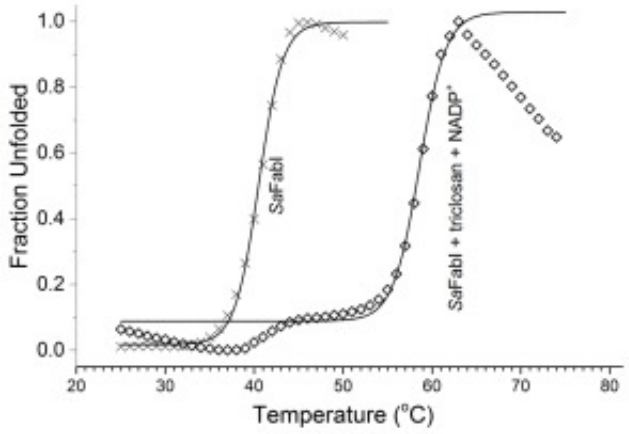
An efficient and economical assay to screen for triclosan-resistant Staphylococcus aureus

Triclosan is an effective inhibitor for an essential bacterial enzyme, the enoyl acyl carrier protein reductase (ENR/FabI), in fatty acid biosynthesis. Due to its value in inhibiting bacterial growth, including methicillin resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) strains, triclosan has been widely used to prevent infections. Species resistance to triclosan can arise from mutations in ENR/FabI. For example, triclosan-resistant *S. aureus* mutants have been reported, suggesting that other *S. aureus* mutants may also survive triclosan treatment, and need to be identified.



The standard method for identifying a triclosan-resistant mutant is to perform an enzyme activity inhibition assay. However, the enzyme assays for *S. aureus* are challenging due to the need to prepare the substrate, enoyl acyl carrier protein. Consequently, screening for triclosan-resistant mutants is also challenging. We have developed a simple thermal shift assay, which does not use protein-linked substrates, to determine the binding ability of triclosan to ENR/FabI active site, and thus can be used for screening for triclosan-resistant mutants. This method may also be applied to select effective triclosan analogues that inhibit ENR/FabI activity.

By following the fluorescence intensity of a probe (Sypro Orange) in *S. aureus* ENR/FabI sample as a function of temperature, a characteristic transition temperature, T_m, is obtained from the protein unfolding profile. When a compound binds to a protein molecule, it will stabilize the protein to give a higher transition temperature. We determine the transition temperatures of *S. aureus* ENR/FabI in the absence and presence of triclosan and $NADP^+$. If triclosan and $NADP^+$ do not bind ENR/FabI, the T_m is about 40 ^oC. If triclosan and $NADP^+$ bind ENR/FabI, the T_m is about 60 ^oC. A 20 ^oC temperature shift is detected. The thermal shifts are observed only when both triclosan and $NADP^+$ are present, but not when only one is present. Thus the binding we observed is the active-site binding.

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