Role of *rsbU* and Staphyloxanthin (SFX) in Intracellular Growth of *Staphylococcus aureus* in Human Phagocytes (THP-1 macrophages)

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ABSTRACT

Background and aims: *S. aureus* intracellular survival is critical for persistence of infection. *rsbU* expression stimulates the production of SFX, a yellow pigment that protects *S. aureus* against oxidant damage (Science 2008; 319:1391-1394). We have examined the role of *rsbU* and SFX in phagocytosis and growth of *S. aureus* in phagocytic cells.

Methods: Bacteria: strain 8325-4 (natural deletion in *rsbU*) and SH1000 (isogenic *rsbU*+ construct); USA300. Cells: THP-1 macrophages cultured and infected as previously described (AAC 2008; 52:2797-805). Impairment of SFX synthesis: BPH-652 (3-phenoxy-alpha-phosphono-benzenebutanesulfonic acid [dehydrosqualene inhibitor]).

Results: 8325-4 produced no SFX whereas SH1000 and USA300 were pigmented. BPH-652 impaired pigmentation of both SH1000 and USA300 at 1 µM in MH broth. SH1000 was more resistant to inactivation by hydrogen peroxide; intracellular growth SH1000 and USA300 were more intense than that of 8325-4, and addition of BPH (100 µM 48 h prior to phagocytosis [broth] and during intracellular growth [culture medium]) reduced their growth to the level of that of 8325-4.

Conclusion: *rsbU* functionality and SFX production is an important factor in promoting intracellular growth of *S. aureus* in macrophages. This effect may be due to SFX-mediated resistance to oxidative stress. Inhibition of SFX synthesis may help in controlling intracellular *S. aureus* infection.

INTRODUCTION

- *Staphylococcus aureus* produces several virulence factors that induce immediate local and general damage during infection.
- Their expression is modulated after *S. aureus* has been phagocytized, allowing for growth within the host cells [1]. Understanding this process and interfering with the production of protecting factors may therefore be a useful strategy to better control *S. aureus* infection and reduce its persistence in infected patients.
- rsbU is a phosphatase that positively controls σ^B, which itself down-regulates the expression of *agr* [2]. *agr* is a regulator of *S. aureus* virulence and a transcriptional factor that plays a central role in stress response [2] and persistence of infection, *in vivo* [3].
- The lack of expression *rsbU* is associated with reduced H_2O_2 tolerance related to impairment of the biosynthesis of staphyloxanthin (SFX) [5], a carotenoid pigment that acts as an antioxidant.

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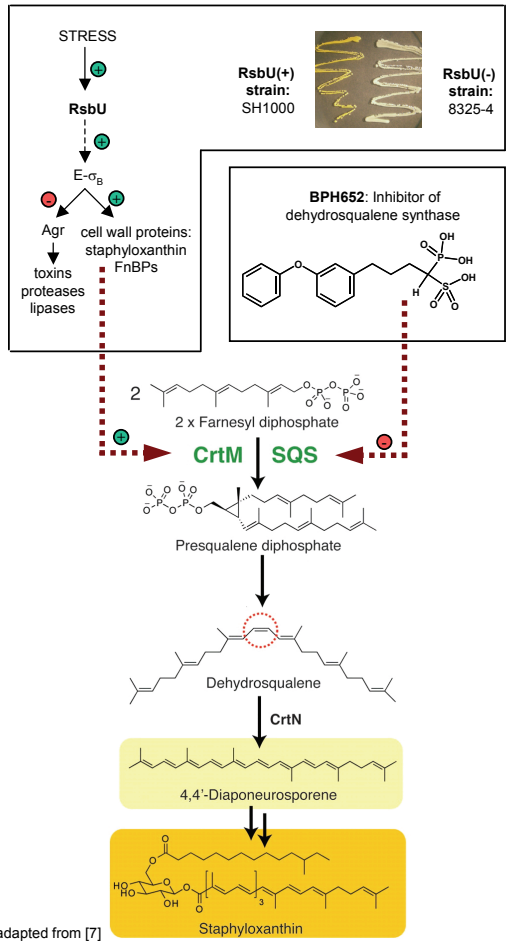
STRATEGIES TO MODULATE STAPHYLOXANTHIN PRODUCTION



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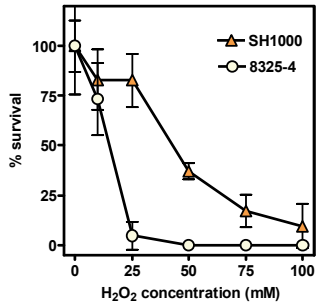
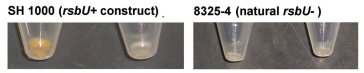
RESULTS

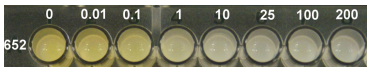
susceptibilities of 8325-4 or SH1000 strains to H_2O_2 in broth.
Bacteria were incubated for 45 min with increasing concentrations of H_2O_2 (0 - 100 mM).



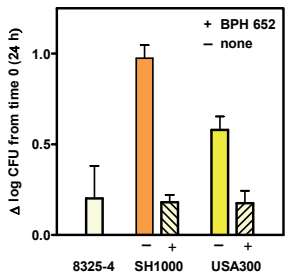
Influence of BPH652 on pigmentation of SH1000 and 8325-4 in broth (100 µM; 48h)



Dose-effect (µM) with SH1000



Intracellular growth of 8325-4, SH1000, or USA300
(24 h; addition or not of 100 µM BPH652 48 h before infection and during infection)



AIMS AND APPROACHES

Our aim was to examine the role of rsbU and staphyloxanthin in intracellular growth of *S. aureus* and in resistance to oxidative stress. This was approached here by using a combination of genomic and pharmacological methods:

- use of strains with different expression of *rsbU* and staphyloxanthin production
- use of the recently described inhibitor of staphyloxanthin synthesis BPH652 [7].

METHODS

- **strains**:
  - *S. aureus* 8325-4 (natural deletion in *rsbU* and weak producer of SFX with a*)*
  - SH1000 (*rsbU*+ restored variant and highly pigmented) [2]
  - US300 (CA-MRSA; pigmented)
- **Resistance of bacteria to hydrogen peroxide** [3]
  - bacteria incubated in PBS containing increasing concentrations of H_2O_2 in the dark at 0°C.
  - after 45 min, reaction stopped by addition of 100 U/ml catalase
  - plating, incubation and counting of colonies

Intracellular infection: [4]
- phagocytosis of opsonized bacteria for 1 h (MOI: 4)
- removal of extracellular bacteria by extensive washing
- intracellular growth allowed in the presence of gentamicin at 1x MIC to minimize growth of extracellular bacteria

RESULTS

- SH1000 is more resistant than 8325-4 to H_2O_2, probably due to large production of SFX.
- BPH652 causes a concentration-dependent decrease of SFX production in SH1000.
- Effects on intracellular growth of SH1000 and USA300 are more important than that for 8325-4.
- Addition of BPH652 brings the intracellular growth of SH1000 and US300 to the same low value seen with 8325-4.

CONCLUSION

- Staphyloxanthin confers a high resistance to oxidative stress to *S. aureus*, which probably contributes to improving its capacity to multiply inside eukaryotic cells.
- Therapeutic strategies targeting staphyloxanthin synthesis or regulators thereof may help in controlling the growth of intracellular *S. aureus* in permissive cells.