

Claims

1. A variant of porcine trypsin comprising or consisting of
5 an amino acid sequence that has at least 90% sequence identity to SEQ ID NO: 1,
wherein said amino acid sequence differs from SEQ ID NO: 1 at least by one or more
amino acid substitutions at one or more positions corresponding to F24, S44, D56, G78,
Y131, S172 and W193 of native porcine trypsin according to SEQ ID NO: 1,
with the proviso that said amino acid sequence is not native porcine trypsin according to
10 SEQ ID NO: 1; and
with the proviso that said amino acid sequence is not porcine variant trypsin S172A
according to SEQ ID NO: 2.
2. The variant of porcine trypsin according to claim 1,
15 wherein the amino acid at the position corresponding to F24 is substituted by an amino
acid selected from the group consisting of Ala, Asn, Arg, Gln, Ile, Leu, Lys, Met,
Ser, Thr, and Val;
wherein the amino acid at the position corresponding to S44 is substituted by an amino
acid selected from the group consisting of Leu and Pro;
20 wherein the amino acid at the position corresponding to D56 is substituted by an amino
acid selected from the group consisting of Ala, Asn, His, and Trp;
wherein the amino acid at the position corresponding to G78 is substituted by an amino
acid selected from the group consisting of Ala, Glu; Pro, Ser, and Tyr;
wherein the amino acid at the position corresponding to Y131 is substituted by an amino
25 acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln; Glu, Gly, His,
Ile, Leu, Met, Ser, Thr, Trp, Val;
wherein the amino acid at the position corresponding to S172 is substituted by an amino
acid selected from the group consisting of Ala, Cys, and Thr; and/or
wherein the amino acid at the position corresponding to W193 is substituted by an amino
30 acid selected from the group consisting of Phe, Ser, Thr, and Tyr.
3. The variant of porcine trypsin according to any one of claims 1 to 2, wherein said amino
acid sequence additionally differs from SEQ ID NO: 1 at least by one or more amino acid
substitutions at one or more positions corresponding to R99, R107, K125, and K170 of
35 native porcine trypsin according to SEQ ID NO: 1.
4. The variant of porcine trypsin according to claim 3,

- wherein the amino acid at the position corresponding to R99 is substituted by an amino acid selected from the group consisting of Ala, Asn, Asp, Glu, Gly, His, Leu, Phe, Thr, Trp, and Tyr;
- wherein the amino acid at the position corresponding to R107 is substituted by an amino acid selected from the group consisting of Asp, Gly, Pro, Ser, and Thr;
- wherein the amino acid at the position corresponding to K125 is substituted by an amino acid selected from the group consisting of Ala, Cys, Gln, Glu, Gly, His, Leu, Ser, and Tyr; and/or
- wherein the amino acid at the position corresponding to K170 is substituted by an amino acid selected from the group consisting of Ala, Asn, Gly, and Tyr.
5. The variant of porcine trypsin according to any one of claims 1 to 4, wherein said amino acid sequence is SEQ ID NO: 3, wherein
- Xaa24 is an amino acid selected from the group consisting of Ala, Asn, Arg, Gln, Ile, Leu, Lys, Met, Phe, Ser, Thr, and Val;
 - Xaa44 is an amino acid selected from the group consisting of Leu, Pro, and Ser;
 - Xaa56 is an amino acid selected from the group consisting of Ala, Asn, Asp, His, and Trp;
 - Xaa78 is an amino acid selected from the group consisting of Ala, Glu, Gly, Pro, Ser, and Tyr;
 - Xaa99 is an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Glu, Gly, His, Leu, Phe, Thr, Trp, and Tyr;
 - Xaa107 is an amino acid selected from the group consisting of Arg, Asp, Gly, Pro, Ser, and Thr;
 - Xaa125 is an amino acid selected from the group consisting of Ala, Cys, Gln, Glu, Gly, His, Leu, Lys, Ser, and Tyr;
 - Xaa131 is an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Ser, Thr, Trp, Tyr, and Val;
 - Xaa170 is an amino acid selected from the group consisting of Ala, Asn, Gly, Lys, and Tyr;
 - Xaa172 is an amino acid selected from the group consisting of Ala, Cys, Ser, and Thr; and/or
 - Xaa193 is an amino acid selected from the group consisting of Phe, Ser, Thr, Trp, and Tyr;
- with the proviso that SEQ ID NO: 3 is not porcine wild-type trypsin according to SEQ ID NO: 1; and

with the proviso that SEQ ID NO: 3 is not porcine variant trypsin S172A according to SEQ ID NO: 2.

6. The variant of porcine trypsin according to any one of claims 1 to 5, wherein said amino acid sequence is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, and SEQ ID NO: 119.
7. The variant of porcine trypsin according to any one of claims 1 to 6, wherein
- (i) said variant is capable of cleaving a peptide with the general formula A-Lys-Thr-Arg-Arg-B to yield a cleavage product of the general formula A-Lys-Thr-Arg-Arg in a yield of at least 80%,
- wherein A is an amino acid sequence consisting of one or more amino acids; and
- wherein B is an amino acid sequence consisting of one or more amino acids;
- and/or
- (ii) the variant exhibits an increased selectivity for the cleavage of a pre-pro-human insulin, pre-pro-insulin glargine, pre-pro-insulin lispro, pre-pro-insulin aspart, or

pre-pro-insulin glulisine at a position C-terminally to position B32-Arg as compared to porcine variant trypsin S172A according to SEQ ID NO: 2.

8. A nucleic acid molecule encoding
- 5 - a variant of porcine trypsin according to any one of claims 1 to 7, or
- a trypsinogen precursor molecule of a variant of porcine trypsin as defined in any one of claims 1 to 7,
- wherein said nucleic acid molecule is optionally contained in a vector or is optionally integrated into a genome.
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9. A host cell containing a nucleic acid molecule according to claim 8.
10. A method of producing a variant of porcine trypsin according to any one of claims 1 to 7 comprising the steps of:
- 15 cultivating a host cell according to claim 9; and
- isolating the trypsinogen precursor molecule of the variant of porcine trypsin from the culture medium or from the host cell; and
- optionally activating the trypsinogen precursor molecule, thereby obtaining said variant of porcine trypsin.
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11. A variant of porcine trypsin according to any one of claims 1 to 7 or a nucleic acid molecule according to claim 8 or a host cell according to claim 9 for use within a process of manufacturing a food ingredient or a feed ingredient.
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12. A use of the variant of porcine trypsin according to any one of claims 1 to 7 in a method for the production of human insulin, an insulin analogue or a derivative of insulin.
13. The use of claim 12, wherein the insulin analogue is selected from the group consisting of insulin aspart, insulin lispro, insulin glulisine, and insulin glargine.
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14. Use of a variant of porcine trypsin according to any one of claims 1 to 7 to cleave a protein or peptide with the general formula A-Lys-Thr-Arg-Arg-B,
- wherein A is an amino acid sequence consisting of one or more amino acids; and
- wherein B is an amino acid sequence consisting of one or more amino acids.
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15. The use of claim 14, wherein the cleavage yields a cleavage product A-Lys-Thr-Arg-Arg.

16. The use of claim 15, wherein the cleavage yields the cleavage product A-Lys-Thr-Arg-Arg in a yield of at least 80.