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RESEARCH HIGHLIGHT

Probiotic fengycins dis(Agr)ee with *Staphylococcus aureus* colonization

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Competition between bacterial species can mediate protection against infection, albeit by mechanisms that are largely unknown. A recent study in *Nature* by Piewngam et al. demonstrates that a *Bacillus* lipopeptide, fengycin, restricts intestinal *Staphylococcus aureus* colonization by inhibiting quorum sensing.

The human pathogen *Staphylococcus aureus* utilizes an extensive arsenal of virulence factors to cause substantial morbidity and mortality worldwide.[1] Although *S. aureus* infections typically arise following asymptomatic nasal or skin carriage, recent studies have proposed that intestinal carriage also serves as an important reservoir.

The intestinal tract harbors a multitude of microbes, collectively known as the microbiota, which confers benefits to the host by promoting a variety of functions including immune development and colonization resistance to pathogens, albeit by mechanisms that are largely unknown.[2] In a recent issue of *Nature*, Piewngam and colleagues identified an inverse correlation between human colonization with *Bacillus* species and with *S. aureus*, and subsequently discovered a primary mechanism by which *Bacillus* species can exclude *S. aureus* from the gut: inhibition of quorum sensing.[3]

The authors initially hypothesized that gut microbiota composition could alter colonization by *S. aureus*. Intriguingly, analysis of fecal samples from a rural Thai population revealed a strong correlation between the presence of *Bacillus* spp. in the gut and the exclusion of both intestinal and nasal colonization by *S. aureus*. The genus *Bacillus* comprises Gram-positive soil bacteria that form endospores. Upon ingestion (e.g., on vegetables), the spores germinate into vegetative cells, allowing the bacteria to temporarily colonize the gut. Of note, some *Bacillus* species are included in probiotic preparations, although the mechanisms by which they exhibit beneficial activities are not well understood. The absence of *S. aureus* colonization in individuals where *Bacillus* was present in the gut led the authors to hypothesize that *Bacillus* species could produce a factor that directly targeted *S. aureus*, akin to bacteriocins secreted by commensal enterococci and to microcins secreted by probiotic *E. coli* Nissle 1917.[4,5] However, culture filtrates from *Bacillus* isolates generally failed to exhibit direct antimicrobial activity against *S. aureus*, indicating that an alternative mechanism of bacterial competition was being employed.

Prompted by, and extrapolating upon, relatively limited information on intestinal colonization by *S. aureus*, the authors hypothesized that *Bacillus* species were inhibiting the *S. aureus* accessory gene regulator (Agr) quorum-sensing system. The ability to sense and to respond to changes in cell density is known as quorum sensing, a function that allows bacteria to coordinate population-level changes in gene expression, including genes involved in pathogenesis.[6] In *S. aureus*, the expression of numerous virulence factors is primarily mediated by Agr. On this front, the authors investigated whether *S. aureus* required Agr in a mouse model of intestinal colonization. Subsequent experiments using wild-type *S. aureus* and an isogenic *agr* mutant revealed that the mutant strain had a severe defect in colonizing the murine intestine, a defect that could be rescued by ectopic expression of the Agr effector, RNAIII. Taken together, these findings revealed an essential role for Agr-dependent quorum sensing during *S. aureus* colonization of the gut.

Next, Piewngam et al. discovered that *Bacillus subtilis* reduced *S. aureus* Agr activity in vitro. Meticulous chromatography and mass spectrometry analyses identified fengycins, specific classes of lipopeptides with antifungal activity,[7] as potential Agr-targeting candidates secreted by *Bacillus* species. Despite the heterogeneity of fengycin profiles among *Bacillus* isolates, the predominant fengycin species was identified as β-OH-C17-fengycin B. Importantly, culture filtrates from a *B. subtilis* strain defective in fengycin synthesis failed to impede Agr signaling, indicating that the lipopeptide accounts for the Agr-inhibiting activity of *B. subtilis*. Induction of *S. aureus* Agr is initiated by interaction of Agr autoinducing peptides (AIPs) with the extracellular quorum-sensing receptor AgrC. As fengycins exhibit structural similarity to AIPs, AgrC interference appeared to be a likely mechanism of quorum-sensing inhibition. In line with this, exogenous addition of AIPs reduced fengycin-mediated Agr inhibition in a dose-dependent fashion in vitro.

To assess whether *Bacillus* fengycins were inhibiting intestinal colonization by *S. aureus*, mice were orally inoculated with wild-type and fengycin-mutant (Δ*fenA*) *B. subtilis* spores, mimicking the typical route of probiotic administration. Levels of *Bacillus* within the murine intestine were comparable between wild-type and the fengycin mutant, indicating that loss of fengycin production did not adversely affect *Bacillus* colonization. As predicted, wild-type *B. subtilis* spores abolished *S. aureus* colonization of the murine intestine (Fig. 1a), whereas spores from the fengycin mutant did not (Fig. 1b). Altogether, the murine model recapitulated the *S. aureus* colonization trend observed in humans and enabled the identification of fengycin-mediated quorum-sensing disruption as a mechanism providing colonization resistance against *S. aureus*. This finding is

