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Revised Sequence of OtrB (Tet347) Tetracycline Efflux Protein from *Streptomyces rimosus*

The sequence of the tetracycline resistance gene designated *tet347* from the tetracycline-producing organism *Streptomyces rimosus* (strain PG3) predicted a protein of 347 amino acids (6) (GenBank accession no. M20370). The *tcrC* gene (also called *tcr3*; GenBank accession no. D38215) from chlorotetracycline-producing *S. aureofaciens* encoded a 512-residue putative tetracycline efflux protein which, starting at residue 222, was 43% identical to the Tet347 protein (3). Whether *tetB*, an *S. rimosus* gene proven to encode tetracycline efflux (5), is the same as *tet347* is unclear (1, 6). The Tet347 protein was predicted to have 7.5 transmembrane helices, and TcrC was predicted to have 14 (TopPred2 software program [2]) (unpublished data). Because even eight transmembrane helices would be unusual for a tetracycline efflux protein, we have resequenced *tet347* (on plasmid pUT1954 [6]) from nucleotide (nt) 110 to near the end of the cloned region at nt 2054 (sequencing was performed at the Tufts Core Facility with PCR cycling/dye terminator and an Applied Biosystems 373 DNA Stretch Sequencer). Sequences were obtained for both strands between nt 110 and 787; overlapping forward sequence was obtained for nt 620 to 1250, 1100 to 1650, and 1590 to 2000. The most serious of the various errors we found in the previous sequence were located upstream of nt 787 and had led to misinterpretation of the open reading frame. Our new sequence shows 216 additional amino acids at the amino terminus of the protein and reidentifies 4 internal amino acids.

Our revised open reading frame begins not at nt 797 but at nt 149 (numbering according to reference 6, with GTG as the translation start codon [see below]). The revised open reading frame ends at nt 1837, in agreement with the earlier study (6). The sequence immediately upstream from the GTG codon is AGGAGAGTGAGGAACC (candidates for Shine-Dalgarno sequences are underlined). The revised sequence specifies a protein of 563 residues, with 14 transmembrane helices predicted by TopPred2. Amino acid residues 12 to 465 of the Tet347 revised sequence are 65% identical to those of a corresponding region in TcrC (Bestfit program of the Genetics Computer Group). TopPred2 hydropathy plots for these two proteins differ principally in the region following putative transmembrane helix 13. As is true for all other tetracycline efflux proteins (4), both TcrC and Tet347 revised have at least one intramembrane negatively charged residue (an aspartate in putative transmembrane helix 1).

An unpublished sequence in the database (GenBank, 4 May 1998 release, accession no. AF061335) for the *S. rimosus* gene *otrB* (derived from strain M15883 [1]) is, in its entirety, identical to nt 290 to 1840 of our revised *tet347* sequence (numbering according to reference 6). However, those authors presumed the translation start to be an ATG codon corresponding to our methionine 28. We favor our upstream GTG translation start because it includes an amino-terminal cytoplasmic tail plus all of putative transmembrane helix 1 (which begins approximately at residue 23) and because our residues 19 to 24 (FTHRQI) are identical to residues 24 to 29 of the homologous TcrC.

We propose that "*otrB*" be used in the future to designate the "*tet347*" gene. Our sequence of *otrB* and the protein product OtrB has been deposited in GenBank under accession no. AF079900, where nt 1 now corresponds to nt 110 of reference 6.

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