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pEOC01: A plasmid from *Pediococcus acidilactici* which encodes an identical streptomycin resistance (*aadE*) gene to that found in *Campylobacter jejuni*

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Abstract

The complete nucleotide sequence of pEOC01, a plasmid (11,661 bp) from *Pediococcus acidilactici* NCIMB 6990 encoding resistance to clindamycin, erythromycin, and streptomycin was determined. The plasmid, which also replicates in *Lactococcus* and *Lactobacillus* species contains 16 putative open reading frames (ORFs), including regions annotated to encode replication, plasmid maintenance and multidrug resistance functions. Based on an analysis the plasmid replicates via a theta replicating mechanism closely related to those of many larger *Streptococcus* and *Enterococcus* plasmids. Interestingly, genes homologous to a toxin/antitoxin plasmid maintenance system are present and are highly similar to the *omega–epsilon–zeta* operon of *Streptococcus* plasmids. The plasmid contains two putative antibiotic resistance homologs, an *ermB* gene encoding erythromycin and clindamycin resistance, and a streptomycin resistance gene, *aadE*. Of particular note is the *aadE* gene which holds 100% identity to an *aadE* gene found in *Campylobacter jejuni* plasmid but which probably originated from a Gram-positive source. This observation is significant in that it provides evidence for recent horizontal transfer of streptomycin resistance from a lactic acid bacterium to a Gram-negative intestinal pathogen and as such infers a role for such plasmids for dissemination of antibiotic resistance genes possibly in the human gut.

Keywords: *Pediococcus acidilactici*; Antibiotic resistant; Multidrug resistant; Plasmid

1. Introduction

Pediococcus acidilactici is widely used as a starter culture to ferment meat (Luchansky et al., 1992; Mattila-Sandholm et al., 1991) and vegetable products (Knorr, 1998). Although, *P. acidilactici* has

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GRAS (Generally Recognized As Space) status and strains of this species have been safely used in animal feeds as probiotic cultures (Vanbelle et al., 1990) and as nutritional enhancers in silage (Cai et al., 1999), certain strains may contain undesirable antibiotic resistance traits. For example, *Pediococcus* species are intrinsically resistant/insensitive to the clinically important antimicrobial agent vancomycin, a property that they share with some other Gram-positive bacteria such as *Leuconostoc* and certain *Lactobacillus* species (Swenson et al., 1990). Interestingly, many antibiotic resistance genes have already been located on pediococcal plasmids (Tankovic et al., 1993; Torriani et al., 1987) suggesting that such traits may be disseminated horizontally between strains. Indeed, pediococci can contain numerous plasmids, ranging in size from 5 to 190 kb (Gindreau et al., 2001; Gonzalez and Kunka, 1983; Raccach, 1987). Some of these plasmids can also encode important functions such as those for carbohydrate utilization, bacteriocin production and immunity, and antibiotic resistance (Giacomini et al., 2000; Motlagh et al., 1994; Raccach, 1987; Tenorio et al., 2001). Previously, 46 kb (Tankovic et al., 1993) and 60 kb (Torriani et al., 1987) plasmids were associated with erythromycin resistant *P. acidilactici* strains.

The possibility of natural pediococcal plasmids transferring into bacterial strains is a concern and has prompted characterisation of these elements. Currently, there are a number of pediococcal plasmids which have been completely sequenced. These include pF8801 (Accession No. AF196967) from *P. damnosus,* a plasmid associated with glucan-production which leads to unacceptable viscosity of wine (Gindreau et al., 2001), and pSMB74 isolated from *P. acidilactici* which encodes for pediocin AcH production (Motlagh et al., 1994). Two *P. pentosaceus* plasmids have also been completely sequenced including the small (3.55 kb) rolling circle replicating plasmid pRS4 (Alegre et al., 2005) and the larger (19.5 kb) pediocin A-producing theta replicating plasmid pMD136 (Giacomini et al., 2000).

Recently, Danielsen et al. (2007) assessed four *Pediococcus* species for their sensitivity to antimicrobial agents. The plasmid discussed here, pEOCO1 is the second *P. acidilactici* plasmid to be sequenced. One strain, *P. acidilactici* NCIMB 6990, was found to be resistant to erythromycin. Furthermore, a plasmid encoded *ermB* determinant was identified by PCR. The aim of this study was to sequence and analyse this erythromycin resistant plasmid, pEOC01. The plasmid was found to encode genes associated with theta replication and plasmid maintenance. In addition to the *ermB* gene, a streptomycin resistant *aadE* gene was identified which is identical to a plasmid encoded *aadE* gene found in *Campylobacter jejuni*. The implications of this latter finding in terms of dissemination of plasmid-borne antibiotic resistance from Gram-positive to Gram-negative bacteria are discussed.

2. Methods

2.1. Strains, media and growth conditions

Strains *P. acidilactici* NCIMB 6990 and *Lactobacillus paracasei* NFBC 338 were routinely cultured in modified MRS (Difco Laboratories) under anaerobic conditions at 37 °C as previously described by (Simpson et al., 2002). *Lactococcus lactis* MG1614 was grown in glucose-M17 (Difco) medium (G-M17) (Terzaghi and Sandine, 1975) at 30 °C. *Escherichia coli* strains XLI-blue (Stratagene) and Top10 cells (Invitrogen™ Life Technologies) were grown at 37 °C in Luria-Bertani broth (Merck) with vigorous agitation. Solid media was prepared by the addition of 1.5% agar.

2.2. Plasmid isolation

Plasmid DNA from *P. acidilactici* was prepared as previously described by Swapan et al. (1989) with minor adjustments. Briefly, the lysis step was performed until a clear cell suspension was evident. Plasmid preparations were resolved in a 0.8% (w/v) vertical agarose gel run overnight in 1 X TAE running buffer at 35 V. The plasmid associated with erythromycin resistance were excised using a QIAquick Gel extraction Kit (Qiagen GmbH) and electroporated into the plasmid free competent *L. lactis* MG1614 strain (Gasson, 1983; Holo and Nes, 1989). Transformants were selected on erythromycin (10 μg ml^{-1}) containing GM17 plates. Plasmid DNA from *L. lactis* MG1614 containing the pediococcal plasmid was isolated using QIAprep Spin Miniprep Kit (Qiagen GmbH) according to manufacturers instructions with the following modifications. The cells were resuspended in a 25% sucrose 20 mg ml^{-1} lysozyme solution and incubated at 37 °C for 30 min prior to plasmid extraction.

2.3. Curing of plasmid

Plasmid loss was induced by growing cells o/n in mMRS containing 0.5 μg/ml novobiocin (McHugh and Swartz, 1977; Tankovic et al., 1993). Cells were serially diluted, spread plated onto mMRS agar and incubated for 48 h. Colonies were transferred onto mMRS agar with and without erythromycin at 50 μg/ml.