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(54) Titre : VARIANTS POLYPEPTIDIQUES DISSOCIANT DES TOXINES DE FUSARIUM, ADDITIF CONTENANT CES VARIANTS ET UTILISATION DE CET ADDITIF ET DE CES VARIANTS ET PROCEDE POUR LA DISSOCIATION DE TOXINES DE FUSARIUM

(54) Title: FUSARIUM TOXIN-CLEAVING POLYPEPTIDE VARIANTS, ADDITIVE CONTAINING THE SAME, AND USE OF THE SAME, AND METHOD FOR CLEAVING FUSARIUM TOXINS

(57) Abrégé/Abstract:
The invention relates to fusarium toxin-cleaving polypeptide variants of a fusarium toxin carboxyl esterase with the SEQ ID NO:46. Each of the polypeptide variants has an amino acid sequence shortened by 47 amino acids at the N terminus, and the amino acid sequences have at least 70%, preferably 80%, in particular 100%, sequence identity, namely SEQ ID NO:1, to the amino acid sequence portion 48 - 540 of the SEQ ID NO:46. The invention also relates to isolated polynucleotides which code for the polypeptide variants, to a fusarium toxin-cleaving additive containing at least one polypeptide variant and optionally at least one auxiliary agent, to the use of the polypeptide variants or the additive, and to a method for hydrolytically cleaving at least one fusarium toxin.

ABSTRACT

The invention relates to fusarium toxin-cleaving polypeptide variants of a fusarium toxin carboxyl esterase with the SEQ ID NO:46. Each of the polypeptide variants has an amino acid sequence shortened by 47 amino acids at the N terminus, and the amino acid sequences have at least 70%, preferably 80%, in particular 100%, sequence identity, namely SEQ ID NO:1, to the amino acid sequence portion 48 - 540 of the SEQ ID NO:46. The invention also relates to isolated polynucleotides which code for the polypeptide variants, to a fusarium toxin-cleaving additive containing at least one polypeptide variant and optionally at least one auxiliary agent, to the use of the polypeptide variants or the additive, and to a method for hydrolytically cleaving at least one fusarium toxin.

ABSTRACT

The invention relates to fusarium toxin-cleaving polypeptide variants of a fusarium toxin carboxyl esterase with the SEQ ID NO:46. Each of the polypeptide variants has an amino acid sequence shortened by 47 amino acids at the N terminus, and the amino acid sequences have at least 70%, preferably 80%, in particular 100%, sequence identity, namely SEQ ID NO:1, to the amino acid sequence portion 48 - 540 of the SEQ ID NO:46. The invention also relates to isolated polynucleotides which code for the polypeptide variants, to a fusarium toxin-cleaving additive containing at least one polypeptide variant and optionally at least one auxiliary agent, to the use of the polypeptide variants or the additive, and to a method for hydrolytically cleaving at least one fusarium toxin.

FUSARIUM TOXIN-CLEAVING POLYPEPTIDE VARIANTS, ADDITIVE CONTAINING THE SAME, AND USE OF THE SAME, AND METHOD FOR CLEAVING FUSARIUM TOXINS

The present invention relates to fusarium toxin-cleaving polypeptide variants, an additive containing the same, and the use of said polypeptide variants and/or said additive, and to methods for cleaving fusarium toxins by said polypeptide variants and/or said additive containing said polypeptide variants.

Mycotoxins very frequently occur in agricultural, plant-based products, causing severe economic damage as a function of the type and concentration of the mycotoxin, in particular in foods or feeds produced from agricultural products and also in humans and animals nourished with such foods or feeds, such damage being extremely manifold. Numerous methods have already been developed, by which it has been attempted to render harmless, i.e. detoxify or degrade, mycotoxins in order to largely prevent any damage caused by mycotoxins in the fields of animal and human nutrition, animal breeding, the processing of feed and food products and the like.

A prominent group of mycotoxins comprises fusarium toxins, wherein the terms “fusarium toxin” or “fusarium toxins” are equivalent and each refer to at least one or several, or the totality of, the fumonisins produced by the mold fungus *Fusarium sp.* as well as derivatives and degradation products thereof, yet in particular to fumonisins A1-2 (FA1-2), fumonisins B1-4 (FB1-4), fumonisins C1, 2, 4 (FC1, FC2, FC4) and HFC1 and to partially hydrolyzed fumonisins FA1-2, FB1-4, FC1-2, FC4 and HFC1. Partially hydrolyzed fumonisins comprise just one tricarballylic acid residue, whereas FA1-2, FB1-4, FC1-2, FC4 and HFC1 comprise two tricarballylic acid residues. Moreover, the structurally similar *Alternaria alternata lycopersici* (AAL) toxins are also encompassed by the group of fusarium toxins, AAL toxins being subdivided into the groups AAL-TA1 (CAS No 79367-52-5), AAL-TA2 (CAS No 79367-51-4), AAL-TB1 (CAS No 176590-32-2) and AAL-TB2 (CAS No 176705-51-4). FA1-2, FB1-4, FC1-2, FC4 and HFC1 have the following structural formula:

Fusarium toxin	R1	R2	R3	R4	R5
FA1	-OH	-OH	-CH2CO	-CH3	-H
FA2	-H	-OH	-CH2CO	-CH3	-H
FB1	-OH	-OH	-H	-CH3	-H
FB2	-H	-OH	-H	-CH3	-H
FB3	-OH	-H	-H	-CH3	-H
FB4	-H	-H	-H	-CH3	-H
FC1	-OH	-OH	-H	-H	-H
FC2	-OH	-H	-H	-H	-H
FC4	-H	-H	-H	-H	-H
HFC1	-OH	-OH	-H	-H	-OH

FB1 is the most frequently occurring toxin from the group of fusarium toxins, yet numerous derivatives and related molecules likewise having toxic effects in humans and animals are known. The diseases caused by the ingestion of mycotoxins in humans or animals are referred to as mycotoxicoses, in the case of fusarium toxins also as fusarium toxin mycotoxicoses. Thus, it is known that fusarium toxins impair the sphingolipid metabolism by interacting with the enzyme ceramide synthase. Sphingolipids not only are components of cell membranes, but also play an important role as signal and messenger molecules in many elementary cellular processes such as cell growth, cell migration and cell binding, in inflammatory processes or intracellular transport processes. Due to the impairment of the sphingolipid metabolism, fusarium toxins have been made

responsible for the toxic effects on various animal species and also humans. It could, thus, be demonstrated that fusarium toxins have immunosuppressive effects, cancerogenically acting in rodents, and they have been associated with esophageal cancer and neural tube defects in humans due to epidemiologic data. They have been held responsible for the typical toxicosis caused by pulmonary edemas in various animal species, for instance in pigs. Examples of fusarium toxin mycotoxicoses include neurotoxic diseases such as the equine leucoencephalomalacia or porcine pulmonary edemas caused by fumonisin intoxication. Since the contamination with fusarium toxins is almost ubiquitous on various cereal crops and, in particular corn, nuts and vegetables, their strongly negative effects on the health of humans and animals are not to be neglected.

The microbial degradation of fumonisins has already been described in EP-A 1 860 954, according to which microorganisms are used to detoxify fumonisins and fumonisin derivatives by adding to feeds detoxifying bacteria or yeasts selected from precisely defined strains for detoxifying fumonisins.

Catabolic metabolic paths for the biological degradation of fumonisins and the genes and enzymes responsible therefor have already been described, too. Thus, EP 0 988 383, for instance, describes fumonisin-detoxifying compositions and methods, wherein the fumonisin-degrading enzymes used are above all produced in transgenic plants in which the detoxification of fumonisins is effected using an amine oxidase that requires molecular oxygen for its enzymatic activity.

Moreover, WO 2004/085624 describes transaminases, deaminases and aminomutases and methods for the enzymatic detoxification of aminated toxins, e.g. fumonisins. In this context, polypeptides possessing deaminase activity are used for detoxification.

The above-identified products or methods involve the drawback of requiring molecular oxygen, and optionally cofactors, for the detoxification of mycotoxins, wherein, in particular, the cited amino oxidases do not show any effect under oxygen-free reaction conditions.

