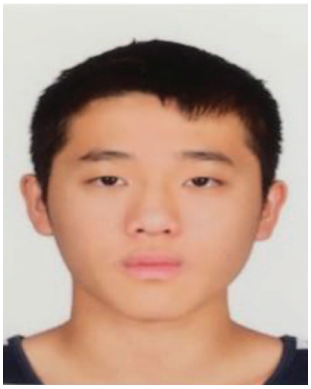
# Amplification of probiotic bacteria in the skin microbiome to combat *Staphylococcus aureus* infection

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**Abstract.** ***Staphylococcus aureus*** **(*S. aureus*) is a Gram-positive bacterium. When pathogenic *S. aureus* colonises onto a skin wound or diabetic ulcer, it can cause a serious infection and lead to amputation or death. The current solutions (e.g. antibiotics and probiotics) are not sufficient enough to be a cure for this infection. To worsen the situation, the *S. aureus* bacteria continue to develop greater resistance towards antibiotics and are becoming more commonplace. An effective solution is to amplify the activity of probiotic bacteria in the skin microbiome by using selective fermentation initiators (SFIs) to induce fermentation. Our data demonstrated that the numbers of *Cutibacterium acnes* (*C. acnes*) and *Staphylococcus epidermidis* (*S. epidermidis*), two major bacteria in skin microbiome, on human skin did not vary significantly over the span of seven days. This stimulates probiotic bacteria such as *S. epidermidis* to produce sufficient short-chain fatty acids (SCFAs) to suppress the growth of *S. aureus*. The development of this new cure to *S. aureus* may reduce hospitalisation greatly as *S. aureus* accounts for the hospitalisation of more than five thousand people per year. Besides antibiotic, probiotics and bacteriophages, SFIs may become novel agents for treatment of infection.**

## Skin microbiome and dysbiosis

The skin microbiome comprises the microbiota in skin that is home to millions of bacteria, fungi and viruses[1]. Skin dysbiosis refers to a condition in which microbial imbalances occur in the skin microbiome[2,3]. Mounting evidence indicates that the probiotic microbes in the human microbiome can employ bacterial interference[4] to rein in the overgrowth of opportunistic pathogens[5,6]. However, little is known about the interactions among probiotic bacteria within the human microbiome for maintaining homeostasis of the microbiome. Bacterial interference, used by probiotic *Staphylococcus epidermidis*, prevents growth of pathogens and has shown to be a promising modality for preventing and/or treating infections. Literature has demonstrated that *Cutibacterium acnes* and *S. epidermidis*, two major bacteria in the skin microbiome[7–9], can fermentatively metabolise

glycerol, a naturally occurring metabolite found in human skin[10], to repel the over-growth of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Our results showed the abundances of both *C. acnes* and *S. epidermidis* on the skin surface of the same person have no significant changes from Day 1 to Day 7 (Figure 1), indicating the stability of commensal bacteria in skin. The stability of abundances of commensal bacteria in skin will make it possible to apply a fixed dose of prebiotic to induce fermentation. SCFAs are one of metabolites of glycerol fermentation of *C. acnes* and *S. epidermidis*. Several SCFAs have been approved by the U.S. Environmental Protection Agency (EPA) or the Food and Drug Administration (FDA) as active compounds for use as antimicrobials[11–13]. It has been illustrated that a specific SCFA, butyric acid, can diminish inflammation via inhibition of histone deacetylase (HDAC) in host cells[14], suggesting the dual antimicrobial and anti-inflammatory abilities of SCFAs.

## *S. aureus* infection in diabetic wounds

Infection of the skin by *S. aureus* is a major cause of hospitalisation and can cause death and organ failure. It is estimated to account for the outpatient visits of 12 million people per year, worldwide, and the problem continues to grow. Furthermore, doctors consistently rely on the use of antibiotics, resulting in the development of MRSA. MRSA is a major issue among people with diabetic ulcers[15]. Diabetic ulcers occur in 15% of people with diabetes, creating wounds that permit pathogens to enter the body, with one of the most common pathogens being MRSA. Already in a frail state, due to poor blood flow in the ulcer, a pathogenic infection impedes the healing of diabetic ulcers, and the spread of such infections to soft tissue or bony structures often results in the need for amputation. Considering these possible outcomes, the estimated 30% of diabetic ulcers that are colonised with MRSA means that MRSA is among the most common causes of amputation. *S. aureus* poses a potent threat not only to diabetic patients, but to healthy, normally functioning people as well. Not only can *S. aureus* enter diabetic ulcers, but also into traumatic skin wounds, which can lead to persistent tissue infection that occasionally progresses to systemic infection and death. Furthermore, MRSA is easily transferred. A mere touch of the infected skin or a touch of even an object that has come in contact with the infected skin can spread this infection. As antibiotics can only serve to be a temporary solution to this problem, scientists continue to propose new solutions to the ongoing issue.

