Ketoreductase enzymes are well known in nature, and numerous genes that encode ketoreductase enzymes and ketoreductase enzyme sequences have been reported. *See, e.g.*, *Candida magnoliae* (Genbank Acc. No. JC7338; GI:11360538) *Candida parapsilosis* (Genbank Acc. No. 10 BAA24528.1; GI:2815409), *Sporobolomyces salmonicolor* (Genbank Acc. No. AF160799; GI:6539734), and *Rhodococcus erythropolis* (Genbank Acc. No. AAN73270.1; GI: 34776951).

Ketoreductase enzymes are being used with increasing frequency to provide alternative synthetic pathways to key compounds. When used, the ketoreductase enzymes may be provided as purified enzymes or as whole cells that express the desired ketoreductase. In view of their promise for improved synthetic pathways, there remains a need to identify additional ketoreductase enzymes that can be used to carry out transformations to prepare specific chiral alcohols.

SUMMARY

The present disclosure relates to ketoreductase enzymes capable of converting ketones to chiral alcohols, particularly as part of nucleotide synthesis. In examples, the subject ketoreductase enzymes described herein are capable of converting ketones to chiral alcohols in the synthesis of fluoronucleotides. In particular, the subject ketoreductase enzymes described herein may be useful in the preparation of fluorinated nucleosides, such as (trisodium O-{[(2*R*,3*S*,4*S*,5*R*)-5-(2-amino-6-oxido-9H-purin-9-yl)-3-fluoro-4-hydroxyoxolan-2yl]methyl} phosphorothioate hydrate (1:6)), also known as 3′-fluoro-thio-guanosine monophosphate or 3′-F-thio-GMP. Such fluorinated nucleosides may be useful as a biologically active compound and or as an intermediate for the synthesis of more complex biologically active compounds.

In one aspect, there is provided a polypeptide, wherein said polypeptide comprises an amino acid sequence having at least a 90% sequence identity to any one of SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

In another aspect, there is provided an expression vector comprising the polypeptide as defined herein, operably linked to one or more control sequences suitable for directing expression of the encoded polypeptide in a host cell.

In another aspect, there is provided a host cell comprising the expression vector as defined herein.

Additional examples describe processes for preparing the subject ketoreductase enzymes and processes for using the subject ketoreductase enzymes.

In another aspect, there is provided a process for precipitating proteins from a cell lysate containing ketoreducase enzymes, comprising treating the cell lysate containing ketoreducase enzymes with >20% isopropanol.

Other embodiments, examples, aspects and features of the present disclosure are either further described in or will be apparent from the ensuing description, examples and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts SDS-PAGE gel shows the removal of IPA insoluble proteins from the lyophilized enzyme preparation.