EP-A 2 326 713 relates to an additive, and a method for preparing the same, by which it is possible to degrade fumonisins in an oxygen-independent and cofactor-free enzymatic reaction. The temperature stability of the enzyme described therein that is mainly resposiblefor the detoxification,

namely a carboxylesterase, is, however, low such that the additive, or carboxylesterase of SEQ ID No. 46, is not suitable for applications at elevated temperatures.

In the food and feed industries, thermal treatments for the production of hygienized products with reduced microbial load are of great importance. In this respect, the pelletization of feeds is particularly wide-spread, already constituting a standardized process, for a plurality of reasons such as enhancing flowability, reducing dust formation, lowering microbial load, in particular of salmonellae. During the pelletizing process, the commodity is usually moistened by hot steaming, heated and subsequently pressed through a matrix under pressure. The use of polypeptides or enzymes as additives for pelletizing foods or feeds constitutes a technological challenge, since the enzymes or polypeptides, as a rule, are sensitive to elevated temperatures. The thermal treatment of enzymes or polypeptides may result in a reduction of their specific activities and/or in irreversible denaturation. A way of counteracting this is the encapsulation or coating of the proteins such as, for instance, described in WO 92/12645. It is thereby possible to protect proteins from thermal influences, yet this approach involves the risk that the proteins will not be released rapidly enough in the mouth-gastrointestinal system, and hence will take effect either too slowly or not at all. Due to their low temperature stability, the hitherto known polypeptides for detoxifying fusarium toxins cannot be admixed to feeds or foods that are to be pelletized without prior encapsulation or prior coating.

Technological processes in which the detoxification of fusarium toxins at elevated temperatures is essential include the production of pasta and other corn products such as polenta, popcorn, cornflakes, corn bread or tortillas, and starch liquefaction processes, saccharification processes or fermentation processes such as, in particular, the mashing or fermentation process in the production of bioethanol. In this respect, it is important to ensure that foods or feeds produced by such processes do not contain fusarium toxins in harmful amounts. Hitherto known polypeptides cannot be used in such processes due to their minimal, or absent, activity at the process temperatures in question.

Hence there is the need for enzymes and/or polypeptides for the specific, safe and reliable cleavage, in particular detoxification, of fusarium toxins, wherein the enzymatic reaction requires neither oxygen nor a cofactor and the enzyme or polypeptide, moreover, exhibits sufficient temperature stability and sufficient temperature activity so as to be usable in technological processes at elevated temperatures.

The present invention, therefore, aims to provide fusarium toxin-cleaving polypeptide variants of a fusarium toxin carboxylesterase of SEQ ID No. 46, by which it is possible to cleave at least one fusarium toxin to non-toxic or less toxic products in an oxygen-independent and cofactor-free manner, wherein the polypeptide variants have increased temperature stabilities and increased temperature activities as compared to fusarium toxin carboxylesterase of SEQ ID No. 46.

To solve this object, the present invention is essentially characterized by fusarium toxin-cleaving polypeptide variants of a fusarium toxin carboxylesterase of SEQ ID No. 46, characterized in that the polypeptide variants each possess an amino acid sequence truncated by 47 amino acids at the N-terminus, the amino acid sequences sharing at least 70%, preferably 80%, particularly preferably 100%, sequence identity, namely SEQ ID No. 1, with the amino acid sequence section 48-540 of SEQ ID No. 46, that the temperature stability (T(50%) of the polypeptide of SEQ ID No. 46 is determined to be 42°C and that of the polypeptide variant of SEQ ID No. 1 is determined to be 45°, or modifications of SEQ ID No. 1 having a relative increase of T(50%) compared to the parental enzyme of SEQ ID No. 1, wherein on at least one position selected from the group consisting of 10, 33, 66, 107, 140, 144, 149, 151, 157, 199, 266, 267, 270, 272, 275, 280, 284, 286, 293, 302, 312, 329, 332, 360, 363, 364, 365, 367, 371, 372, 377, 389, 391, 394, 418, 419, 424, 427, 429, 430, 436, 440, 443, 447, 453, 455, 456, 457, 462, 463, 464, 465, 469, 473, 478, 487 and 490 an amino acid substitution is contained as modification, and that the amino acid substituents at positions 10 and 456 are selected from Q, E, N, H, K and R, at positions 33, 107, 293 and 332 from E, Q, D, K, R and N, at positions 66, 463 and 478 from D, E, K, N, Q and R, at positions 140 and 490 from P, A, S and N, at positions 144 and 367 from I, L, M and V, at positions 149, 270, 312, 329 and 372 from F, Y, W and H, at positions 151 and 453 from D, E, K and R, at positions 157 and 462 from F, H, W and Y, at positions 199, 302, 365 and 464 from I, L, M and V, at positions 266 and 455 and from A, S and T, at positions 267, 394 and 429 from A, N, P and S, at position 272 from H, N, Q and S, at position 275 from A, D, E, G, K, N, Q, R and S, at position 280 from A, D, E, K, N, P, Q, R and S, at position 284 from A, N, P, S, T and V, at position 286 from A, D, E, K, N, P, R and S, at positions 360, 377, 391, 419 and 427 from A, I, L S, T and V, at positions 363, 443 and 457 from A, S, T and V, at position 364 from H, I, L, M, N, Q, S and V, at position 371 from A, I, L, M, S, T and V, at position 389 from I, L, M and V, at positions 418, 430, 447 and 473 from A, G and S, at position 424 from A, D, E, G, K, R and S, at position 436 from A, G, S and T, at position 440 from A, G, S and T, at position 465 from A, G, H, N, Q, S and T, at position 469 from D, E, K and R and/or at position 487 from N, D, Q, H and S. It has surprisingly turned out that an amino acid

sequence truncated by 47 amino acids relative to SEQ ID No. 46 is both significantly more active than said sequence and also has increased temperature stability as compared to said sequence. In that polypeptide variants of SEQ ID No. 1 are formed, in particular polypeptide variants having amino acid sequences sharing at least 70% sequence identity with SEQ ID No. 1, and comprising amino acid substitutions on at least one position selected from the group consisting of 10, 33, 66, 107, 140, 144, 149, 151, 157, 199, 266, 267, 270, 272, 275, 280, 284, 286, 293, 302, 312, 329, 332, 360, 363, 364, 365, 367, 371, 372, 377, 389, 391, 394, 418, 419, 424, 427, 429, 430, 436, 440, 443, 447, 453, 455, 456, 457, 462, 463, 464, 465, 469, 473, 478, 487 and 490, a temperature stability enhancement by at least 4% as compared to that of the fusarium toxin carboxylesterase of SEQ ID No. 1 has been achieved. The use of such polypeptide variants having fusarium toxin-cleaving properties has enabled the detoxification of fusarium toxins at elevated temperatures, for instance during technological processes. This is, in particular, enabled by the temperature activity being also enhanced in addition to the temperature stability. In particular, it will thereby be safeguarded that the enzymatic activity of the polypeptide variants will even be maintained at elevated temperature stresses as might, for instance, also occur during transport in containers.

According to the invention, a particularly strong increase in the temperature stability is achieved in that the amino acid substituents at positions 10 and 456 are selected from Q, E, N, H, K and R, at positions 33, 107, 293 and 332 from E, Q, D, K, R and N, at positions 66, 463 and 478 from D, E, K, N, Q and R, at positions 140 and 490 from P, A, S and N, at positions 144 and 367 from I, L, M and V, at positions 149, 270, 312, 329 and 372 from F, Y, W and H, at positions 151 and 453 from D, E, K and R, at positions 157 and 462 from F, H, W and Y, at positions 199, 302, 365 and 464 from I, L, M and V, at positions 266 and 455 and from A, S and T, at positions 267, 394 and 429 from A, N, P and S, at position 272 from H, N, Q and S, at position 275 from A, D, E, G, K, N, Q, R and S, at position 280 from A, D, E, K, N, P, Q, R and S, at position 284 from A, N, P, S, T and V, at position 286 from A, D, E, K, N, P, R and S, at positions 360, 377, 391, 419 and 427 from A, I, L S, T and V, at positions 363, 443 and 457 from A, S, T and V, at position 364 from H, I, L, M, N, Q, S and V, at position 371 from A, I, L, M, S, T and V, at position 389 from I, L, M and V, at positions 418, 430, 447 and 473 from A, G and S, at position 424 from A, D, E, G, K, R and S, at position 436 from A, G, S and T, at position 440 from A, G, S and T, at position 465 from A, G, H, N, Q, S and T, at position 469 from D, E, K and R, and/or at position 487 from N, D, Q, H and S, whereat the amino acids originally present on the cited positions having been substituted in any event.

