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Comparative analysis of eight *Arthrobacter* plasmids

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Abstract

Despite the prevalence of *Arthrobacter* in the environment little is known about their plasmids, or the capacity of *Arthrobacter* plasmids to mediate horizontal gene transfer. In this study, we compared eight plasmids from five *Arthrobacter* strains in order to identify putative core maintenance genes for replication, segregation, and conjugation. Iteron like sequences were identified on some of the plasmids; however, no genes with obvious similarity to known replication sequences such as an origin of replication, or *rep* genes were identified. All eight plasmids contained a putative conjugation system. Genes with similarity to a relaxase, coupling protein, and various components of a type IV secretion system were identified on each plasmid; it appears that three different systems may be present. Putative *parA* partitioning genes were found in all of the plasmids. Each of the *Arthrobacter* strains examined contained a putative *parB* gene; however, of the three plasmids in *Arthrobacter* strain FB24 only one plasmid had a putative *parB* gene. Cluster analysis of many of the *Arthrobacter* genes suggested that they often formed branches within existing families of plasmid maintenance genes. Comparison of a concatenation of all the maintenance genes from each plasmid suggests that the eight *Arthrobacter* plasmids represent multiple evolutionary pathways.

Keywords: *Arthrobacter*; Plasmids; Conjugation; Partitioning

1. Introduction

Horizontal gene transfer (HGT), by transduction, conjugation, or natural transformation, is one of the driving forces of bacterial adaptation and evolution with significant numbers of bacterial genomes showing evidence of HGT (Gogarten and Townsend, 2005; Smalla and Sobecky, 2002). Chromosomal recombination in bacteria is too infrequent to account for adaptive evolution; bacteria are thought to use the vast, phylogenetically diverse gene pool available to them by HGT (Levin and Bergstrom, 2000). The role of plasmids in the dissemination of genes for antibiotic resistance (Taylor et al., 2004), metal resistance (Borremans et al., 2001; Lebrun et al., 1994; Nucifora et al., 1989), and for organic compound catabolism have been extensively studied (Sayler et al., 1990; Top et al., 2000). However, most of the studied plasmids reside within a few restricted bacterial clades; nearly 70% of the sequences in the Plasmid Genome Database

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(http://www.genomics.ceh.ac.uk/plasmiddb/) are from Proteobacteria and Firmicutes. It is not clear that plasmid maintenance functions from other clades (e.g., Actinobacteria) will be similar to previously identified genes.

Plasmids are genetic mosaics and often carry genes that confer novel phenotypic properties upon cells; most attention is paid to these accessory genes. However, stable persistence within individual cells requires a set of core maintenance genes. The essential characteristic of a plasmid is that it is a self-replicating, extrachromosomal element; therefore, it must contain mechanisms to ensure faithful replication and control of copy number (Thomas, 2000). Plasmids also need to ensure that they are segregated between dividing cells; whereas high-copy number plasmids appear to rely on random segregation, large low-copy number plasmids utilize active partitioning systems. Circular plasmids can form multimeric structures (Hallet et al., 2004); to ensure proper segregation, multimer resolution systems are required.

Some plasmids are self-transmissible via conjugation. Conjugation is mediated by three separate groups of genes: the Mpf (mating pair formation) system which is comprised of genes that form the pilus and channel for DNA transfer, the Dtr (DNA transfer) system comprised of genes for DNA replication and processing to form a covalently bound DNA protein complex, and the coupling protein which serves as a bridge between the Mpf and Dtr systems (Cabezón et al., 1997; Gilmour et al., 2003; Llosa et al., 2003; Schroder et al., 2002; Schroder and Lanka, 2005). The ability to transfer to new hosts enhances plasmid survival; movement to a diverse set of hosts with varied fitness characteristics will reduce the chance of plasmid extinction (Bergstrom et al., 2000).

The genus *Arthrobacter* belong to the high GC class of Actinobacteria and are ubiquitous in soils (Jones and Keddie, 1992) being found in both pristine and contaminated environments. As a group, they possess a broad range of interesting physiological functions, including resistance to desiccation and ionizing radiation (Boylen, 1973; Fredrickson et al., 2004; Labeda et al., 1976), metal resistance (Beasley, 2004; Margesin and Schinner, 1996) and catabolism of organic pollutants and pesticides (Eaton, 2001; Igloi and Brandsch, 2003; Overhage et al., 2005; Sajjaphan et al., 2004). Despite their significance in the environment relatively little is known about plasmids in *Arthrobacter*, in particular their core maintenance functions.

In this study, we have used a comparative genomics approach to look at the core maintenance genes from eight *Arthrobacter* plasmids from five *Arthrobacter* strains. Three of these plasmids, pAO1 (Igloi and Brandsch, 2003), pTC1 (Mongodin et al., 2006; Sajjaphan et al., 2004) and pTC2 (Mongodin et al., 2006) have been previously described while the remaining five plasmids have been recently sequenced. This study is the first comparative analysis of *Arthrobacter* plasmids, and can therefore provide valuable insight into the genetics, physiology, and ecology of *Arthrobacter*. While the analysis of sequence data cannot substitute for molecular and biochemical experiments, these data can serve as basis for generating hypotheses for future research.

2. Materials and methods

2.1. Arthrobacter strains and plasmids

The plasmid pSI-1 was from the lead resistant *Arthrobacter* sp strain AK-1 isolated directly (Jerke, 2006) from contaminated soils collected in Seymour, Indiana (Joynt et al., 2006). *Arthrobacter* sp. strain FB24 harbored three plasmids (pFB24-104, pFB24-105, and pFB24-136). FB24 was isolated from a xylene and chromate enriched microcosm (Nakatsu et al., 2005) constructed using contaminated soils from Seymour, Indiana (Joynt et al., 2006). The *Arthrobacter* sp. strain Chr15 was isolated from soil collected from the Cannelton Industries Superfund site, Marie, MI (Zhou et al., 2002) by plating serially diluted soil onto PYT80 agar media (80 mg L^{-1} each of peptone, yeast extract and tryptone) amended with 10 mM K_2CrO_4 (Nakatsu et al., 2001). The *Arthrobacter aurescens* TC1 strain was isolated from atrazine contaminated soil from South Dakota (Strong et al., 2002). *Arthrobacter nicotinovorans* P-34 was isolated from a soil enriched with nicotine (Hochstein and Rittenberg, 1959).

2.2. Plasmid Isolation

The plasmid pSI-1 was isolated during log growth phase from large scale cultures (10–20 L) grown in xenobiotic basal media (Konopka et al., 1989), which was modified to increase lead solubility (Jerke, 2006). In order to maintain selective pressure AK-1 was grown in the presence of 75 μM $PbNO_3$. Chr15 was cultured in nutrient broth (1.5–3 L) with 1% glycine and amended with 5 mM K_2CrO_4 (Beasley, 2004). Plasmid DNA was isolated by a modified alkaline lysis procedure (Sambrook et al., 1989) as described elsewhere (Jerke, 2006). The isolated plasmid DNA was purified by ethidium bromide CsCl density gradient ultra-centrifugation (Sambrook et al., 1989) to remove contaminating genomic DNA. Plasmid sizes of pSI-1 and pChr15 were calculated based on comparison