Determination of Phenazine Specificity for Clinically Significant Efflux Pumps in *Pseudomonas aeruginosa* PA14

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Abstract

Phenazines are redox-active metabolites that are synthesized and secreted by the Gram-negative bacterium *Pseudomonas aeruginosa*. These molecules function in quorum sensing, virulence, and iron sequestering. There are several phenazines synthesized by the bacterium: all of which have key differences in the molecular structure, ranging from the blue phenazine pyocyanin to red phenazines such as 1-hydroxyphenazine. Research suggests that phenazines are secreted into the local environment via efflux pumps such as MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM, otherwise known as Resistance-Nodulation-Division (RND) efflux pumps. There is, however, no information on phenazine-pump specificity. The present study therefore aims to determine if phenazines are selectively secreted by specific RND efflux pumps. Sequencing of the strains used in this study has revealed mutations in the *pvdD*, *nfxB,* and *lasR* genes. The *nfxB* gene produces a regulator for the MexCD-OprJ efflux pump, while *lasR* is a global transcription regulator. The siderophore pyoverdine is produced via nonribosomal synthetases, which the gene *pvdD* encodes. Efflux pump inhibition has revealed that pyocyanin may be secreted by the RND pumps. RT-qPCR has confirmed pump abundance for the 4 RND pumps. Structure elucidation has revealed several techniques that do not work to purify the pigments. Taken together, these results suggest that the red phenazines may be secreted via a different set of efflux pumps compared to pyocyanin which appears to be secreted via the RND pumps. This information can be used to produce an antibiotic which stops the efflux of both pyocyanin as well as antibiotics.