The term “carboxylesterase” refers to any enzyme, polypeptide or polypeptide variant capable of cleaving carboxylic ester compounds to the respective alcohol compounds and carboxylic acid compounds by means of water. The term “fusarium toxin carboxylesterase” refers to any enzyme, polypeptide or polypeptide variant capable of hydrolyzing at least one fusarium toxin by hydrolytically cleaving at least one tricarballylic acid (propane-1,2,3-tricarboxylic acid) off the same. A “fusarium toxin-cleaving property” as described herein refers to the capability of hydrolytically cleaving off at least one tricarballylic acid residue from a least one fusarium toxin, in particular from FA1-2, FB1-4, FC1-2, FC4, HFC1, AAL-TA1-2 und AAL-TB1-2 or derivatives or degradation products thereof.

The terms used below are taken from the technical terminology and, unless otherwise stated, are each used in their conventional meanings. Thus, the term “polynucleotide” refers to any kind of genetic material of any length and sequence, such as single-stranded and double-stranded DNA and RNA molecules, including regulatory elements, structural elements, groups of genes, plasmids, entire genomes and fragments thereof. The term “polypeptide” comprises proteins such as enzymes, antibodies and polypeptides with up to 500 amino acids, such as peptide inhibitors, domains of proteins, yet even short polypeptides with small sequence lengths, e.g. less than 10 amino acids, such as receptors, ligands, peptide hormones, tags and the like. The term “position” in a polynucleotide or polypeptide refers to a single, specific base or amino acid in the sequence of said polynucleotide or polypeptide, respectively. The names of the amino acids are abbreviated by the one- or three-letter codes familiar to the skilled person in the art.

The term “unit” or “U” refers to the measure of the catalytic activity of an enzyme, polypeptide or polypeptide variant and is defined as the number of micromoles (µmol) of substrate, i.e. fumonisin B1 in this case, that are reacted or cleaved per minute under defined conditions. Thus, if at least one tricarballylic acid residue is cleaved off from 60 µmol FB1 within 15 min, this corresponds to a catalytic activity of 4 units. For FB1 cleavage, the reaction conditions are defined as follows: The reaction is carried out in 20 mM Tris-HCl buffer (pH 8.0) with 0.1 mg/ml bovine serum albumin at a temperature of 30°C for 30 min. The substrate concentration in the reaction is 100 µM FB1.

By “catalytic activity”, “catalytic enzyme activity” or “activity” of an enzyme or polypeptide solution or solution of a polypeptide variant, the enzymatic concentration of the enzyme or polypeptide solution, or solution of the polypeptide variant, is defined, indicated in units per milliliter of solution.

The term “specific activity” is defined as the catalytic activity per milligram of enzyme, polypeptide or polypeptide variant and is calculated from the ratio of the catalytic activity of an enzyme solution to the mass concentration (mass per unit volume) of the enzyme in said solution. If, for instance, an enzyme solution has a catalytic activity of 50 U/ml and a mass concentration of 1 mg/ml, its specific activity is 50 U/mg.

The term “temperature stability” refers to the property of enzymes, polypeptides or polypeptide variants to maintain their catalytic activities after temporary exposure to elevated temperatures (preincubation). The temperature stability is determined by measuring, and comparing, the activity of an enzyme or polypeptide solution, or solution of a polypeptide variant, after a 5-minute heat treatment and without heat treatment at identical, defined conditions. The temperature stability is thus a measure for the resistance of enzymes to temporal heat exposure. The temperature at which the residual activity of the heat-treated enzyme is 50% as compared to the non-heat-treated 100% control is the measure for the temperature stability and is abbreviated by T(50%). If, for instance, the activity of an enzyme solution is 50 U/ml without preincubation and 25 U/ml after a 5-minute preincubation at 50°C, the temperature stability of the enzyme is 50°C, or the enzyme is temperature-stable up to 50°C. The increases in the T(50%) of polypeptide variants relative to the parental polypeptide of SEQ ID No. 1 is defined as a measure for the increased temperature stability and can be indicated relatively as a percentage value or absolutely in degree Celsius.

The term “temperature activity” defines the temperature at which the enzyme, polypeptide or polypeptide variant exhibits the highest activity, the catalytic activity being measured over a period of 30 minutes.

The terms “polypeptide variant’” or “variant”, in particular, refer to polypeptide sequences possessing at least one amino acid substitution as compared to SEQ ID No. 46, wherein the enzymatic function, i.e. the fusarium toxin-cleaving property, remains maintained. Moreover, a “polypeptide variant” may additionally comprise amino acid insertions or deletions, in particular a C- or N-terminally extended or truncated sequence, relative to the polypeptide sequence of SEQ ID No. 46. An enzymatic function is substantially maintained if the enzymatic reaction mechanism remains unchanged, i.e. the fusarium toxin is hydrolyzed on the same site and the specific activity of the variant is at least 10%, preferably at least 50%, more preferably at least 90%, yet in particular >100%, based on the original, parental polypeptide of SEQ ID No. 46.

Amino acid substitutions at defined positions are described by the following nomenclature: original amino acid; position; new amino acid. If, for instance, glycine substitutes for a proline at position 134, this is indicated by Pro134Gly or P134G. Multiple mutations are separated by a plus sign or a slash. If, for instance, proline is substituted by glycine at position 134, and arginine is substituted by lysine at position 136, this is indicated by Pro134Gly+Arg136Lys or Pro134Gly/Arg136Lys or P134G+R136K or P134G/R136K, respectively. If an amino acid is substituted by two or several alternative amino acids at one position, the alternative amino acids are separated by a comma or a slash. If, for instance, proline is substituted at position 134 not only by glycine, but also by serine, valine and methionine, this is indicated by Pro134Gly,Ser,Val,Met or Pro134Gly/Ser/Val/Met or P134G,S,V,M or P134G/S/V/M. If, for instance, a substitution or exchange of an amino acid at a defined position is not defined in detail, this is to be interpreted such that said amino acid may be replaced by any other amino acid. If, for instance, a mutation of proline at position 134 is not defined in detail, the proline can be substituted by one of the following amino acids: A, R, N, D, C, Q, E, G, H, I, L, K, M, F, S, T, W, Y or V.

The term “sequence identity” refers to a percentual sequence identity. For amino acid sequences and nucleotide sequences, the sequence identity can be determined visually, yet preferably calculated by a computer program. The amino acid sequence having the sequence SEQ ID No. 1 is defined as reference sequence. A sequence comparison is also performed within sequence sections, a section meaning a continuous sequence of the reference sequence. Normally, the length of the sequence sections for nucleotide sequences is 18 to 600, preferably 45 to 200, more preferably 100 to 150, nucleotides. Normally, the length of the sequence sections for peptide sequences is 3 to 200, more preferably 15 to 65, most preferably 30 to 50, amino acids. There is a plurality of purchasable or costlessly available bioinformatic programs that can be used for the determination of homology and are constantly updated. Examples include GCG Wisconsin Bestfit package (Devereux et al. 1984), BLAST (Altschul et al. 1990) or BLAST 2 (Tatusova und Madden 1999). Due to the different adjustment options of these algorithms, it may happen that different results are output at identical input sequences. It is, therefore, necessary to define the search algorithm and its respective settings. In the present case, the sequence identity was assessed using the programs NCBI BLAST (Basic Local Alignment Search Tool), in particular BLASTP for polypeptides and BLASTN for polynucleotides, which are available on the website of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/). It is thereby possible to compare two or several sequences with one another according to the algorithm of Altschul et al., 1997 (Nucleic Acids Res., 25:3389-3402). In this case, the program versions of August 12, 2014,

were used. As program setings, the base settings were applied, in particular for the amino acid comparison: “max target sequence“ = 100; “expected threshold“ = 10; “word size“ = 3; “matrix” = BLOSOM62; “gap costs“ = “Existence: 11; Extension: 1”; “computational adjustment“ = “Conditional compositional score matrix adjustment“; and for the nucleotide sequence comparison: Word Size: 11; Expect value: 10; Gap costs: Existence = 5 , Extension = 2; Filter = low complexity activated; Match/Mismatch Scores: 2,-3; Filter String: L; m.