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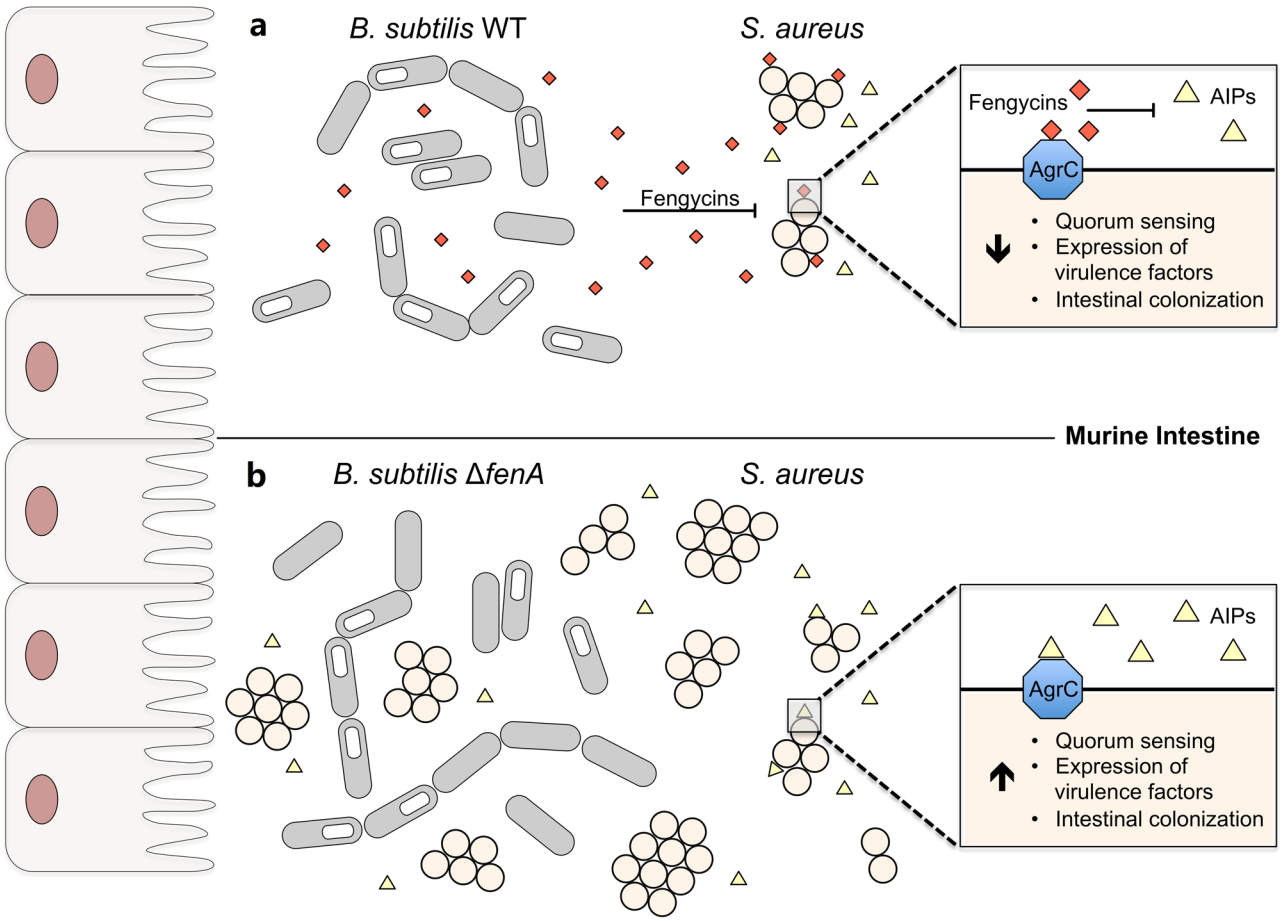

Fig. 1 *Bacillus* eliminates intestinal *Staphylococcus aureus* colonization. **a** Fengycins produced by wild-type *B. subtilis* compete with Agr autoinducing peptides (AIPs) and block AgrC-dependent quorum sensing in *S. aureus*, thus reducing the expression of virulence factors and eliminating intestinal colonization by the pathogen. **b** A *B. subtilis* mutant defective in fengycin synthesis (Δ*fenA*) fails to inhibit *S. aureus* quorum sensing, virulence factor expression, and colonization

particularly relevant for human health, as fengycins also exhibited activity against the high-risk methicillin-resistant USA300 clone.

The rapid emergence of antibiotic resistance worldwide necessitates the development of alternative therapeutic strategies. The study by Piewngam and colleagues provides an attractive method for treating infections as well as raises intriguing possibilities, such as leveraging probiotics to decolonize asymptomatic carriers of a pathogen. Moving forward, it will be important to determine how limited fengycins are in their spectrum of activity, as well as the propensity for fengycin resistance to emerge in the context of therapeutic administration. Separately, it is essential to continue investigations into additional mechanisms of bacterial competition that can be harnessed to target *S. aureus*. Previously identified colonization resistance mechanisms against *S. aureus* in the skin and in the nasal mucosa include quorum-sensing inhibitors and other antimicrobial molecules produced by commensal staphylococci.[8–10] It remains to be determined whether any of these mechanisms also function to hinder *S. aureus* intestinal colonization of individuals without detectable *Bacillus*. Nevertheless, the current findings by Piewngam et al. provide valuable insights into the importance of bacterial competition during infection, which may be utilized for the development of novel narrow-spectrum therapeutics.

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