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# 109-1 STRUCTURE AND DELIVERY OF A NOVEL QUORUM SENSING MOLECULE FROM DSF FAMILY

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Resumo:

The ability of changing to a social behavior confer advantages to the colonization and survival of microorganisms in the environment and host. Cell-cell communication is mediated by extracellular signaling molecules known as autoinducers (AI). At low cell density the AI production is basal and insufficient to activate the quorum sensing (QS) cascade, but as the bacterial population increases the AI accumulated in the environment overcome the threshold required for activation. Once triggered, the bacteria change the expression pattern of genes creating a positive feedback system for AI production and initiates the synthesis of a broad range of virulence factors that aid in the microbial competitions and host invasion or colonization. In *Xanthomonas* spp, phytopathogens that colonizes and develops several diseases in a broad range of plants representing a risk for the global agriculture, this communication is mediated by molecules from the *Diffusible Signal Factors* (DSF) family, which are fatty acids with a typical unsaturation at the second carbon and chain length from 10 up to 16 carbons. QS activation initiates the production of the major exopolysaccharide known as xanthan gum and expression of virulence factors such as endoglucanases, both essential for the establishment of the disease. In addition to QS, X. citri counts with many others virulence factors such as the type 4 pilus, secretion systems and outer membrane vesicles (OMVs), the latter one has been described to be an important way to delivery toxins, eliminate misfolded proteins, and acts as siderophores to capture iron from the media. In this work, we explored the QS in Xanthomonas citri pv citri by the DSF characterization and its delivery mechanism. OMVs from plate-growing bacterium were purified by a series of centrifugation followed by density gradient and we demonstrated to have DSF, once it could revert the endoglucanase secretion phenotype of a reporter mutant ΔrpfF, deficient in the production of this molecule but capable of responding to exogenous addition of DSF. The addition of OMVs purified from Δ*rpfF* could not complement the phenotype. Since there are more environmental bacteria that share this QS system, we investigated the possibility of cross-communication and curiously we found that, in agreement with the literature, *X. campestris* can recognize the DSF from *Stenotrophomonas maltophilia*, *Burkholderia seminalis*, *X. citri*, and *Xylella fastidiosa*. Nevertheless, *X. citri* is activated only by its own specie and by *X. campestris*. To evaluate if the vesicles produced from these other bacteria also carry DSF, we purified and tested against a reporter strain of *X. campestris*, which was activated by all the OMVs. Once *X. citri* showed a specific DSF recognition system we decided to determine the structure of this molecule. For this, we developed a protocol for DSF purification. In summary, a large-scale purification of DSF was performed using Δ*rpfC* cells, a mutant that overexpress DSF. Cells from 50 plates were scraped and resuspended in sodium acetate buffer pH 4.8 and DSF extracted with ethyl acetate. The molecule was fractionated using two reverse phase columns, C-18 and phenyl-hexyl, in a Shimadzu HPLC. We obtained 2.9 mg pure molecule that was lyophilized and resuspended in deuterated methanol and analyzed by nuclear magnetic resonance (NMR) for the obtention of unidimensional $^{1}H$, $^{13}C$, and bidimensional HSQC spectra, which will be used to solve the DSF structure.

Palavras-chave:

Xanthomonas, Quorum Sensing, Nuclear Magnetic Resonance, Structural Biochemistry, Outer Membrane Vesicles

Agência de fomento:

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)