According to a preferred further development of the invention, the amino acid substitution is selected from the group consisting of 10Q, 33E, 66D, 107E, 140P, 144M, 149F, 151R, 157Y, 199I, 266S, 267P, 270F, 272H, 275E, 275A, 280D, 280P, 284T, 284P, 286P, 286R, 293E, 302I, 312F, 329F, 332E, 360V, 363T, 364H, 364L, 365I, 367H, 371V, 371M, 372F, 377V, 389L, 391V, 394P, 418A, 419V, 424A, 424K, 427V, 429P, 430A, 436A, 436S, 440G, 440S, 443T, 447A, 453R, 455S, 456Q, 457T, 462Y, 463D, 464I, 465H, 465S, 465G, 469K, 473A, 478D, 487N und 490P. In that the polypeptide variants comprise at least one such substitution, the temperature stability and the temperature activity can even be more selectively enhanced, wherein other enzyme parameters such as the specific activity, pH stability or pH activity can likewise be improved, which, however, at least exhibit the values of the fusarium toxin carboxylesterase of SEQ ID No. 1.

According to a further development of the invention, the polypeptide variants on at least one position selected from the group consisting of 66, 199, 302, 377, 394, 424, 430 and 463 each comprise an amino acid substitution and a temperature stability increased by at least 6% as compared to that of fusarium toxins carboxylesterase of SEQ ID No. 1. Such polypeptide variants enable the cleavage of fusarium toxins during technological processes at elevated temperatures, such as the production of pasta and other corn products such as polenta, popcorn, cornflakes, corn bread or tortillas, and starch liquefaction processes, saccharification processes or fermentation processes such as, in particular, the mashing or fermentation process in the production of bioethanol.

According to a preferred further development of the invention, the amino acid substitution is selected from the group consisting of 66D, 199I, 302I, 377V, 394P, 424A, 430A und 463D. Such a substitution enables an increase of the temperature stability and of the temperature activity of the polypeptide variants by at least 3°C relative to the fusarium toxin carboxylesterase of SEQ ID No. 1.

According to a further development of the invention, the polypeptide variants, on at least two, in particular three, positions of the amino acid sequence, selected from the group consisting of 10, 33, 66, 107, 140, 144, 149, 151, 157, 199, 266, 267, 270, 272, 275, 280, 284, 286, 293, 302, 312, 329, 332, 360, 363, 364, 365, 367, 371, 372, 377, 389, 391, 394, 418, 419, 424, 427, 429, 430, 436, 440, 443, 447, 453, 455, 456, 457, 462, 463, 464, 465, 469, 473, 478, 487 and 490, each comprise an amino acid substitution, wherein the amino acid substitution being selected from the group consisting of 10Q, 66D, 144M, 151R, 199I, 266S, 267P, 272H, 275E, 275A, 280D, 284T, 286P, 286R, 293E, 302I, 360V, 363T, 364H, 364L, 365I, 367H, 371V, 371M, 372F, 377V, 389L, 391V, 394P, 418A, 419V, 424A, 424K, 427V, 429P, 430A, 436A, 436S, 440G, 440S, 443T, 447A, 453R, 455S, 456Q, 457T, 462Y, 463D, 464I, 465H, 465S, 465G, 469K, 473A, 478D, 487N and 490P, and exhibit a temperature stability increased by at least 15% as compared to the fusarium toxin carboxylesterase of SEQ ID No. 1. It has surprisingly turned out that by substituting several amino acids their positive effects on the temperature stability are approximately additive, the temperature stability being increased by more than 7°C by inserting at least three amino acids different from the amino acids originally present in the sequence. Such an increase is sufficient to use the enzymes, for instance, for mash resting at 55°C in the production of bioethanol or to pelletize feeds at moderate temperatures of about 65 to70°C.

According to a further development of the invention, the amino acid sequence of the polypeptide variants comprises combinations of several amino acid substitutions, the combinations of the positions being selected from the group consisting of 66/199/302/394/424/430, 66/199/302/377/394/424/430, 66/199/302/377/394/424/430/463, 66/144/199/302/360/372/377/394/424/430/443/463, 199/302/377/394/424/430/463, 66/199/302/377/394, 66/199/302/364/377/394/424/430/463, 66/199/302/377/394/424/430/463/465, 66/199/302/377/394/424/430/440/463, 66/199/302/377/394/424/430/447/463, 66/199/302/377/394/418/424/430/463, 66/199/302/377/394/424/436/430/463, 66/199/302/364/377/394/424/430/463, 66/199/302/377/394/424/430/463/490, 66/199/302/377/394/424/430/463/469, 66/199/302/377/389/394/424/430/463, 66/199/302/377/394/424/430/463/465, 66/199/302/377/394/424/430/463/464, 66/199/302/377/394/424/430/463/465, 66/199/302/377/394/424/430/440/463, 66/199/302/377/394/424/430/457/463, 66/199/302/377/394/424/430/436/463, 66/199/302/363/371/377/394/424/430/463, 66/199/302/377/394/424/430/447/453/463, 66/199/302/377/394/424/430/456/462/463, 66/199/302/377/394/419/424/427/430/463, 66/199/302/365/377/394/424/430/463/487 und 66/199/302/371/377/394/424/430/463/487.

Polypeptide variants substituted in such a manner exhibit a temperature stability increased by at least 25% as compared to the fusarium toxin carboxylesterase of SEQ ID No. 1. Such combinations of substitutions enable a further increase of not only the temperature stability but also the temperature activity of the polypeptide variants.

According to a preferred further development of the invention, the amino acid sequences of the polypeptide variants are selected from the group consisting of SEQ ID Nos. 2 to 29. Such polypeptide variants have a temperature stability increased by at least 11°C, preferably at least 13°C, and even more preferably at least 15°C, as compared to the enzyme of SEQ ID No. 1, thus guaranteeing the activity of the respective polypeptide, and hence the detoxification of fusarium toxins, during or after technological processes at elevated temperature loads, such as mash resting at 65°C in the production of bioethanol or pelletizing at temperatures of about 75-80°C.

According to a further development of the invention, each of the amino acid sequences of the polypeptide variants comprises combinations of several amino acid substitutions, the combinations of the positions being selected from the group consisting of
66/99/302/364/377/389/394/419/424/427/430/447/463/465/469,
66/199/302/377/389/394/419/424/427/430/447/463/465/469,
66/199/302/363/364/371/377/389/394/419/424/427/430/447/463/464/465/469,
66/199/302/363/371/377/389/394/419/424/427/430/447/463/464/465/469,
66/199/302/364/367/371/377/389/394/418/419/424/427/430/436/440/447/463/464/465/
469/490, 66/199/302/367/371/377/389/394/418/419/424/427/430/436/440/447/463/464/465/469/
490,
66/199/302/363/367/371/377/394/424/430/463/490,
66/199/302/377/394/418/419/424/427/430/436/440/447/463,
66/199/302/377/389/394/424/430/457/463/464/465/469,
66/199/302/363/371/377/389/394/419/424/427/430/440/447/457/463/464/469/490,
66/199/302/377/394/424/430/463/447/490/469/465,
66/199/302/377/394/424/430/463/490/469/465/419/427/371/487,
66/199/302/371/377/394/419/424/427/430/447/453/463/465/469/487/490,
66/199/302/367/371/377/389/394/418/419/424/427/429/430/436/440/447/457/463/464/465/469/490
, 66/199/302/371/377/389/394/419/424/427/430/436/447/453/456/462/463/465/469/490/ 487
and
66/199/302/367/371/377/389/394/418/419/424/427/429/430/436/440/447/453/456/457/462/463/464

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/465/469/487/490. These polypeptide variants exhibit a temperature stability increased by at least 40%, and an increased temperature activity, as compared to the fusarium toxin carboxylesterase of SEQ ID No. 1 so as to be usable in a plurality of methods requiring elevated temperatures.

According to a preferred further development of the invention, the amino acid sequences of the polypeptide variants are selected from the group consisting of SEQ ID Nos. 30 to 45. Such polypeptide variants exhibit temperature stabilities increased by at least 18%, preferably at least 22°C, and more preferably at least 27°C, as compared to the enzyme of SEQ ID No. 1, thus ensuring an activity of the polypeptide, and hence the detoxification of fusarium toxins, during or after technological processes at high temperature loads, such as pelletizing at temperatures above 80°C, in particular above 85-90°C. Pelletizing at high temperatures of about 90°C is of great importance, in particular, in the poultry industry in order to ensure a satisfactory reduction of the salmonella load on feed.

