Type Four Pilus Biogenesis Proteins Interactions Network – The Same as Type Two Secretion System?

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Bacteria use a diverse array of secretion systems, sensory transduction pathways, motility and adhesion mechanisms in order to interact and respond to their environment, many of which have a role as virulence factors. Among such systems, the type II secretion (T2SS) system stands out as the main route for the export of folded proteins the extracellular medium \(^1\). Type IV pilus (T4P) are large transmembrane multi-protein complexes that are homologous to T2SS and have important roles in DNA uptake, adhesion to surfaces and motility. GspL, a T2SS accessory protein, interacts with the ATPase GspE \(^2\), but whether such interaction occurs in T4P between the accessory protein PilM and the ATPases PilB and PilT/U remains to be proved \(^3\).

To test whether this interaction actually exists in T4P, we performed size exclusion chromatography (SEC) and nuclear magnetic resonance (NMR) \(^{15}\)N-TROSY assays of the Xanthomonas citri’s PilM ortholog (XAC3385) and the ATPase protein PilB (XAC3239). XAC3385 was found to be a FtsA family member, it has the ability to bind ATP and to interact with another T4P accessory protein PilN (XAC3384), with small shifts of their elution volumes in SEC experiments. However, we could not detect any interaction with PilB complexed with T4P regulatory proteins PilZ (XAC1133) nor FimX (XAC2398).

Also, our NMR experiments showed that XAC3385 is a well folded protein presenting a \(^{15}\)N-TROSY spectra with sharp and disperse peaks. Moreover, although XAC3385 does bind both ATP and the citossolic region of XAC3384, it does not suffer great conformational changes upon biding of neither of these ligands, as shown by discrete changes of some peaks in these spectra. These results show that XAC3385 is a good target for NMR experiments and suggest that, if there is any interaction with XAC3239, this is transient and weak, since it could not be detected by our methods.

Key words: Type IV Pilus, Type II Secretion System, Xanthomonas citri

References

