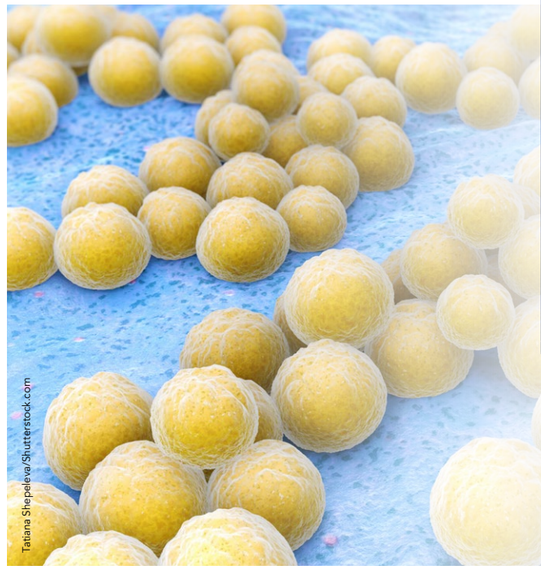
Bio
Dr Greg Hillebrand is a biochemist and currently Senior Principal Research Scientist at Amway, and Adjunct Professor of Cosmetic Science at the University of Cincinnati. With over 35 years of experience in the cosmetic industry, he leads research and development for Amway's microbiome-based skincare technology and products.

Funding
Amway Corporation, Ada, MI, USA

Collaborators
- Seitaro Nakagawa (University of Michigan)
- Gabriel Núñez (University of Michigan)



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Personal Response

What would be the next best steps to test the clinical efficacy of these compounds?

"We would develop several different topical rosemary formulation(s) to give eczema sufferers a variety of convenient solutions for their personal eczema symptoms, including both leave-on (creams and lotions) and wash-off products (body shampoos, bath additives, etc). Efficacy would be demonstrated in well-designed vehicle-controlled randomised double-blind studies for both treating and preventing flares, itch, and skin irritation in both adults and children."