The term "conservative mutation" refers to the substitution of amino acids by other amino acids that are considered as conserved by a person skilled in the art, i.e. have similar specific properties, or the properties of the amino acid is maintained, i.e. conserved. Specific properties of amino acids are, for instance, their sizes, polarities, hydrophobicities, charges or pKa values. Amino acids can be classified in groups based on their properties, and the groups can be illustrated in the Venn Diagram. Amino acids from the same group, and preferably from the same subgroup, may be substituted for each other. The classification of amino acids according to the properties: hydrophobicity, polarity and size in groups and subgroups can be taken from Taylor W.R. (1986). By a conservative or conserved mutation, a substitution of an acidic amino acid for another acidic amino acid, a basic amino acid for another basic amino acid, a polar amino acid for another polar amino acid and the like are, for instance, understood. The polypeptide variants, in particular, may additionally contain at least one conservative amino acid substitution on at least one position, said conservative amino acid substitution being selected from the group of substitutions: G for A, A for G/S, V for I/L/A/T/S, I for V/L/M, L for I/M/V, M for L/I/V, P for A/S/N, F for Y/W/H, Y for F/W/H, W for Y/F/H, R for K/E/D, K for R/E/D, H for Q/N/S, D for N/E/K/R/ Q, E for Q/D/K/R/N, S for T/A, T for S/V/A, C for S/T/A, N for D/Q/H/S and Q for E/N/H/K/R

If a substitution in a polypeptide variant according to the invention at a defined position results in that, for instance, a polar amino acid such as Asp is replaced by a hydrophobic amino acid such as Ala, conserved mutations will also include any mutations leading to another hydrophobic amino

acid (e.g. glycine, leucine, phenylalanine, valine) at that position. Such further polypeptide variants containing alternative conserved mutations are likewise encompassed.

The present invention further aims to provide polynucleotides encoding a fusarium toxin- cleaving polypeptide variant of a fusarium toxin carboxylesterase of SEQ ID No. 1, which enable the cleavage of at least one fusarium toxin to non-toxic or less toxic products in an oxygen-independent and cofactor-free manner and which exhibit an increased temperature stability as compared to the fusarium toxin carboxylesterase of SEQ ID No. 1.

To solve this object, the invention is characterized in that the polynucleotide comprises a nucleotide sequence encoding a fusarium toxin-cleaving polypeptide variant of a fusarium toxin carboxylesterase having the amino acid sequence SEQ ID No. 1, the polypeptide variants comprising an amino acid sequence sharing at least 70% sequence identity with the amino acid sequence SEQ ID No. 1, and that the polypeptide variants on at least one position selected from the group consisting of 10, 33, 66, 107, 140, 144, 149, 151, 157, 199, 266, 267, 270, 272, 275, 280, 284, 286, 293, 302, 312, 329, 332, 360, 363, 364, 365, 367, 371, 372, 377, 389, 391, 394, 418, 419, 424, 427, 429, 430, 436, 440, 443, 447, 453, 455, 456, 457, 462, 463, 464, 465, 469, 473, 478, 487 comprise an amino acid substitution, yet in particular the substitutions 10Q, 33E, 66D, 107E, 140P, 144M, 149F, 151R, 157Y, 199I, 266S, 267P, 270F, 272H, 275E, 275A, 280D, 280P, 284T, 284P, 286P, 286R, 293E, 302I, 312F, 329F, 332E, 360V, 363T, 364H, 364L, 365I, 367H, 371V, 371M, 372F, 377V, 389L, 391V, 394P, 418A, 419V, 424A, 424K, 427V, 429P, 430A, 436A, 436S, 440G, 440S, 443T, 447A, 453R, 455S, 456Q, 457T, 462Y, 463D, 464I, 465H, 465S, 465G, 469K, 473A, 478D, 487N and 490P or combinations thereof. Such an isolated polynucleotide, by using an expression vector, enables the generation of a transgenic host cell for the production of the polypeptide variants according to the invention.

The term “expression vector” refers to a DNA construct that is able to express a gene *in vivo* or *in vitro*. In particular, it encompasses DNA constructs suitable for transferring the polypeptide-encoding nucleotide sequence into the host cell so as to be integrated in the genome or freely located in the extrachromosomal space, and to intracellularly express the polypeptide-encoding nucleotide sequence and, optionally, transport the polypeptide out of the cell.

The term “host cell” refers to any cell that contains either a nucleotide sequence to be expressed, or an expression vector, and which is able to produce an enzyme or a polypeptide according to the

invention. In particular, this refers to prokaryotic and/or eukaryotic cells, preferably *P. pastoris*, *E. coli, Bacillus subtilis, Streptomyces, Hansenula, Trichoderma, Lactobacillus, Aspergillus,* plant cells and/or spores of *Bacillus*, *Trichoderma* or *Aspergillus.* The name *Pichia pastoris* used herein is synonymous with the name *Komagataella pastoris*, *Pichia pastoris* being the older and *Komagataella pastoris* the systematically newer name (Yamada et al., 1995).

The present invention further aims to provide a fusarium toxin-cleaving additive containing at least one fusarium toxin-cleaving polypeptide variant of a fusarium toxin carboxylesterase having the amino acid sequence SEQ ID No. 46, the respective polypeptide variant cleaving at least one fusarium toxin to non-toxic or less toxic products in an oxygen-independent and cofactor-free manner and having an increased temperature stability as compared to the fusarium toxin carboxylesterase of SEQ ID No. 46.

To solve this object, the invention is characterized in that the fusarium toxin-cleaving additive comprises at least one polypeptide variant of a fusarium toxin carboxylesterase according to the invention and optionally at least one supplement material. By adding such an additive to fusarium toxin-contaminated feed, it has become possible to detoxify the fusarium toxins, which can be significantly measured by a reduction of the sphinganine to sphingosine ratio in the plasma and/or kidney and/or lung and/or liver of a subject fed with the additive.

The sphinganine to sphingosine ratio in various organs and in the plasma of animals is a generally accepted and sensitive biomarker for the toxic effects of fusarium toxins, in particular FB1. Disorders of the sphingolipid metabolism caused by fusarium toxins are *inter alia* associated with brain diseases of horses or lung edemas of pigs. The relevance of the sphinganine to sphingosine ratio as a biomarker and its analytical measurement is described in Grenier et al. (Biochem. Pharmaceuticals Vol. 83 (2012) p. 1465-1473) and in the EFSA Journal (2014; 12(5):3667).

According to a further development of the invention, the additive is formed such that the supplement material is selected from the group consisting of inert carriers, vitamins, minerals, phytogenic substances, enzymes and other components for detoxifying mycotoxins, such as mycotoxin-degrading enzymes, in particular aflatoxin oxidases, ergotamine hydrolases, ergotamine amidases, zearalenone esterases, zearalenone lactonases, zearalenone hydrolases, ochratoxin amidases, fumonisin aminotransferases, aminopolyol aminoxidases, deoxynivalenol epoxide hydrolases, deoxynivalenol dehydrogenases, deoxynivalenol oxidases, trichothecene

dehydrogenases, trichothecene oxidases; mycotoxin-degrading microorganisms; and mycotoxin-binding substances, for instance microbial cell walls or inorganic materials such as bentonite. The use of such additives, for instance, in feed or food products, ensures that possibly contained amounts of fusarium toxins are reliably cleaved, in particular detoxified, to such an extent as to prevent any harmful effect on the organism of the subject ingesting such a feed or food product.

Further fields of application of the invention comprise additives containing, in addition to at least one polypeptide variant according to the invention, at least one enzyme which, for instance, participates in the degradation of proteins, e.g. proteases, or which is involved in the metabolism of starch or fibers or fat or glycogen, e.g. amylase, cellulose or glucanase, as well as, for instance, hydrolases, lipolytic enzymes, mannosidases, oxidases, oxidoreductases, phytases or xylanases.

