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審査研究科	人間総合科学研究科
学位論文題目	Study on Accessory Gene Regulator (AGR) Variants in *Staphylococcus aureus* (黄色ブドウ球菌の病原性マスターレギュレータ Agr 系の相変異の研究)

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Abstract of thesis

Staphylococcus aureus is responsible for a broad range of infections and uses a vast arsenal of virulence factors that facilitate its invasion into and survival within the human host. However, avirulent strains are frequently isolated as the cause of clinical infections. The transition from a commensal to a pathogenic state is driven by the induced expression of virulence factors, controlled by the accessory gene regulator (Agr) system. In this doctoral dissertation, the author Vishal Samir Gor attempts to dissect the underlying mechanism of how Agr genes are reactivated to establish virulence. The author screened Agr-revertant strains derived from Agr-negative avirulent strains and sequenced the revertant strains' genome to uncover underlying genetic mechanisms for Agr shutdown. The author showed that a fraction of Agr-negative strains could repair the mutations in the *agr* locus either by alterations within short sequence repeats or by site-specific rearrangements. The author further determined that Agr-revertant strains sustain an Agr-OFF state as long as they exist as a minority in the population. However, it can activate their Agr system on a solid medium or upon phagocytosis. Based on findings, the author suggests that revertant cells might function as a cryptic insurance strategy to survive immune-mediated host stress during infection.

Purpose: *Staphylococcus aureus* is a Gram-positive coccoid bacterium that creates a heavy economic and healthcare burden for humans. *S. aureus* is an opportunistic pathogen that can proficiently establish infections in various organs

causing superficial skin abscesses to severe bacteremia and toxic shock syndrome in humans. With its rapid development of antimicrobial resistance, *S. aureus* is a significant health burden globally. The ability for *S. aureus* to survive in multiple environments is primarily due to its impressive array of virulence factors. The expression of these virulence factors is controlled by the central virulence regulatory network called the Agr system. Although the Agr system plays critical roles in virulence regulation, it is known to be genetically unstable. The Agr-negative strains are frequently isolated from patients and are linked to more severe infection outcomes, associated with higher mortality and increased bacteremia duration. Previous reports have documented Agr mutations reminiscent of phase variation, a bacterial mechanism of reversible gene expression. In this study, the author hypothesized that the phenomenon of Agr dysfunction emerges from reversible phase variation and constitutes a mechanism of heterogeneity, resulting in the adaptation of *S. aureus* beyond the stress response.

Materials and Methods: Agr-negative variants were generated from two *S. aureus* strains: MW2 and s0437. The author screened for Agr revertant strains by subjecting Agr-negative variants to successive liquid cultures before plating on Sheep Blood Agar (SBA) and assayed for haemolytic colonies. The Agr status of haemolytic colonies was confirmed by a modified CAMP test and by semi-quantitative RT-PCR. A total of 61 of the 173 clinical isolates were also characterized as being Agr negative by the CAMP test. Phenotypic Agr revertant strains were sequenced at their Agr locus to identify any newly-acquired mutations. A fluorescent reporter construct was used to monitor Agr activity in populations growing in planktonic and solid structured media. The same reporter construct was also used to monitor Agr activity during phagocytosis.

Results: Agr revertant strains were generated from two laboratory strains (MW2 and s0437) and two clinical strains (66r and 3082). Underlying genetic phase variation mechanisms responsible for the phenotypic reversion were identified amongst MW2, s0437, and 66r. By serially passaging Agr-negative strains and screening for phenotypic reversion of hemolysis and subsequent sequencing of the genome, the author identified two recombination events responsible for generating a reversion phenotype; a genetic duplication plus inversion event in *agrC* locus, and a poly(A) tract alteration in *agrA* locus. The author also showed that one clinical Agr-negative methicillin-resistant *S. aureus* isolates could reproducibly generate Agr-revertant colonies with a poly(A) tract alteration in *agrA* locus. Using fluorescent reporter system to monitor Agr activity, author demonstrated that while revertant cells are generated at a low frequency, allowing them to sustain an Agr OFF state in planktonic culture, they can activate their Agr system upon phagocytosis by macrophages or growth in sold media.

Discussion: In this doctoral dissertation thesis, the author demonstrated that a fraction of Agr negative strains could activate the Agr system with underlying genetic mechanisms reminiscent of phase variation. This is the first report demonstrating that Agr-negative strains can revert their Agr activity. The Agr revertant cells are generated at a low frequency in discrete local microenvironments upon growth on solid structured media, suggesting that local Agr activation could be important for the exodus from biofilms. Furthermore, the author suggests that the ability of revertant strains to activate their Agr system upon phagocytosis could enable *S. aureus* to survive from host immune attack and disseminate to other sites of the body for continued infection.

Abstract of assessment result

General Comments: Based on the results, the applicant proposes a model whereby Agr phase variation acts as a predictive adaptation mechanism to ensure against host-mediated immune stress. The applicant showed that *S. aureus* might undergo phase variation to activate Agr gene expression from Agr deficient strains through genetic recombination events within the *agr* locus. The finding that *agr* revertant activates the Agr system upon phagocytosis in the macrophage suggests that *S. aureus* could escape the host immune response to infect other parts of the body. Although the applicant was unable to describe the prevalence of Agr phase variants in the clinical setting, he also identified strains that could revert their haemolytic phenotype independently of Agr function. This work provides new insights into understanding the molecular mechanism of staphylococcal virulence.

Assessment: The final examination committee conducted a meeting as a final examination on December 22, 2020. The applicant provided an overview of a dissertation, addressed questions and comments raised during the Q&A session. All of the committee members reached a final decision that the candidate has passed the final examination.

Conclusion : The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.