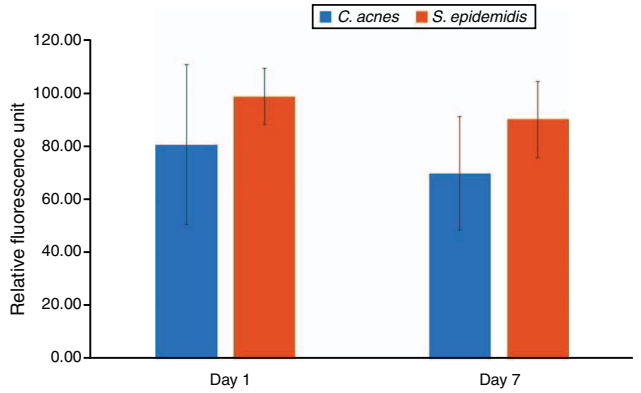


Figure 1. The abundance of *C. acnes* and *S. epidermidis* on the skin surface. Skin swabs from the arm skin surface (5 cm x 5 cm) were collected on Day 1 and Day 7 and submerged into 100 µl Saliva DNA lysis buffer (Norgen Biotek Corp., ON, Canada) immediately. The sample was diluted 10x with distilled water, loaded onto a GeneScan™ chip for bacterial identification using the 16s RNA sequencing. The fluorescence reading on the y-axis was generated by the GeneScan™ software based on fluorescence signal detected by the system (www.ameridx.com). The data was plotted manually by Excel software. Primers pairs for specific 16S rRNA gene amplification were GGGTTGTAAACCGCTTTCGCCT and GGCACACCCATCTCT GAGCAC for *C. acnes* and GCACGTAGTTAGCCGTGGCTTTCTG and CTTATAGATGGATCCGCGCCGCATT for *S. epidermidis*. The mean ± standard derivation for three separate samples was calculated. A two-tailed t-test was used for statistical analysis.

## Possible problems of antibiotic, probiotic and bacteriophage for treatment of *S. aureus* skin infection

The use of antibiotics has provided an accessible and successful solution to almost all bacterial infections. However, antibiotics, if overused, can result in the development of antibiotic-resistant bacteria, which deems antibiotics to be undesirable for long-term management of bacterial infections. The emergence of MRSA provides a clear example of the shortcoming of this approach. The problems of antimicrobial resistance are discussed in the May 2019 issue of *Microbiology Australia*, while '*S. aureus*' drug resistance was part of the theme in September 2008. The use of probiotics represents a potential solution to this problem. Probiotics are essentially symbiotic microorganisms that outcompete pathogenic bacteria[16]. Adding probiotic bacteria to human skin will shift the course of infection leading to the balanced ratio of bacteria. As addressed earlier, *S. aureus* is an infection on the skin. However, the FDA prohibits the application of probiotics on the skin because probiotics are live bacteria and entrance of live bacteria into the bloodstream can cause other infections leading to death. Thus, probiotics can only be present in edible items such as yogurt and currently, does not represent a viable treatment for *S. aureus* infection. The last of the current solutions to combat dysbiosis would be the use of bacteriophage. Bacteriophage are viruses that selectively kill certain bacterial species[17]. Although this represents a creative approach to replace antibiotics, it has been reported that there are certain limitations inherent in bacteriophage therapy[18].

## Prebiotic as a bacteria-specific carbon source for fermentation

The use of prebiotics represents a potential solution to the existing problems facing the management of MRSA infection. This approach essentially consists of assisting the beneficial or probiotic bacteria, while weakening pathological or undesirable bacteria. The fact that not all people who come in contact with *S. aureus* get an infection implies the existence of endogenous mechanisms preventing infection. In general, commensal bacteria use a carbon source derived from human cells (e.g. fibre or glucose) to make SCFAs such acetic acid and butyric acid via fermentation[19,20]. Among other things, these SCFAs can serve as 'microbial weapons' by which certain bacterial strains can inhibit the growth of competing species. If harmful bacteria overwhelm the probiotic bacteria, this may result in an infection or injury from pathogens. If the probiotic bacteria overwhelm the pathogens, the person would be safe from injury. The imbalance of bacteria in the microbiome is referred to as dysbiosis, resulting in pathologic infection. As current treatments proved ineffective against *S. aureus*, a new solution (Figure 2) to this problem would be to provide a defined prebiotic as a carbon source, also named a selective fermentation initiator (SFI), to selectively induce fermentation of probiotic bacteria. Pathogens and the probiotic bacteria in humans each have different enzymes to yield different SCFAs. This results from the fact that there are certain carbon sources that only the probiotic bacteria can ferment to combat pathogens. Due to differences in the enzymes of probiotics and pathogens, there are certain sources in which only the probiotics can utilise to ferment and produce SCFAs. Such carbon sources would be SFIs.