The present invention, moreover, aims at the use of an additive according to the invention for cleaving at least one fusarium toxin in, in particular, pelletized food or feed products, in particular for pigs, poultry, cattle, horses, fishes or aquaculture. Any foods or feeds, in particular also distillers dried grains with solubles (DDGS), that are suitable for human or animal nutrition, in particular also for domestic animals, sheep or goats, can be used as foods or feeds.

The present invention, moreover, aims at the use of an additive according to the invention for cleaving at least one fusarium toxin in a process, in particular at temperatures of at least 50°C, for the production or processing of food or feed products. Such use of the additive ensures the detoxification of fusarium toxins, e.g. during food technological processes in which treatments at elevated temperatures are important, for instance in the processing of corn or grain, in starch liquefaction processes, in saccharification processes, or in fermentation processes such as the mashing or fermentation process in, in particular, the production of bioethanol. It will thereby be safeguarded that no relevant, in particular health-damaging, amounts of fusarium toxins will remain intact in any product originating from such a process, such as feed pellets, pasta, polenta, popcorn, cornflakes, corn bread, tortillas, DDGS or starch.

The present invention further aims to provide a polypeptide variant for use in a preparation for the prophylaxis and/or treatment of fusarium toxin mycotoxicoses. In the case of prophylaxis, it has become possible by the use of such a polypeptide variant or additive, despite the ingestion of fusarium toxins, to substantially maintain the health status of humans and animals at the level corresponding to that without, or reduced, oral ingestion of fusarium toxins. As regards the

treatment of fusarium toxin mycotoxicoses, it has become possible to alleviate the symptoms of such a disease and, in particular, significantly improve the sphinganine to sphingosine ratio in organs and/or plasma. Moreover, such use will enable an enhancement of the capacity of livestock, in particular the feed conversion ratio and the gain in weight, as well as a reduction of the mortality rate.

Furthermore, the invention aims to provide a method for enzymatically cleaving at least one fusarium toxin, by which at least one fusarium toxin is hydrolytically cleaved by a polypeptide in an oxygen-independent, specific, safe and reliable manner to non-toxic or less toxic products, the hydrolytic cleavage occurring either during or after a temperature treatment.

To solve this object, the method is carried out such that at least one tricarballylic acid is hydrolytically cleaved off from the fusarium toxin by a polypeptide variant according to the invention, or an additive according to the invention. In doing so, the at least one fusarium toxin is mixed with at least one polypeptide variant according to the invention, or at least one additive according to the invention, at least one polypeptide variant hydrolytically cleaves at least one tricarballylic acid from the at least one fusarium toxin thus detoxifying the fusarium toxin, wherein the mixture of the respective polypeptide variant and the fusarium toxin is subjected to a temperature treatment of at least 50°C, preferably at least 70°C, and the hydrolytic cleavage is performed either during or after the temperature treatment.

In a preferred further development of the invention, the method is performed such that the polypeptide variant, or the additive, is mixed with a feed or food product contaminated with at least one fusarium toxin, and the temperature treatment is optionally performed by a pelletizing process. This will ensure that the fusarium toxins contained in the contaminated and optionally pelletized feed or food product will be cleaved as soon as the mixture of the polypeptide variant and the fusarium toxin has been contacted with moisture. With moist feeds or foods such as mashes or pulps, the hydrolysis of the fusarium toxins takes place in the moist feed or food prior to its oral ingestion. It will thereby be ensured that the harmful effects of fusarium toxins on humans and animals will be largely eliminated or at least reduced. By moisture, the presence of water or water-containing liquids is understood, this also including saliva or other liquids present in the digestive tract. The digestive tract is defined to comprise the mouth cavity, the pharynx (throat), the esophagus and the gastrointestinal tract or equivalents thereof, wherein different designations may

be found with animals, or individual components may not be present in the digestive tracts of animals.

In a preferred further development of the invention, the method is conducted such that the polypeptide variant is used at a concentration range from 5 U to 500 U, preferably from 10 U to 300 U, and more preferably from 15 U to 100 U, per kilogram of feed or food product. By adding such amounts of the polypeptide variant, it has become possible, as a function of the concentration of the fusarium toxins, to cleave the latter in the food or feed product, in particular in DDGS, and thereby detoxify the same to such an extent that at least 70%, preferably at least 80%, in particular at least 90%, of the at least one fusarium toxin will be cleaved.

Unless otherwise specified, singular designations like “a” or “the” are to be understood as examples and shall comprise a plurality of options. If it is, for instance, referred to “a gene”, “an enzyme” or “a cell”, this shall always encompass the plural.

In the following, the invention will be explained in more detail by way of examples.

Polypeptide variants

Example 1: Modification, cloning and expression of polynucleotides encoding fusarium toxin-cleaving polypeptides

Amino acid substitutions, insertions or deletions were performed by mutations of the nucleotide sequences by means of PCR using the QuikChange site-directed mutagenesis kit (Stratagene) according to instructions. Alternatively, also complete nucleotide sequences were synthetized (GeneArt). The nucleotide sequences generated by PCR mutagenesis and those obtained from GeneArt were integrated by standard methods in expression vectors for the expression in *E. coli* or *P. pastoris*, were transformed in *E. coli* or *P. pastoris*, and were expressed in *E. coli* or *P. pastoris*, respectively (J.M. Cregg, Pichia Protocols, second Edition, ISBN-10: 1588294293, 2007; J. Sambrook et al. 2012, Molecular Cloning, A Laboratory Manual 4th Edition, Cold Spring Harbor), wherein any other suitable host cell may also be used for this task.

The term “expression vector” refers to a DNA construct capable of expressing a gene *in vivo* or *in vitro*. In particular, it encompasses DNA constructs suitable for transferring the polypeptide-

encoding nucleotide sequence into the host cell so as to be integrated in the genome or freely located in the extrachromosomal space, and to intracellularly express the polypeptide-encoding nucleotide sequence and, optionally, transport the polypeptide out of the cell. The term "host cell" refers to any cell that contains either a nucleotide sequence to be expressed or an expression vector and is able to produce an enzyme or polypeptide according to the invention. In particular, this refers to prokaryotic and/or eukaryotic cells, preferably *P. pastoris*, *E. coli, Bacillus subtilis, Streptomyces, Hansenula, Trichoderma, Lactobacillus, Aspergillus,* plant cells and/or spores of *Bacillus*, *Trichoderma* oder *Aspergillus.* The soluble cell lysate in the case of *E. coli* and the culture supernatant in the case of *P. pastoris*, respectively, were used to determine the catalytic properties of the polypeptide variants.

Example 2: Determination of the catalytic activity and specific activity of fusarium toxin-degrading polypeptides

The corresponding genes encoding fusarium toxin-degrading polypeptides were cloned in *Escherichia coli* using standard methods, intracellularly expressed, and subsequently lyzed by ultrasonic treatment and centrifuged. The clear supernatant was diluted with 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 mg/l bovine serum albumin (about 10^{-3} to 10^{-5}) and used in the FB1-degradation mixture so as to degrade 10% to 90% of the amount of FB1 contained in the degradation mixture by the polypeptide.

In order to determine the catalytic enzyme activity, tests on the hydrolytic cleavage of fumonisin B1 (FB1) were carried out, the tests having been performed in a 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 mg/l bovine serum albumin at a temperature of 30°C for 30 minutes. In addition, the mixture contained a substrate concentration of 100 µM FB1 (Biopure Referenzsubstanzen GmbH Tulln, Austria, BRM 001007) and one of the polypeptides to be tested. After an incubation of 30 minutes, the mixture was heat-inactivated at 99°C for 5 min to stop the reaction.

In order to determine the enzymatic activity of feed samples, the fusarium toxin-transforming polypeptide variants have to be extracted from the feed samples prior to testing. To this end, 10 grams of feed were dissolved in 100 ml 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 mg/ml bovine serum albumin and shaken at 150 rpm for 1 hour at 20°C. After this, the samples were centrifuged at 4000 g for 15 min, and the clear supernatant was diluted as required (10^{-2} to 10^{-3}) and used in the FB1 solution.

The quantification of FB1 was performed by LC-MS (liquid chromatography – mass spectroscopy) according to the method of Heinl et al. (J. of Biotechnology, 2010, 145, pp. 120-129, 2.6.3. "Liquid chromatography - mass spectrometry"). To this end, a calibration with FB1 standards additionally containing a complete ^{13}C-labeled, internal FB1 standard (Biopure Referenzsubstanzen GmbH Tulln, Austria) was done. As opposed to Heinl et al. (2010), only the degradation of FB1 was measured to determine the catalytic enzyme activity of the polypeptide solutions used. The catalytic enzyme activity of the used polypeptide solutions is indicated in units per ml, one "unit" being defined as reduction of 1 µmol FB1 per minute under the above-identified reaction conditions in the test.

