

## Complete nucleotide sequence of the fosfomycin resistance transposon Tn2921

Sir,

Fosfomycin is a cell wall-active antibiotic introduced into clinical practice around 1970 [1]. Despite its broad spectrum of activity and good pharmacological properties, its use has been somehow hampered by the high number of spontaneous resistant mutants isolated following antibiotic challenge in many bacterial pathogens [2], most of them carrying chromosomal mutations impairing drug transport.

Fosfomycin has been used steadily for the treatment of lower urinary tract infections. Recently, the emergence of resistance to newer antibiotics has resulted in a renewed interest in fosfomycin as a therapeutic agent [3].

The existence of plasmids conferring resistance to fosfomycin was demonstrated in strains of *Serratia marcescens* isolated in the Hospital 'Nuestra Señora de Covadonga' (Asturias, Spain) [4]. The fosfomycin resistance gene *fosA* in some of those plasmids was found to reside on a transposable element called Tn2921 [5]. The *fosA* gene was shown to encode a fosfomycin-inactivating mechanism [6] that consisted of enzymatic coupling with glutathione. The transposable element Tn2921 was estimated to be ca. 12.5 kb long, but its composition was unknown except for the presence of the *fosA* gene and the two terminal copies of insertion sequence IS2921, which are closely related to IS10 (1.3 kb each) [7]. To gain further insight into the gene content of

Tn2921 and the possible mechanisms leading to the assembly of the transposon, the complete nucleotide sequence of Tn2921 in plasmid pSU912 was determined. The transposon was flanked by identical 1329-bp long insertion sequences, with only 1 bp difference from IS10-right (IS10R) and 14 bp with IS10-left (IS10L). A single sequence of 12 452 bp containing the complete 12 434 bp of the transposon and the two target 9-bp direct repeats has been deposited in GenBank (accession no. FJ829469). The numbering used in the text makes reference to this GenBank entry.

The first open-reading frame (ORF) next to IS10L (1347–1701) contains a 120-amino acid N-terminal fragment of a tryptophanyl-tRNA synthetase II truncated by the insertion of IS10. The 9-mer Tn10 target site sequence GGCGAAGCC (1339–1347) signals the limit of the gene remnant and indicates that IS10 was integrated here by transposition.

The *fosA* gene (1901–2326) is the fosfomycin resistance gene. Genes similar to *fosA* are relatively frequent in the chromosomes of many bacteria, where they play functions related or not with antibiotic resistance.

Protein BLAST searches identified three complete ORFs in the region (2312–6033) contiguous to *fosA*. The first ORF encoded a transcriptional regulator belonging to the LacI family. The next encoded a conserved hypothetical protein with a possible function as a glycoside hydrolase. The final ORF encodes a sugar transporter belonging to the major facilitator superfamily (MFS). This

gene arrangement is suggestive of a system operating to sense, transport and hydrolyse some sugar molecules for further use in bacterial metabolism.

The next two ORFs in Tn2921 (6068–8172) form part of a 5-methylcytosine GTP-dependent restriction system. The *mcrC* gene is complete, whilst the *mcrB* gene is 5' truncated and is probably not functional, rendering the *mcrC* gene useless because both proteins are required for function.

The sequence from here to the right end of the transposon consists of nested remnants of transposable elements following a 'transposon in transposon' design type. Next to *mcrB* (8172–9872), a non-functional fragment of the Tn3 transposase gene was identified. The sequence from 9874 to 11 114 corresponded to the left end of the transposon Tn7 including the left IR and the carboxyl-terminal end of the site-specific recombinase Int12. The presence of the left IR of the transposon Tn7 suggests that Tn7 could have transposed into Tn3. Tn7 was interrupted by integration of *IS10R*, which constitutes the right end of Tn2921 (Fig. 1).

The complete central region of Tn2921 was not syntenic with any sequence deposited in the public sequence databases. However, the 5.6 kb DNA region between *IS10L* and the *mcr* genes, including *fosA* (1339–5967), showed 90% identity with a genomic segment from *Enterobacter cancerogenus* ATCC 33516 (GenBank accession no. ABWM00000000). A chromosomal region like this could be the origin of the transposon. The deletion of a gene (ENTCAN\_00725)

and integration of IS 10 into ENTCAN\_00726 will exactly produce the left half of Tn2921 (Fig. 1).

In conclusion, Tn2921 provides a good example of evolution of an antibiotic resistance composite transposon. We can assume that an enterobacterial gene encoding a protein of the glyoxalase family was deregulated and subsequently incorporated as a fosfomicin resistance gene in a transposable element by successive integration of two identical ISs. Furthermore, the presence of genes unrelated to resistance and of many gene remnants in the central part of Tn2921 suggests that this transposon may have evolved recently under the selective pressure of fosfomicin usage.

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### **Competing interests**

None declared.

### **Ethical approval**

Not required.

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Asunción Seoane

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**Fig. 1.** Gene map of transposon Tn2921. Genes that are inactive due to truncation are filled with dots. The *fosA* gene is in black. The lower part of the figure shows the segment from the *Enterobacter cancerogenus* genome that is homologous and co-linear with the left half of the transposon (grey blocks). Deletion of gene ENTCAN\_00725 and insertion of IS 10L at the indicated point (black arrowhead) of ENTCAN\_00726 could be the origin of the transposon. Segments from Tn3 and Tn7 are also indicated.

