Lindberg, D., et al. (J. Biotechnol. 147 (2010) 169-171) reported deep eutectic solvents (DESs) are viable co-solvents for enzyme-catalyzed epoxide hydrolysis. Gorke, J.T., et al. reported hydrolase-catalyzed biotransformations in deep eutectic solvents (Chem. Commun. (2008) 1235–1237).

In US 8,247,198 enzymatic processing in deep eutectic solvents is reported.

Bellucci, J.J., et al. report the use of lysine as nucleophile (Angew. Chem. Int. Ed. Engl. 53 (2014) 1-6).

WO 2013/016653 provides methods for detecting the concurrent presence of at least two targets within a biological sample. The method includes contacting said biological sample with a first binding agent, said first binding agent operably linked to a first sortase molecule, wherein said first binding agent specifically binds to a first target; contacting said biological sample with a second binding agent, said second binding agent operably linked to a first sortase recognition sequence peptide, wherein said second binding agent specifically binds to a second target; adding a sortase substrate under conditions where a first sortase-mediated ligation of the sortase substrate to the first sortase recognition sequence will produce a ligation product, and detecting the ligation product, wherein detection of said ligation product indicates the concurrent presence of the first target and the second target in the biological sample.

Schmohl, L. and Schwarzer, D., reported about sortase-mediated ligations for the site-specific modification of proteins (Curr. Opin. Chem. Biol. 22 (2014) 122-128).

Summary

It has been found that a transpeptidation reaction can be performed in deep eutectic solvents. It has further been found that the reduction or even absence of water is not detrimental to the reaction but suppresses hydrolysis side reaction.

As reported herein is a method for the enzymatic production of a polypeptide comprising the following step

- incubating

  i) a first polypeptide (optionally comprising within the 100 C-terminal amino acid residues) the amino acid sequence LPXTG (SEQ ID NO:

01, wherein X can be any amino acid residue) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

ii) a second polypeptide comprising i) a glycinyl, an alaninyl, or a cysteinyl compound at its N-terminus (i.e. a compound that comprises a cysteine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group, which is part of a peptide bond, at position 1, or a compound that comprises an alanine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group, which is part of a peptide bond, at position 1, or a compound that comprises a glycine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group, which is part of a peptide bond), at position 1, or ii) an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, or iii) a lysine amino acid residue within its 5 N-terminal amino acid residues, and

iii) a third polypeptide with sortase A activity (i.e. a third polypeptide that is a sortase A or a catalytically active fragment thereof, i.e. that has sortase A activity),

in a deep eutectic solvent

and thereby producing a polypeptide.

One aspect as reported herein is a method for the enzymatic production of a polypeptide comprising the following step

- incubating

i) a first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

ii) a second polypeptide that has i) a glycinyl, an alaninyl, or a cysteinyl compound at its N-terminus, or ii) an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, or iii) a lysine amino acid residue within its 5 N-terminal amino acid residues, and

iii) a third polypeptide with sortase A activity,

in a deep eutectic solvent

and thereby producing a polypeptide.

In one embodiment the third polypeptide with sortase A activity is derived from Staphylococcus aureus sortase A, or from Streptococcus pyogenes Sortase A, or from Listeria monocytogenes Sortase A.

In one embodiment the third polypeptide is derived from Staphylococcus aureus sortase A, or from Streptococcus pyogenes Sortase A, or from Listeria monocytogenes Sortase A.

In one embodiment the third polypeptide with sortase A activity is derived from Staphylococcus aureus sortase A, or from Streptococcus pyogenes Sortase A, from Listeria monocytogenes Sortase A, or a catalytically active fragment thereof.

In one embodiment the third polypeptide comprises the amino acid sequence of SEQ ID NO: 05, SEQ ID NO: 06, or SEQ ID NO: 38. In one preferred embodiment the third polypeptide comprises the amino acid sequence of SEQ ID NO: 38.