For determining the specific activities, the enzyme concentrations were determined by quantitative Western blot or ELISA. The specific enzyme activities were calculated by the activities (units) having been based on the used amounts of enzyme and are indicated in units per mg.

Example 3: Temperature stability of fusarium toxin-degrading polypeptides

The expression and quantification of the fusarium toxin-degrading polypeptides were performed as described in Examples 1 and 2. Prior to the determination of the activity, the amount of cell lysate was divided into several portions (of 60 µl each). Two to 10 portions were subjected to a heat treatment for 5 min in a commercially available PCR cycler (e.g. Eppendorf Matercycler Gradient), each portion having been incubated at different temperatures. Meanwhile, another portion of the cell lysate, the 100% control, was incubated on ice. Following the heat treatment, all of the samples/test mixtures were incubated at 10°C for 1 minute to equalize the temperatures. The enzymatic activity of both the heat-treated samples and the 100% control were determined as described in Example 2. The activity remaining after the heat treatment is referred to as residual activity. The temperature at which the residual activity is 50% as compared to the non-heat-treated 100% control, is abbreviated by T(50%), constituting the measure for the temperature stability of the polypeptide.

The increases of T(50%), indicated in degree Celsius, of polypeptide variants relative to the polypeptide of SEQ ID No. 46 or SEQ ID No. 1, respectively, is a measure for the increased temperature stability. The increase in the T(50%) value can be indicated in °C, yet also in percent relative to the T(50%) value of the parental polypeptide. The following example serves for

illustration: If the parental enzyme had a catalytic activity of 50 U/ml after a 5-minute incubation on ice and a catalytic activity of 25 U/ml after a 5-minute incubation at 48°C, the T(50%) value would be 48°C. If a polypeptide variant had a T(50%) value of 51°C, the relative increase in the temperature stability (T(50%)) would be 6.25. This results from the difference between the two T(50%) values of 3°C, divided by the T(50%) value of the parental starting enzyme of 48°C, multiplied by 100.

Instead of the catalytic activity, the specific activity may also be used for determining the temperature stability.

The determination of the temperature stability may also be performed by alternative enzymatic assays and even without determining the catalytic activity of the FB1 reaction. What is important in this respect, is that equal amounts of thermally treated polypeptide and of the 100% control are used, which is, for instance, ensured by the use of equal volumes of cell lysate.

Instead of the catalytic activity, also the measurement signals of enzymatic degradation assays (e.g. MS signal, extinction, etc.) may be used for determining the temperature stability. If the measurement signal is directly proportional to the enzymatic activity (e.g. peak surface of reacted FB1), the T(50%) value of a polypeptide is the temperature at which the value of the measurement signal of the heat-treated polypeptide comprises 50% of the value of the measurement signal of the 100% control of the polypeptide.

The temperature stability (T(50%)) of the polypeptide of SEQ ID No. 46 was determined to be 42°C, that of the polypeptide of SEQ ID No. 1 to be 45°C. Thus, the relative increase in the temperature stability that could be achieved by truncating the N-terminal sequence was about 7%. Moreover, an increase in the enzymatic activity of the polypeptide of SEQ ID No. 1 relative to the parental polypeptide of SEQ ID No. 46 could also be determined.

The temperature stability of the polypeptide of SEQ ID No. 1 could be further increased by the selective substitution of individual amino acids. The relative increases in the temperature stability of these polypeptide variants relative to the parental polypeptide of SEQ ID No. 1 are illustrated in Table 1.

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Table 1: Modifications of the polypeptide variants and their relative increases in the temperature stability in percent as compared to the parental enzyme of SEQ ID No. 1

Modification(s) of SEQ ID No. 1	Relative increase in T (50%)	SEQ ID No. of the polypeptide containing the modifications
H10Q	4.4 %	-
A33E	4.4 %	-
N66D	6.7 %	-
G107E	4.4 %	-
I140P	4.4 %	-
L144M	4.4 %	-
L149F	4.4 %	-
K151R	4.4 %	-
V157Y	4.4 %	-
L199I	6.7 %	-
R266S	4.4 %	-
Q267P	4.4 %	-
K270F	4.4 %	-
R272H	4.4 %	-
G275E	4.4 %	-
G275A	4.4 %	-
G280D	4.4 %	-
G280P	4.4 %	-
R284T	4.4 %	-
R284P	4.4 %	-
L286P	4.4 %	-
L286R	4.4 %	-
K293E	4.4 %	-
L302I	6.7 %	-
A312F	4.4 %	-
L329F	4.4 %	-

Q332E	4.4 %	-
F360V	4.4 %	-
S363T	4.4 %	-
Q364H	4.4 %	-
Q364L	4.4 %	-
F365I	4.4 %	-
N367H	4.4 %	-
L371V	4.4 %	-
L371M	4.4 %	-
L372F	4.4 %	-
A377V	6.7 %	-
T389L	4.4 %	-
I391V	4.4 %	-
A394P	6.7 %	-
S418A	4.4 %	-
M419V	4.4 %	-
E424A	6.7 %	-
E424K	4.4 %	-
A427V	4.4 %	-
A429P	4.4 %	-
S430A	6.7 %	-
T436A	4.4 %	-
T436S	4.4 %	-
A440G	4.4 %	-
A440S	4.4 %	-
V443T	4.4 %	-
V447A	4.4 %	-
Q453R	4.4 %	-
T455S	4.4 %	-
K456Q	4.4 %	-
S457T	4.4 %	-
F462Y	4.4 %	-
E463D	6.7 %	-

R464I	4.4 %	-
R465H	4.4 %	-
R465S	4.4 %	-
R465G	4.4 %	-
M469K	4.4 %	-
S473A	4.4 %	-
G478D	4.4 %	-
K487N	4.4 %	-
Q490P	4.4 %	-
L199I/A394P	13.3 %	-
N66D/L199I	11.1 %	-
L199I/L302I	13.3 %	-
L199I/A377V	15.6 %	-
L199I/E424A	11.1 %	-
L199I/S430A	11.1 %	-
L199I/E463D	13.3 %	-
L199I/L302I/A394P	20.0 %	-
L199I/L302I/A377V	17.8 %	-
L199I/L302I/E424A	17.8 %	-
L199I/A377V/A394P	22.2 %	-
L199I/A394P/A429P	17.8 %	-
L372F/A394P/V443T	17.8 %	-
L199I/L302I/L372F	15.6 %	-
L144M/L199I/L302I	20.0 %	-
F360V/A394P/V443T	17.8 %	-
H10Q/K151R/A302I	15.6 %	-
R266S/A377V/E424K	20.0 %	-
Q267/A394P/T436S	17.8 %	-
R272H/G280D/E463D	20.0 %	-
G275A/L302I/F360V	17.8 %	-
N66D/L286P/N367H	15.6 %	-
R284T/L286R/S430A	15.6 %	-
K293E/E424A/M469K	20.0 %	-

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S363T/A377V/K456Q	20.0 %	-
Q364H/L371V/S430A	17.8 %	-
L199I/Q364L/Q490P	15.6 %	-
F365I/A394P/R464I	15.6 %	-
L371M/A377V/A429P	17.8 %	-
L302I/L372F/Q453R	15.6 %	-
T389L/M419V/E463D	17.8 %	-
I391V/A394P/A440G	20,0 %	-
S418A/S430A/F462Y	20.0 %	-
N66D/A427V/V443T	17.8 %	-
A440S/S457T/E463D	20.0 %	-
L199I/V447A/T455S	20.0 %	-
A377V/R465H/K487N	17.8 %	-
L302I/R465S/G478D	15.6 %	-
A377V/R465G/S473A	20.0 %	-
N66D/L199I/L302I/A394P/E424A/S430A	26.7 %	SEQ ID No. 2
N66D/L199I/L302I/A377V/A394P/E424A/S430A	31.1 %	SEQ ID No. 3
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D	37.8 %	SEQ ID No. 4
N66D/L144M/L199I/L302I/F360V/L372F/A377V/A394P/E424A/S430A/V443T/E463D	33.3 %	SEQ ID No. 5
L199I/L302I/A377V/A394P/E424A/S430A/E463D	31.1 %	SEQ ID No. 6
N66D/L199I/L302I/A377V/A394P	26.7 %	SEQ ID No. 7
N66D/L199I/L302I/Q364H/A377V/A394P/E424A/S430A/E463D	35.5 %	SEQ ID No. 8
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D/R465H	40.0 %	SEQ ID No. 9
N66D/L199I/L302I/A377V/A394P/E424A/S430A/A440G/E463D	37.8 %	SEQ ID No. 10
N66D/L199I/L302I/A377V/A394P/E424A/S430A/V447A/E463D	35.5 %	SEQ ID No. 11

