(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. For example, Kosjek, B. *et al.* disclosed the asymmetric synthesis of a chiral precursor 4,4-dimethoxy-2H-pyran-3-ol with a ketoreductase. *Organic Process Research & Development*, 2008, **12**. 584-588. 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 certain chemical transformations to prepare specific chiral alcohols.

SUMMARY

The present disclosure relates to ketoreductase enzymes capable of converting ketones to chiral alcohols, particularly on a substituted indane scaffold. In some examples, the subject ketoreductase enzymes described herein are capable of converting hydroxy indanones to diastereomerically pure 1,3-indane diols useful for the synthesis of belzutifan, 3-[[(1S,2S,3R)-2,3-difluoro-2,3-dihydro-1-hydroxy-7-(methylsulfonyl)-1H-inden-4-yl]oxy]-5-fluorobenzonitrile. This agent is a hypoxia-inducible factor inhibitor and recently received approval from the Food and Drug Administration in the United States for the treatment of adult patients with von Hippel-Lindau disease who require therapy for associated renal cell carcinoma, central nervous system hemangioblastomas, or pancreatic neuroendocrine tumors, not requiring immediate surgery.

In one aspect, there is provided a polypeptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 2.

In another aspect, there is provided a polynucleotide encoding the polypeptide as defined herein.

In another aspect, there is provided an expression vector comprising the polynucleotide 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 a polypeptide comprising an amino acid sequence having at least a 90% sequence identity to SEQ ID NO: 2, comprising treating the cell lysate containing the polypeptide to result in a cell lysate composition comprising >20% isopropanol.

Other 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 iPrOH insoluble proteins from the lyophilized enzyme preparation.

DETAILED DESCRIPTION OF THE INVENTION

Definitions