The use of synthetic biology to produce industrially relevant compounds is receiving increased interest. New pathways can be introduced into easily grown host organisms to demonstrate this approach. This study describes the use of a thermophilic bacterium, *Thermus thermophilus* as a host to develop enzyme cascades carried out at elevated temperatures both *in vivo* and *in vitro* conditions. *T. thermophilus* can easily be grown under aerobic conditions and is naturally competent being able to take up foreign DNA using a dedicated DNA translocator structure made up of pilin-like proteins, within the bacterial cell wall. These advantageous properties have resulted in the development of a variety of genetic tools that have been used in this study.

T. thermophilus is being used as a host for production of both natural small molecules such as extremolytes and for non-natural compounds used as drug intermediates. The construction of non-natural synthetic operons has used a BioBricks cloning approach. The non-natural pathway was composed of three genes, an esterase, a carboxylic acid reductase and an alcohol dehydrogenase which were codon optimised for the high G-C content of *T. thermophilus* DNA. While all of the enzymes could be expressed in the thermophilic host, the presence of native aldehyde ferrodoxin dehydrogenase enzymes has currently limited the synthetic pathway to the first step, the hydrolysis of the substrate methyl *p*-toluate by the esterase. However the *in vitro* reaction cascade was achieved successfully and the reaction has been modelled using additional enzymes to regenerate the required NAD(P)H and ATP.