Plasmids – potential tool for the investigation of gene transfer in *Mycobacterium tuberculosis*

Changes in the genetic repertoire by the acquisition of new genetic material through horizontal gene transfer is an important phenomenon in the evolution of many bacteria (Ochman & Moran, 2001). Transferred genes may either provide small but evolutionary important contributions to fitness or confer condition-specific advantages, facilitating adaptation to a new environment (Pal *et al.*, 2005). Many bacterial pathogens have evolved the capacity to produce virulence factors that are directly involved in infection and disease. Acquiring virulence through horizontal gene transfer is the most common mechanism of gaining new genetic information (Ochman *et al.*, 2000; Morschhauser *et al.*, 2000; Hacker & Kaper, 2000; Ziebuhr *et al.*, 1999). The need for information regarding gene transfer has prompted further investigation of different pathogenic organisms. Understanding gene transfer will explain the pathogenicity of the organism in the host.

Mycobacterium tuberculosis is a globally successful pathogen causing tuberculosis (TB), a leading cause of death worldwide (Drobniewski *et al.*, 2003). It was estimated that there were 8·8 million new TB cases in the world in 2003, with a prevalence of 15·4 million and 2 million deaths from TB (World Health Organization, 2005). In spite of significant research into the understanding of the pathogenicity of the organism, important gaps in our knowledge remain. *M. tuberculosis* harbours 19 genes of eukaryotic origin and has the highest number of eukaryotic–prokaryotic inter-kingdom gene fusions of all sequenced bacterial genomes (Wolf *et al.*, 2000; Gamielidien *et al.*, 2002). It would be reasonable to speculate that this organism may have obtained virulence gene(s) from other organisms, so the investigation of gene transfer may lead to a significant improvement in the understanding of the pathogenicity of this organism. Identifying a suitable tool that will allow gene transfer investigations among mycobacteria will be extremely useful.

Screening of plasmids from mycobacteria has been performed in various studies in order to demonstrate an epidemiological marker in mycobacteria (Gangadharam *et al.*, 1988; Dam *et al.*, 2000). Sequence analysis of the plasmid pCLP from *Mycobacterium celatum* revealed the presence of a similar sequence in *M. tuberculosis* that includes the putative resolvase and transposase of IS1535 (Dantec *et al.*, 2001). As genes harboured in plasmids are more prone to transfer than chromosomally located genes, a plasmid can serve as a vehicle for transferring genes among mycobacteria. This is further evidenced in another report that showed the presence of an 18 kb plasmid in the *M. tuberculosis* H₃₇Rv strain (Katti, 2001). This finding provides direct evidence of gene transfer in *M. tuberculosis* although an earlier study reported the absence of plasmids in *M. tuberculosis* (Zainuddin & Dale, 1990). The presence of the same plasmid (18 kb) in several other mycobacterial species further supports the gene transfer phenomenon among mycobacteria.

Post-genomic tools are continuously generating information on mycobacteria. The fatty acid biosynthetic pathway, especially mycolic acid (targeted by many drugs), has been extensively modified in *M. tuberculosis* by horizontal gene transfer as shown by phylogenetic analysis of the *M. tuberculosis* genome with 58 complete bacterial genomes (Kinsella *et al.*, 2003). However, in the absence of more direct evidence, the plasmid is clearly a promising tool for the investigation of gene transfer among mycobacteria, and the 18 kb plasmid of *M. tuberculosis* has potential as such a tool.

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Dam, T., Bose, M., Isa, M. & Viridi, J. S. (2000). Isolation of plasmids from *Mycobacterium avium*–intracellular complex (MAC) strains from India. *J Med Microbiol* **49**, 393–394.

Dantec, C., Le Winter, N., Gicquel, B., Vincent, V. & Picardeau, M. (2001). Genomic sequence and transcriptional analysis of a 23-kilobase mycobacterial linear plasmid: evidence for horizontal transfer and identification of plasmid maintenance systems. *J Bacteriol* **183**, 2157–2164.

Drobniewski, F. A., Caws, M., Gibson, A. & Young, D. (2003). Modern laboratory diagnosis of tuberculosis. *Lancet Infect Dis* **3**, 141–147.

Gamielidien, J., Ptitsyn, A. & Hide, W. (2002). Eukaryotic genes in *Mycobacterium tuberculosis* could have a role in pathogenesis and immunomodulation. *Trends Genet* **18**, 5–8.

Gangadharam, P. R. J., Perumal, K., Crawford, J. T. & Bates, J. H. (1988). Association of plasmids and virulence of *Mycobacterium avium* complex. *Am Rev Respir Dis* **137**, 212–214.

Hacker, J. & Kaper, J. B. (2000). Pathogenicity islands and the evolution of microbes. *Annu Rev Microbiol* **54**, 641–679.

Katti, M. K. (2001). Plasmids of mycobacteria. *J Med Microbiol* **50**, 575–576.

Kinsella, R. J., Fitzpatrick, D. A., Creevey, C. J. & Mcinerney, J. O. (2003). Fatty acid biosynthesis in *Mycobacterium tuberculosis*: lateral gene transfer, adaptive evolution, and gene duplication. *Proc Natl Acad Sci U S A* **100**, 10320–10325.

Morschhauser, J., Kohler, G., Ziebuhr, W., Blum-Oehler, G., Dobrindt, U. & Hacker, J. (2000). Evolution of microbial pathogens. *Philos Trans R Soc Lond Ser B* **355**, 695–704.

Ochman, H. & Moran, N. A. (2001). Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis. *Science* **292**, 1096–1099.

Ochman, H., Lawrence, J. G. & Groisman, E. A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**, 299–304.

Pal, C., Papp, B. & Lercher, M. J. (2005). Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. *Nat Genet* **37**, 1372–1375.

Wolf, Y. I., Kondrashov, A. S. & Koonin, E. V. (2000). Interkingdom gene fusions. *Genome Biol* **1**, Research 0013.1–13.13.

World Health Organization. (2005). *Global Tuberculosis Control: Surveillance, Planning, Financing*. http://www.who.int/tb/publications/global_report/2005/en/

Zainuddin, Z. F. & Dale, J. W. (1990). Does *Mycobacterium tuberculosis* have plasmids? *Tubercle* **71**, 43–49.

Ziebuhr, W., Ohlsen, K., Karch, H., Korhonen, T. & Hacker, J. (1999). Evolution of bacterial pathogenesis. *Cell Mol Life Sci* **56**, 719–728.