

A novel thymidylate synthase from the *Vibrionales*, *Alteromonadales*, *Aeromonadales*, and *Pasteurellales* (VAAP) clade with altered nucleotide and folate binding sites

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ABSTRACT

Thymidylate synthase (TS, E.C. 2.1.1.45) is a crucial enzyme for *de novo* deoxythymidine monophosphate (dTMP) biosynthesis. The gene for this enzyme is *thyA*, which encodes the folate-dependent TS that converts deoxyuridine monophosphate group (dUMP) into (dTMP) using the cofactor 5,10-methylenetetrahydrofolate (mTHF) as a carbon donor. We identified the *thyA* gene in the genome of the *Vibrio parahaemolyticus* strain FIM-S1708+ that is innocuous to humans but pathogenic to crustaceans. Surprisingly, we found changes in the residues that bind the substrate dUMP and mTHF, previously postulated as invariant among all TSs known (*Finer-Moore, Santi & Stroud, 2003*). Interestingly, those amino acid changes were also found in a clade of microorganisms that contains *Vibrionales*, *Alteromonadales*, *Aeromonadales*, and *Pasteurellales* (VAAP) from the *Gammaproteobacteria* class. In this work, we studied the biochemical properties of recombinant TS from *V. parahaemolyticus* FIM-S1708+ (VpTS) to address the natural changes in the TS amino acid sequence of the VAAP clade. Interestingly, the K_m for dUMP was $27.3 \pm 4.3 \mu\text{M}$, about one-fold larger compared to other TSs. The K_m for mTHF was $96.3 \pm 18 \mu\text{M}$, about three- to five-fold larger compared to other species, suggesting also loss of affinity. Thus, the catalytic efficiency was between one or two orders of magnitude smaller for both substrates. We used trimethoprim, a common antibiotic that targets both TS and DHFR for inhibition studies. The IC_{50} values obtained were high compared to other results in the literature. Nonetheless, this molecule could be a lead for the design antibiotics towards pathogens from the VAAP clade. Overall, the experimental results also suggest that in the VAAP clade the nucleotide salvage pathway is important and should be investigated, since the *de novo* dTMP synthesis appears to be compromised by a less efficient thymidylate synthase.

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Declarations can be found on
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