

Orf1/SpcS Chaperones ExoS for Type Three Secretion by *Pseudomonas aeruginosa*¹

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Objective *Pseudomonas aeruginosa* is a ubiquitous and opportunistic pathogen that uses the type III secretion system (TTSS) to inject effector proteins directly into the cytosol of target cells to subvert the host cell's functions. Specialized bacterial chaperones are required for effective secretion of some effectors. To identify the chaperone of ExoS, the representative effector secreted by the TTSS of *P. aeruginosa*, we analyzed the role of a postulated chaperone termed Orf1. **Methods** By allelic exchange, we constructed the mutant with the deletion of gene *Orf1*. Analysis of secreted and cell-associated fractions was performed by SDS-PAGE and Western blotting. Using strain expressing *in trans* *Orf1*, tagged by V5 polypeptide and histidine, protein-protein interaction was determined by affinity resin pull-down assay in combination with MALDI-TOF. The role of *Orf1* in the expression of *exoS* was evaluated by gene reporter analysis. **Results** Pull-down assay showed that *Orf1* binds to ExoS and ExoT. Secretion profile analysis showed that *Orf1* was necessary for the optimal secretion of ExoS and ExoT. However, *Orf1* had no effect on the expression of *exoS*. **Conclusion** *Orf1* is important for the secretion of ExoS probably by maintaining ExoS in a secretion-competent conformation. We propose to name *Orf1* as SpcS for "specific *Pseudomonas* chaperone for ExoS".

Key words: Type III secretion system; *Pseudomonas aeruginosa*; Chaperone

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