

P036 Carbon-Carbon bond formation: DOXP Synthase (1-deoxy-D-xylulose-phosphate synthase) and comparison of its biocatalytic potential with transketolase

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Transketolase has been widely studied as a carbon-carbon bond forming biocatalyst, as it has a broad substrate specificity for the aldehyde acceptor. The enzyme plays a key role in the pentose phosphate pathway, exchanging two-carbon units between sugars. It has been used to add a two-carbon ketol group on to non-native aldehyde substrates using the 2-carbon ketol donor substrate, hydroxypyruvate. A biocatalyst that could use pyruvate as this 2-carbon donor would give access to a large range of products without the terminal hydroxyl that transketolase products have. The *Escherichia coli* enzyme 1-deoxy-D-xylulose-phosphate synthase (DOXP-S), which uses pyruvate as its substrate in an analogous reaction to transketolase, could be an alternative enzyme for these biotransformations. DOXP-S has been cloned, expressed and characterised as a biocatalyst. The substrate range, assessed with high performance liquid chromatography (HPLC), is compared with that of transketolase. DOXP-S is shown to be much more selective over acceptor substrates than transketolase. Sequence comparisons with transketolase and existing transketolase mutants have been used to inform site-directed mutagenesis on DOXP-S, to combine the broader substrate range of transketolase with the donor specificity of DOXP-S.