ABSTRACT

Staphylococcus aureus is a remarkably adaptable pathogen that expresses an arsenal of virulence factors including secreted toxins such as hemolysins, PSMs, leukocidins, and cell wall-associated adhesins like clumping factors, autolysins, protein A, which together promote its adaptation and colonization under adverse stress conditions, encountered during infections. The expression of these virulence factors is regulated at distinct levels by various two-component systems, sigma factors, RNA binding proteins and small RNAs as an important regulatory molecule. The small RNAs (sRNAs) fine-tune the complex regulatory network and coordinate the expression of multiple virulence factors involved in biofilm formation, antibiotic resistance and cell wall metabolism in *S. aureus*. The sRNA SprX is an important riboregulator involved in the regulation of surface-associated clumping factor B, secreted virulence factors delta hemolysin, glycopeptides resistance and complement binding protein in *S. aureus*. This study describes the regulatory role of sRNA SprX in *S. aureus* in the regulation of autolysins.

S. aureus strains containing *sprX* overexpression or knockdown plasmids were employed for studying the expression of autolysins, and the associated physiology. A comparative proteomic study revealed the down-regulation of Immunodominant staphylococcal antigen A (IsaA) under the influence of increased levels of SprX. Expression of *isaA* and other autolysins exhibited a differential regulation under modified levels of SprX. Reduced levels of SprX in the knockdown strain resulted in the down-regulation of multiple autolytic bands corresponding to major autolysin AtlA and its process intermediates, as observed by cell wall degradation zymography. This was paralleled by a 0.1 to 0.2- fold reduction in the transcripts of autolysin regulator WalR and its downstream genes, *atlA*, *lytM* and *isaA* in qRT-PCRs. Under the overexpression of SprX, expression of *isaA* transcripts were reduced to 0.6-0.9 fold where as *lytM* transcripts showed 1.2 - 2.2- fold up-regulation. Autolysin deficient *S. aureus* strains are reported to have impaired cell division leading to high cell aggregation and resistance to Triton X-100 induced lysis. The *sprX* knockdown strain also exhibited altered phenotypes of high cell aggregation as analyzed by scanning electron microscopy, decreased biofilm formation and increased resistance to Triton X-100-induced lysis, all indicating that SprX influences the expression of autolysins.

A potential RNA-RNA interaction was indicated *in silico* between SprX and WalR at the 5’ coding region, and at *isaA* at 3’ coding sequence. These target mRNA-SprX

interactions were further confirmed by *in vitro* RNA-RNA interaction in electrophoretic mobility shift assays. In conclusion the study unveils a novel role for the small RNA SprX in fine regulation of autolysins, mediated through the regulator WalR and identified IsaA and WalR as new targets.