compared to other N- or C-terminally truncated variants of the same novel Listeria monocytogenes Sortase A.

As reported herein is a polypeptide comprising the amino acid sequence of SEQ ID NO: 38. In this polypeptide only the amino acid sequence of SEQ ID NO: 38 is/has been obtained from Listeria monocytogenes. In one embodiment the amino acid sequence of SEQ ID NO: 38 is the sole Listeria monocytogenes derived amino acid sequence in the polypeptide.

One aspect as reported herein is use of a polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domain that has the amino acid sequence of SEQ ID NO: 38 and any of the further domains are polypeptides from different organisms or synthetic/artificial, wherein the domains are conjugated to each other either directly or via a peptidic linker, for the conjugation of a sortase-motif comprising polypeptide with a polypeptide comprising 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.

In one embodiment the polypeptide comprises additionally a tag at its N- or C-terminus either conjugated directly or via an intervening linker.

In one embodiment the polypeptide is consisting of the amino acid sequence of SEQ ID NO: 38 and the C-terminal tag of SEQ ID NO: 32. In one embodiment the polypeptide is consisting of the amino acid sequence of SEQ ID NO: 38.

In one embodiment the polypeptide is a sortase A.

As reported herein is a (fusion) polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domains are conjugated to each other either directly or via a peptidic linker. In one embodiment the domain that has the amino acid sequence of SEQ ID NO: 38 and (each/any of) the further domains are (obtained from) polypeptides from different organisms and/or are synthetic/artificial polypeptides. In one embodiment the domain that has the amino acid sequence of SEQ ID NO: 38 and the one or more further domains are from different organisms/do not exist in this combination in nature.

Another aspect as reported herein is a polypeptide consisting of SEQ ID NO: 38.

ii) a second polypeptide that comprises 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 polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domain that has the amino acid sequence of SEQ ID NO: 38 and any of the further domains are polypeptides from different organisms or synthetic/artificial, wherein the domains are conjugated to each other either directly or via a peptidic linker,

and thereby producing a polypeptide.

Another 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 comprises 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 polypeptide as defined herein,

  and thereby producing a polypeptide.

In one embodiment the method is for the enzymatic conjugation of two polypeptides.

In one embodiment the second polypeptide has at its N-terminus the amino acid sequence GGG, AAA, CGG, CAA, KGG or KAA.

In one embodiment the first polypeptide comprises at its C-terminus 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). In one embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 04) or LPETA (SEQ ID NO: 42) or LPKTG (SEQ ID NO: 43) or LPKTA (SEQ ID NO: 44).

In one embodiment the first polypeptide and the second polypeptide are independently of each other selected from an antibody variable domain, an antibody heavy chain Fab-fragment, an antibody Fc-region, a tag, and a peptide or polypeptide, a linker and a non-sortase motif moiety, each first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be

Patent Claims

1. Use of a polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domain that has the amino acid sequence of SEQ ID NO: 38 and any of the further domains are polypeptides from different organisms or synthetic/artificial, wherein the domains are conjugated to each other either directly or via a peptidic linker, for the conjugation of a sortase-motif comprising polypeptide with a polypeptide comprising 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.

2. A polypeptide consisting of SEQ ID NO: 38.

3. Use of a polypeptide according to claim 2 for the conjugation of a sortase-motif comprising polypeptide with a polypeptide comprising 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.

4. 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 comprises 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 polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domain that has the amino acid sequence of SEQ ID NO: 38 and any of the further domains are polypeptides from different organisms or synthetic/artificial, wherein the domains

are conjugated to each other either directly or via a peptidic linker,

and thereby producing a polypeptide.

5. 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 comprises 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 polypeptide according to claim 2,

and thereby producing a polypeptide.

6. The method according to claim 4 or 5, wherein the method is for the enzymatic conjugation of two polypeptides.

7. The method according to any one of claims 4 to 6, wherein the second polypeptide has at its N-terminus the amino acid sequence GGG, AAA, CGG, CAA, KGG or KAA.

8. The method according to any one of claims 4 to 7, wherein the first polypeptide comprises at its C-terminus 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).

9. The method according to any one of claims 4 to 8, wherein the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 04) or LPETA (SEQ ID NO: 42).

10. The method according to any one of claims 4 to 9, wherein the first polypeptide and the second polypeptide are independently of each other selected from an antibody variable domain, an antibody heavy chain Fab-fragment, an antibody Fc-region, a tag, and a peptide 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), a linker and a non-sortase motif moiety.