Different bacterial species make different enzymes that ferment specific carbon sources. All *S. aureus*, *S. epidermidis* and *C. acnes* can ferment glucose to SCFAs[21–23]. To gain maximum survival advantage, *S. aureus* and *S. epidermidis*/*C. acnes* that co-exist within a diabetic ulcer[24,25] exclude each other via production of SCFAs by fermentation of glucose. When *S. aureus* survives after competitive bacterial interference the infection *will* proceed to continue to damage the host. However, polyethylene glycol dimethacrylate (PEG-DMA) has been developed as a SFI that can specifically intensify fermentation activity of *S. epidermidis*, but not *S. aureus*[26,27]. The exclusive induction of the fermentation of *S. epidermidis* by PEG-DMA amplified the probiotic activity of *S. epidermidis* against *S. aureus*.

In a skin wound or diabetic ulcer, the microbiome is comprised of probiotic bacteria and *S. aureus* where probiotic bacteria act to inhibit the proliferation of *S. aureus*. The prebiotic strategy would result in the cultivation of fermentation specifically in probiotic bacteria such as *S. epidermidis*, amplifying their activity against *S. aureus* within diabetic ulcers. The probiotic bacteria metabolising these SFIs will create SCFAs via fermentation that prevent pathogens from entering skin wounds. SFIs do not eliminate all bacteria like antibiotics, therefore it would not leave the wound susceptible to opportunistic pathogens. Furthermore, since SFIs do not kill the pathogens directly, pathogens cannot develop resistance. SFIs also represent a more feasible solution compared to probiotics, since SFIs are not live entities, would not cause infection and therefore could be applied on the skin. Therefore, SFIs could be the most plausible solution to MRSA infections in diabetic ulcers. SFIs can potentially reduce hospitalisation, the need for amputations, and delays for healing diabetic ulcers.

## Conclusion

The technology of bacterial fermentation has been widely employed in the development of various products including yogurt, wine, and vinegar. The concept of using SFI to activate the fermenting probiotic bacteria against *S. aureus* and restore the dysbiotic skin microbiome not only may inspire the next generation probiotic/prebiotic-based medicine but also defines novel roles of

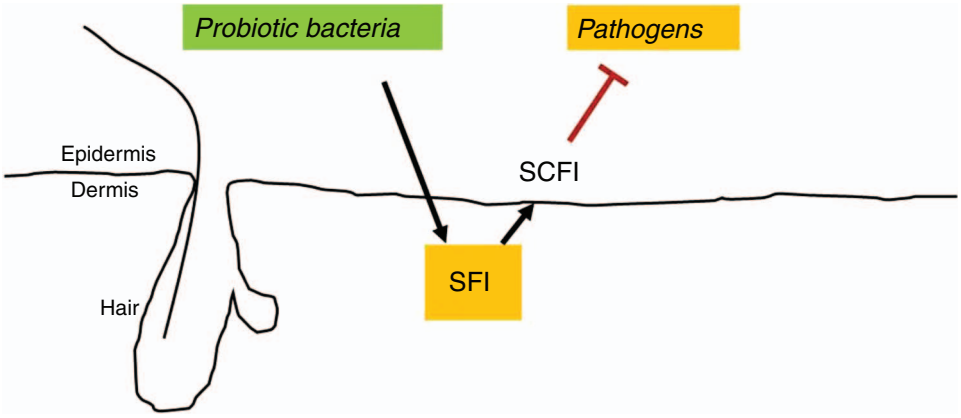


Figure 2. Probiotic bacteria mediate SFI fermentation to produce SCFA to decolonise pathogens in skin. Fermenting bacteria in skin can use SFI as a carbon source to undergo fermentation and produce SCFA which has antimicrobial activity to eliminate pathogens in the skin.

probiotic bacteria and their associated prebiotics in the innate immunity of the skin against *S. aureus* infections.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgements

We thank Christopher M Bates, an Assistant Professor at Materials Department, University of California, Santa Barbara, for discussing the feasibility of using PEG-based compounds as a skin probiotic. This research did not receive any specific funding.

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## Biographies

**Tristan Yusho Huang** is a rising senior student at Canyon Crest Academy and conducting research as an intern at Department of Dermatology, University of California, San Diego. His project is working on the use of prebiotic as a carbon source for bacterial fermentation and electricity production.

**Deron Raymond Herr** is an Assistant Professor at Department of Pharmacology, National University of Singapore. His research focused on the metabolism and signal transduction of bioactive lipids, specifically, sphingosine 1-phosphate and lysophosphatidic acid. He is working on validation of lipids as a skin prebiotic.

**Chun-Ming Huang** is a Professor at Department of Biomedical Sciences and Engineering, National Central University and Department of Dermatology, University of California, San Diego. His research is centering on the skin microbiome and its association with skin diseases such as acne vulgaris. He has isolated various skin probiotic bacteria for establishment of a skin probiotic bank, which is used as a platform to screen bacteria-specific prebiotic.

**Yong Jiang** is a CEO at America Diagnosis, Inc., San Diego. Dr Jiang has developed a DNA chip for high-throughput screening the relative abundance of microbes in the human microbiome.