Major Transcriptional Modulations in *Staphylococcus aureus* Induced by the *Candida albicans* Secreted Quorum Sensing Molecule Farnesol

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OBJECTIVES

In polymicrobial infections the presence of one microorganism may contribute to increase the pathogenesis of another. *Candida albicans* and the bacterial pathogen *Staphylococcus aureus* are the most frequent combination of microorganisms isolated from the human host. Our preliminary studies had described the complex and dynamic interactions between these two pathogens with important clinical and therapeutic implications. In this study, we aimed to elucidate the mechanisms orchestrating inter-species interactions with pathogenic implications, namely those mediated by quorum sensing molecules. Specifically, we investigated the impact of the *C. albicans* secreted quorum sensing molecule farnesol on *S. aureus* on a molecular level.

METHODS

Cells exposed to farnesol were stained for intracellular ROS. A *S. aureus* farnesol-sensitized phenotype was generated through sequential passaging in farnesol supplemented growth media. The sensitized strain was then desensitized by gradual removal of the exogenous farnesol. Passaged cells and their respective controls were recovered and RNA was extracted from cells. Comprehensive transcriptional analysis was performed on the farnesol-sensitized and desensitized cells and controls by RNA sequencing followed by functional categorization of differentially expressed genes.

RESULTS

Fluorescence microscopy demonstrated accumulation of intracellular ROS in the farnesol treated cells (Fig. 1). Comparative transcriptional analysis of sensitized and desensitized cells relative to each other and the respective controls demonstrated major transcriptome alterations involving key regulatory pathways. A total of 769 genes were found to be differentially regulated in the farnesol-sensitized cells compared to control cells and 492 in the de-sensitized compared to sensitized cells. Most notable among the up-regulated genes in the farnesol sensitized cells are redox sensors and global regulators involved in ROS detoxification *via* the thiol-specific redox system (Fig. 2, 3), specifically, the oxidant sensors SarA and Spx that induce transcription of antioxidant enzymes such superoxide dismutases (SODs), catalase (KatA) and alkyl hydroperoxide reductase (Ahp). In *S. aureus*, once ROS mediated cell damage occurs, cells express several enzymes including thioredoxin/thioredoxin reductase (Trx) and methionine sulfoxide reductases (Msr) to repair protein damage. Interestingly, in desensitized cells, expression of the global regulators SpxA and SarA remained high, even after the external pressure with farnesol is removed. Functional categorization of genes in sensitized cells indicated down-regulation of various metabolic and biosynthesis pathways, as well as pathogenesis associated genes. In contrast, in desensitized cells, genes involved in *S. aureus* virulence such as hemolysins, toxins, host tissue binding proteins and cell wall adhesins were significantly upregulated compared to the sensitized cells, as well as their control cells not previously exposed to farnesol.

Fig.1. Farnesol induced Intracellular ROS accumulation

Fig. 2. Farnesol induced up-regulation of ROS detoxification enzymes under control of redox sensors and global regulators.

Fig. 4. Gene ontology enrichment analysis of genes (A) down-regulated in farnesol sensitized cells compared to control cells and (B) up-regulated in farnesol desensitized compared to sensitized cells.

Fig. 3. Thiol-based redox system

CONCLUSIONS AND FUTURE DIRECTIONS:

The findings from this study demonstrate that farnesol induces a stress response by the bacterial cell *via* the thiol-based redox system under control of major global regulators of virulence. Collectively, the findings support the hypothesis that farnesol-induced transcriptional modulations of key regulatory networks and pathways may impact *S. aureus* fitness and virulence, and, in turn, the pathogenesis of mixed *C. albicans*-*S. aureus* infections. Functional analyses and *in vivo* studies to evaluate the pathogenic potential of the farnesol sensitized cells are underway using mouse models of systemic staphylococcal infection.