In-vitro evolution of dihydropteroate synthase: Effect of amino acid changes on enzyme function and development of resistance.

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Antifolates are a class of drugs that have been very successful as antibacterial and antiprotozoal. The drugs target the folate biosynthesis pathway of the parasite. Folates are necessary cofactors of various life processes like DNA synthesis, RNA repair. The fact that humans lack the enzymes for folate biosynthesis makes the pathway a very attractive target. The drugs are relatively cheap to produce, due to which, there has been a wide spread use of antifolates, which has now made the parasites resistant to the drug. The project was divided into two parts, focusing on the folate biosynthesis of malaria parasite (*Plasmodium* species) and *Streptococcus mutans* (which causes tooth decay) respectively.

In the first project, we focused on an enzyme in *P. falciparum* called DHPS. DHPS is involved in the folate biosynthesis pathway of the parasite and is present with another enzyme called HPPK. In previous studies from the same group, it has been shown that one portion of the HPPK (HPPK-2) and DHPS (DHPS-2) part is not needed for the enzyme complex to function properly. We started this study to analyze another region of the DHPS part (DHPS-1), to see the effects on the enzyme activity. We created four mutant sequences by deleting various portions of DHPS-1 and combining it with a sequence, HD2A, that already lacks two parts (named DHPS-2 and HPPK-2). Another sequence was created by deleting part of the linker region that joins the two enzymes. These mutant sequences were then transferred into bacteria that cannot produce the HPPK-DHPS enzyme complex. The rationale was that if a bacterium has to grow in a medium that lacks folate, it would have to make its own; and if it does not have its own enzyme complex, it would rely on the enzyme complex that has been transferred into it. Therefore, a bacteria culture that grows fast has an enzyme complex that is functional and vice-versa. The growth rate measurements showed that a longer deletion grew much faster than all other mutations. The run was repeated again and there were significant differences between the two runs. However, due to lack of time further experiments could not be carried out. When these bacteria were cultured on solid plates, they showed quite good growth. This suggests that the mutant enzyme is capable of substituting for the lack of bacterial enzyme.

The second part of the project focused on dental caries (commonly known as tooth decay) and its potential to act as a storehouse of antibiotic resistance. *Streptococcus mutans* are bacteria from the Viridans Group Streptococci (VGS) that colonize the mouth and are responsible for tooth decay. The major interest in VGS and especially *S. mutans*, has largely been due to the fact that they are capable of transferring their genetic elements to related species like *S. pneumoniae*. These genetic elements could include elements that confer antibiotic resistance. In Uganda, for example, Cotrimoxazole (SXT) is highly prescribed in dental practice and also recommended for HIV/AIDS prophylaxis. Due to this, large-scale resistance is observed in *S. mutans* against SXT. The present study was a continuation from 2009, when several clinical isolates were studied. Two isolates were selected that had differed in 3 amino acids. We started by changing the three amino acids in one isolate to match the other. Antibiotic susceptibility testing was performed to check how resistant the mutants were to SXT. This was done by growing the mutants on a solid medium which had antibiotic incorporated in it. The lowest concentration at which the mutants were not able to grow was considered as the Minimal Inhibitory Concentration (MIC). It was observed that MIC decreased with increasing mutations (that is the isolate became more susceptible to the antibiotic). Based on these results, it can be interpreted that *S. mutans* relies on point mutations to generate resistance. However, since these results are from the comparison of only two clinical isolates, further studies would be needed to reach to a conclusive proof. We also need to consider whether other mechanisms of resistance are involved. For example, some microbes use pumps that push out the antibiotic once it enters the microbial cell.