**dfrA25, a novel trimethoprim resistance gene from Salmonella Agona isolated from a human urine sample in Brazil**

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**Introduction**

Trimethoprim competitively inhibits the enzyme dihydrofolate reductase, which is responsible for the reduction of dihydrofolate to tetrahydrofolate.1,2 Acquired resistance to trimethoprim includes mutations in the promoter region or in the dihydrofolate reductase structural gene,3,4 but the most common trimethoprim resistance mechanism is the supplementation of a trimethoprim-sensitive dihydrofolate reductase with a trimethoprim-resistance-containing dihydrofolate reductase, resulting in high-level trimethoprim resistance in various bacteria. Until now more than 30 different trimethoprim-resistance-mediating dihydrofolate reductase (dfr) genes, subdivided into two major types, 1 and 2 (referred to as dfrA and dfrB), have been observed.3,4 A new trimethoprim resistance gene is defined when the dihydrofolate reductase protein (DHFR) encoded by the gene has <95% identity at the amino acid level to known DHFR proteins.5 The association of these genes with mobile genetic elements such as transposons or plasmids has often been observed, and the presence of these genes in bacteria as part of integrons is also common.5,6

In Salmonella various dfr genes have been detected.6 In a previous study, the prevalence of integrons and resistance genes in Salmonella spp. isolates from Brazil, trimethoprim resistance encoded as gene cassettes in class 1 integrons was found in 41% of the isolates and a putative trimethoprim resistance gene cassette was detected within a class 1 integron in Salmonella Agona (no. 32) isolated from human urine.6 In the present study we characterized this novel trimethoprim resistance gene, designated as dfrA25.

**Materials and methods**

The DNA sequence of the gene cassette containing the putative trimethoprim resistance gene dfrA25 of Salmonella Agona (no. 32) was analysed by use of vector NTI Suite 8. In order to determine whether dfrA25 conferred resistance to trimethoprim, the gene was cloned into Escherichia coli MT102. A PCR product was amplified by use of the primers Salm32Ecor1 (5'-GCCGGAATTCTAACC-CAGGACGAGTACC-3') and Salm32BamH1 (5'-CCGGATCCC-GTCAATTGCATAGCTTTG-3') flanking the gene cassette. The PCR product was digested with EcoRI and BamHI and ligated into the plasmid pLOW1, which was partially digested with the same enzymes.7 The ligation product was electroporated into E. coli MT102 and presumptive clones were selected on Luria–Bertani (LB) agar supplemented with chloramphenicol 20 mg/L. Plasmids were purified from selected colonies using the QIAprep Spin Miniprep Kit (Qiagen, Germany). A 1166 bp fragment was sequenced with the primers M13F (5'-GCGGATAACAATTTCACACAGG-3') and M13R (5'-GCGGATAACAATTTCACACAGG-3') to make sure that the right gene was inserted. The clone (YA32) was checked for resistance to tetramethoprim, the gene was cloned into Escherichia coli MT102 and resistance to 10 different antimicrobial drugs was measured. A phylogenetic tree was constructed based on representative trimethoprim-resistance-containing DfrA proteins retrieved from GenBank. Filter-mating experiments and Southern blots of plasmid preparations were performed with the donor and selected transconjugants.

**Results and conclusions**

The gene was cloned into Escherichia coli MT102 and resistance to 10 different antimicrobial drugs was measured. A phylogenetic tree was constructed based on representative trimethoprim-resistance-containing DfrA proteins retrieved from GenBank. Filter-mating experiments and Southern blots of plasmid preparations were performed with the donor and selected transconjugants.

Keywords: integrons, gene cassettes, dihydrofolate reductase
to ten different antimicrobial drugs (ampicillin, chloramphenicol, gentamicin, nalidixic acid, neomycin, spectinomycin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim) by use of a commercially dehydrated panel, Sensititre (Trek Diagnostic Systems, UK), as described by the NCCLS.8

A phylogenetic analysis at the amino acid level was performed by making a phylogenetic tree based on one representative microorganism for each trimethoprim-resistance-mediating DfrA protein. The multiple alignments were made by use of the program ClustalX (version 1.81); an N-J tree was made based on this alignment, which was used as a guide tree for the final alignment used to produce the final bootstrap N-J tree.

Filter-mating experiments with the donor Salmonella Agona (no. 32) to the recipients E. coli 1000R and Salmonella Typhimurium

Figure 1. Phylogenetic tree of the DfrA proteins involved in trimethoprim resistance. Each type of DfrA protein was retrieved from GenBank indicated with species name and GenBank accession no. The number of each major branch node refers to the number of times that a particular node was found in 1000 bootstrap replications. The arrow indicates the placement of DfrA25 in the tree. A. salmonicida, Aeromonas salmonicida; B. subtilis, Bacillus subtilis; M. profunda, Moritella profunda; P. mirabilis, Proteus mirabilis; P. multocida, Pasteurella multocida; S. epidermidis, Staphylococcus epidermidis; S. haemolyticus, Staphylococcus haemolyticus; S. Typhimurium, Salmonella enterica serovar Typhimurium; V. cholerae, Vibrio cholerae.
Salmonella Agona no. 32 and tet(A)-2, respectively.

The blots were hybridized with two digoxigenin-labelled DNA probes for the presence of the 3' segment of class 1 integrons using the PCR product (225 bp) amplified by the primers qacE\textit{D}\textsubscript{1-F} and qacE\textit{D}\textsubscript{1-B} (5'-CAACATTGCTT-3').

**Results and discussion**

This gene cassette contained a 471 bp ORF with 85% identity at the amino acid level to the trimethoprim-resistant dihydrofolate reductase DfrA5 (GenBank accession no. DQ267940). Downstream of the dihydrofolate reductase gene a putative 59 bp element (nt 507–593) was found with 92% identity to the corresponding sequence of a putative 59 bp element found downstream of a dfrA14 gene cassette in \textit{E. coli} (Z50805) and on the plasmid pRSB107 (AJ851089). No other gene cassettes were observed within this class 1 integron.

The clone (YA32) that contained the dfrA25 gene ligated into pLOW1 conferred resistance to chloramphenicol (marker for the plasmid pLOW1), streptomycin (chromosomal) and trimethoprim 32 mg/L or tetracycline 16 mg/L, respectively. Presumptive transconjugants were selected on tetracycline (TET) 32 mg/L and rifampicillin (RIF) 50 mg/L.  

**GenBank submission**

The sequence of 593 bp was submitted to GenBank (accession no. DQ267940).

**Acknowledgements**

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**Transparency declarations**

None to declare.

**References**


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**Table 1.** Filter-mating experiments to two recipients: \textit{E. coli} 1000R and \textit{Salmonella} Typhimurium JEO3817

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Transfer frequency\textsuperscript{a}</th>
<th>Transfer frequency\textsuperscript{b}</th>
</tr>
</thead>
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<tr>
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<td>\textit{E. coli}, 1000R</td>
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<td>7.14 × 10\textsuperscript{-3}</td>
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<tr>
<td>\textit{Salmonella} Agona no. 32</td>
<td>\textit{Salmonella} Typhimurium, JEO3817</td>
<td>ND\textsuperscript{c}</td>
<td>3.4 × 10\textsuperscript{-7}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Transconjugants per donor, transconjugants were selected on tetracycline (TET) 16 mg/L and rifampicillin (RIF) 50 mg/L.

\textsuperscript{b}Transconjugants per donor, transconjugants were selected on trimethoprim (TMP) 32 mg/L and rifampicillin (RIF) 50 mg/L.

\textsuperscript{c}No transconjugants detected.


