Characterisation and transfer studies of plasmids from the marine environment

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Abstract Plasmids are important in bacterial communities. By their ability to spread genetic information horizontally, *i.e.* by transfer between different cell lineages, rather than by vertical transfer from "mother"- to "daughter" cells, they contribute to bacterial adaptation and evolution. They commonly carry antibiotic and heavy metal resistance genes and are often responsible for spread of antibiotic resistance even between unrelated bacteria. Transfer capabilities and host range of the conjugative plasmid pBF1, isolated from the marine environment have been investigated with a novel detection system for gene transfer based on the green fluorescent protein (GFP) where transconjugants can be detected by epifluorescence microscopy on a single-cell level. Indigenous seawater bacteria with different morphologies were shown to receive the plasmid from *Pseudomonas putida*. In addition, pBF1 was transferred from *P. putida* to several bacteria from the proteobacteria α group and even to the very distantly related *Planctomyces maris*.

The complete nucleotide sequences of plasmids pBF1 (62 689 bp) and pBF6 (66 729 bp) were determined and analysed in order to reveal their identity. The plasmids surprisingly turned out to be identical except for their mercury resistance transposons. pBF1 carries the mercury resistance transposon Tn*5053* and pBF6 carries Tn*5058*. Both are flanked by 5bp direct repeats and for Tn*5058* this is the first report indicating a transposition event. The most remarkable insertions are three genes with high similarity to *mexEF* and *oprN* which are part of a tripartite multi-drug resistance system in *Pseudomonas aeruginosa*. This is the first report that *mexEF* and *oprN* are carried by a plasmid. Interestingly, the pBF1/pBF6 backbone was shown to lack an insert between the replication initiation gene (*trfA*) and the vegetative replication origin (*oriV*), that all known IncP plasmids have. Lack of this insertion suggests that the pBF1/pBF6 represent an ancestral IncP and a "missing link" in the IncP plasmid evolution.

The DNA sequences of the identical *trfA* genes from the plasmids pBF1 and pBF6 were used to further analyse a collection of mercury resistance plasmids to which pBF1 and pBF6 belong. Analysis of the remaining plasmids using *trfA* specific probes revealed that 9 of the 12 plasmid groups represented in the collection showed homology to the IncP family of these plasmids and 3 plasmids (pBF1, pBF6 and pB3) formed a new subgroup.

Finally a replication proficient fragment was isolated from the 24kb plasmid pBFp1, that was one of three plasmids that did not hybridise to the *trfA* derived probe from pBF1/pBF6. Sequencing of the plasmid replicon (rep-pBFp1) revealed a putative open reading frame encoding a RepA protein and an *oriV*-like region containing an A+T rich sub-region, iterons, and DnaA boxes. Sequence comparisons showed significant similarities to the incW plasmid pSa both for the RepA protein and in the *oriV* sequence.

Keywords: Plasmid, conjugation, host range, GFP, IncP, sequencing, *mex EF*, Tn*5053*, Tn*5058*, replicon, *oriV*, *trfA*, *repA*, pBF1, pBF6, pBFp1

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