

Analysis of conjugative staphylococcal plasmid and its relaxosome

Bacterial conjugation transfers plasmid DNA from a donor to a recipient following cell to cell contact. Plasmid pWBG4 is the reference plasmid for a family of conjugative staphylococcal plasmids that transfers at very high frequencies and encodes production of a water-soluble, orange, diffusible pigment (DiP) and resistance to several antibiotics. This plasmid has been cloned into *E. coli* vectors and was completely sequenced in 2007 by Quang Ngyuen, an Honours student. The sequence analysis has revealed a unique plasmid carrying several genetic elements that have not been reported before and may contribute new insights into the mechanisms of conjugation and virulence factors of staphylococci. This project will continue the analysis of this plasmid and aims to identify the gene(s) for the production of the orange diffusible pigment, the transfer genes responsible for conjugation and, in particular, the genes that lead to the formation of its relaxation complex. During conjugation a protein, called a relaxase, nicks a single strand of plasmid DNA at an origin of transfer (oriT) site and becomes covalently attached to an exposed 5' DNA end to form a 'tadpole' – a DNA 'tail' linked to a protein 'head'. This complex is then transported across a conjugation bridge to the recipient cell. Many plasmids are known to form transient relaxation complexes during the conjugation process and their DNA is usually isolated in the covalently-closed circular (CCC) form indicating that the relaxation complex does not usually nick one of the strands. Plasmid pWBG4 has only ever been isolated in open-circular (OC) form indicative of a relaxation complex that is very active or unstable and always nicks one of the strands of plasmid DNA. A sequence in plasmid pWBG4 has been identified that can act as an oriT site and should therefore contain the relaxation site. This sequence will be further analyzed to identify the relaxation genes.

This project addresses fundamental mechanisms of gene transfer and evolution of multi-resistant bacteria. The molecular techniques in the project include DNA sequencing, sub-cloning PCR products, manipulation of shuttle vectors and transposon mutagenesis. The recombinant strains are classified as GMOs and must be manipulated in a PC2 laboratory certified by the Office of the Gene Technology Regulator.

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