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Sequence analysis of the plasmid genome of the probiotic strain *Lactobacillus paracasei* NFBC338 which includes the plasmids pCD01 and pCD02

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Abstract

Lactobacillus paracasei NFBC338 is a probiotic strain that was isolated from the human gastrointestinal tract (GIT) and contains a plasmid genome of $\geqslant$80 kb. Using a shotgun sequencing approach, two of the plasmids, pCD01 (19,882 bp) and pCD02 (8554 bp) have been completely sequenced, and four contiguous sequences (Contigs) have been assembled. Bioinformatic analysis of pCD01 revealed that it contains 23 putative open reading frames (ORFs) and that it contains regions characterised by potential replication functions and multidrug resistance (MDR). In contrast, the content of pCD02 is mainly cryptic, although, it does contain two insertion sequence (IS) elements. Indeed, up to 17% of the entire plasmid genome encodes putative transposable elements. In addition, there are a number of interesting ORFs distributed over the four Contigs that show significant homology to genes such as those involved in adherence and biotin metabolism, which may prove beneficial to *Lb. paracasei* NFBC338 under certain environmental conditions. This study provides a novel insight into the rich plasmid complement of this probiotic *Lactobacillus* strain, which may potentially be exploited as the basis for development of improved genetic tools for probiotic lactobacilli.

Keywords: Plasmid; Probiotic; Replication; Lactobacilli; Transport

1. Introduction

Lactobacilli generally appear to contain multiple plasmids which can vary in size from 1.2 to 169 kb (Mayo et al., 1989). Generally 1–10

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plasmids have been found in lactobacilli, and in the case of *Lactobacillus plantarum* LPC25, 16 plasmids have been identified (Ruiz-Barba et al., 1991). *Lactobacillus* plasmids were first isolated from *Lb. casei* (Chassy et al., 1976) and then from a variety of other lactobacilli. While 23 have been sequenced to date, many still remain cryptic with regard to their role in cellular performance and functioning. A few plasmid-encoded functions have been discovered and applied to vector construction, strain identification, detection, and modification (Wang and Lee, 1997). According to Wang and Lee (1997), *Lactobacillus* plasmid functions can be divided into four main groups; (1) hydrolysis of proteins, (2) metabolism of carbohydrates, amino acids, and citrate, (3) production of bacteriocins, exopolysaccharides, and pigments, and (4) resistance to antibiotics, heavy metals, and phages.

The first *Lactobacillus* strain to be efficiently and reproducibly transformed was *Lb. casei*, with the vector pSA3 by Chassy and Flickinger (1987). Since then, many other strains of lactobacilli have been successfully transformed with different electroporation protocols. Kullen and Klaenhammer (2000) reported that the plasmid vectors most widely used for lactobacilli are of three types: (i) plasmids based on rolling circle replication (RCR) replicons, (ii) plasmids with two origins of replication (one for *Escherichia coli* and a second for gram-positive bacteria), and (iii) *Lactobacillus* vectors with an alternative replication origin for gram-negative bacteria. This growing interest in the characterisation of *Lactobacillus* replicons themselves as potential useful vectors, has for example, led to the development of a *Lactobacillus–E. coli* shuttle vector that was designed based on the replicon of the *Lactobacillus fermentum* plasmid (Pavlova et al., 2002). This vector was transformed and stably maintained in several *Lactobacillus* strains and was used to successfully express the s-layer protein gene (*slpA*) of *Lactobacillus acidophilus* in a heterologous *Lactobacillus* strain. In a more recent study, a derivative of pRV500 from *Lactobacillus sakei* was constructed that carried the pRV500 replicon (Alpert et al., 2003). This vector was also found to be maintained at a reasonable rate over 20 generations in several lactobacilli, making this plasmid another potentially useful tool for different applications in lactobacilli (Alpert et al., 2003). Genetic engineering using these plasmid vectors has also led to the development of lactobacilli designed for therapeutic purposes, such as the delivery of antigens like the B subunit of cholera toxin, α-amylase, or an epitope from human immunodeficiency virus (HIV) at the mucosal surface (Perdigon et al., 2001).

Lactobacillus paracasei NFBC338 is a human probiotic strain that was originally isolated from the GIT, and has since been used for the manufacture of Cheddar cheese (Gardiner et al., 1998; Stanton et al., 1998) and spray-dried powders (Desmond et al., 2001, 2002; Gardiner et al., 2000). Due to the commercial importance of this strain, the plasmid complement was isolated and by a combination of sequence and restriction digest analysis found to amount to approximately 88,643 bp of DNA. In this study, we describe the complete sequence and genetic organisation of two of its plasmids, pCD01 (19,882 bp) and pCD02 (8554 bp), and also, that of the four unassembled Contigs, 1 (31,138 bp), 2 (14,160 bp), 3 (12,010 bp), and 4 (2899 bp).

2. Materials and methods

2.1. Plasmid isolation

Plasmid DNA from *Lb. paracasei* NFBC338 was isolated by the method of Anderson and McKay (1983), with one minor adjustment at the lysis step. Briefly, the culture was inoculated (2% v/v) and grown for approximately 4 h. The cells were harvested (6000*g* for 20 min) and resuspended in lysis solution 1 [(sucrose (6.7% w/v), Tris (50 mM), and EDTA (1 mM) at pH 8)], supplemented with lysozyme (Sigma Chemical, Poole, UK) and mutanolysin (Sigma) (20 mg/ml each), and incubated for 30 min at 37 °C. All subsequent steps in the plasmid isolation procedure and vertical gel electrophoresis are identical to those described by Anderson and McKay (1983). Plasmids isolated from *Lactococcus lactis* DRC3 were used as standard molecular weight sizes as