N66D/L199I/L302I/A377V/A394P/S418A/E424A/S430A/E463D	35.5 %	SEQ ID No. 12
N66D/L199I/L302I/A377V/A394P/E424A/T436A/S430A/E463D	35.5 %	SEQ ID No. 13
N66D/L199I/L302I/Q364L/A377V/A394P/E424A/S430A/E463D	35.5 %	SEQ ID No. 14
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D/Q490P	37.8 %	SEQ ID No. 15
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D/M469K	37.8 %	SEQ ID No. 16
N66D/L199I/L302I/A377V/T389L/A394P/E424A/S430A/E463D	40.0 %	SEQ ID No. 17
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D/R465S	35.5 %	SEQ ID No. 18
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D/R464I	40.0 %	SEQ ID No. 19
N66D/L199I/L302I/A377V/A394P/E424A/S430A/E463D/R465G	35.5 %	SEQ ID No. 20
N66D/L199I/L302I/A377V/A394P/E424A/S430A/A440S/E463D	33.3 %	SEQ ID No. 21
N66D/L199I/L302I/A377V/A394P/E424A/S430A/S457T/E463D	37.8 %	SEQ ID No. 22
N66D/L199I/L302I/A377V/A394P/E424A/S430A/T436S/E463D	35.5 %	SEQ ID No. 23
N66D/L199I/L302I/S363T/L371V/A377V/A394P/E424A/S430A/E463D	40.0 %	SEQ ID No. 24
N66D/L199I/L302I/A377V/A394P/E424A/S430A/V447A/Q453R/E463D	35.5 %	SEQ ID No. 25
N66D/L199I/L302I/A377V/A394P/E424A/S430A/K456Q/F462Y/E463D	40.0 %	SEQ ID No. 26
N66D/L199I/L302I/A377V/A394P/M419V/E424A/A427V/S430A/E463D	35.5 %	SEQ ID No. 27
N66D/L199I/L302I/F365I/A377V/A394P/E424A/S430A/E463D/K487N	33.3 %	SEQ ID No. 28

N66D/L199I/L302I/L371M/A377V/A394P/E424A/S430A/E463D/K487N	33.3 %	SEQ ID No. 29
N66D/L199I/L302I/Q364L/A377V/T389L/A394P/M419V/E424A/A427V/S430A/V447A/E463D/R465S/M469K	51.1 %	SEQ ID No. 30
N66D/L199I/L302I/A377V/T389L/A394P/M419V/E424A/A427V/S430A/V447A/E463D/R465S/M469K	46.7 %	SEQ ID No. 31
N66D/L199I/L302I/S363T/Q364L/L371V/A377V/T389L/A394P/M419V/E424A/A427V/S430A/V447A/E463D/R464I/R465S/M469K	60.0 %	SEQ ID No. 32
N66D/L199I/L302I/S363T/L371V/A377V/T389L/A394P/M419V/E424A/A427V/S430A/V447A/E463D/R464I/R465S/M469K	57.8 %	SEQ ID No. 33
N66D/L199I/L302I/Q364L/N367H/L371V/A377V/T389L/A394P/S418A/M419V/E424A/A427V/S430A/T436A/A440S/V447A/E463D/R464I/R465S/M469K/Q490P	62.2 %	SEQ ID No. 34
N66D/L199I/L302I/N367H/L371V/A377V/T389L/A394P/S418A/M419V/E424A/A427V/S430A/T436A/A440S/V447A/E463D/R464I/R465S/M469K/Q490P	62.2 %	SEQ ID No. 35
N66D/L199I/L302I/S363T/N367H/L371V/A377V/A394P/E424A/S430A/E463D/Q490P	42.2 %	SEQ ID No. 36
N66D/L199I/L302I/A377V/A394P/S418A/M419V/E424A/A427V/S430A/T436A/A440S/V447A/E463D	46.7 %	SEQ ID No. 37
N66D/L199I/L302I/A377V/T389L/A394P/E424A/S430A/S457T/E463D/R464I/R465S/M469K	48.9 %	SEQ ID No. 38
N66D/L199I/L302I/S363T/L371V/A377V/T389L/A394P/M419V/E424A/A427V/S430A/A440S/V447A/S457T/E463D/R464I/M469K/Q490P	55.5 %	SEQ ID No. 39
N66D/L199I/L302I/A377V/A394P/E424A/S430A/V447A/E463D/R465S/M469K/Q490P	46.7 %	SEQ ID No. 40

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N66D/L199I/L302I/L371M/A377V/A394P/M419V/E424A/A427V/S430A/E463D/R465S/M469K/K487N/Q490P	51.1 %	SEQ ID No. 41
N66D/L199I/L302I/L371M/A377V/A394P/M419V/E424A/A427V/S430A/V447A/Q453R/E463D/R465S/M469K/K487N/Q490P	55.5 %	SEQ ID No. 42
N66D/L199I/L302I/N367H/L371V/A377V/T389L/A394P/S418A/M419V/E424A/A427V/A429P/S430A/T436A/A440S/V447A/S457T/E463D/R464I/R465S/M469K/Q490P	64.4 %	SEQ ID No. 43
N66D/L199I/L302I/L371M/A377V/T389L/A394P/M419V/E424A/A427V/S430A/T436A/V447A/Q453R/K456Q/F462Y/E463D/R465S/M469K/K487N/Q490P	62.2 %	SEQ ID No. 44
N66D/L199I/L302I/N367H/L371V/A377V/T389L/A394P/S418A/M419V/E424A/A427V/A429P/S430A/T436A/A440S/V447A/Q453R/K456Q/S457T/F462Y/E463D/R464I/R465S/M469K/K487N/Q490P	65.2 %	SEQ ID No. 45

Example 4: Temperature-dependent activity (temperature activity) of fusarium toxin-degrading polypeptides

The fusarium toxin-degrading polypeptide variants to be tested for their temperature-dependent activities were purified prior to carrying out the tests. To this end, the polypeptide variants were purified from fermentation supernatants in a two-step process using anion exchange chromatography and subsequently size exclusion chromatography. The polypeptide variants were adjusted to concentrations of 1 mg/ml and used in the reaction mixture at dilutions of 10^{-5} to 10^{-6} in reaction volumes of 1 ml. Activity determinations were performed by tests as described in Example 3, by FB1 hydrolysis and subsequent quantification of FB1 by LC-MS, the tests have been carried out at different temperatures. Incubation was performed using two heating blocks (Eppendorf, ThermoMixer) at temperatures of 10 °C, 20 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C and 70 °C. Thirty minutes after the beginning of the heat exposure, 100 µl of the reaction

mixture were each taken and heat-inactivated at 99°C for 5 min. The test performed at 30°C in the heating block served as a 100% control. Exemplary results are indicated in Table 2.

Table 2: Temperature-dependent activities of fusarium toxin-degrading polypeptides. <LOQ values are below detection limits (level of quantification: <0.15 U/l in the test preparation)

Sequence ID incl. amino acid substitutions	**Temperature**	**Relative activity, based on the activity at 30 °C [%]**
SEQ ID No. 1	10 °C	35
	20 °C	59
	30 °C	100
	35 °C	123
	40 °C	100
	45 °C	105
	50 °C	90
	55 °C	26
	60 °C	< LOQ
	65 °C	< LOQ
	70 °C	< LOQ
SEQ ID No. 1 with L199I/L302I/A394P	30 °C	100
	40 °C	100
	50 °C	95
	60 °C	62
	70 °C	16
SEQ ID No. 1 with L144M/L199I/L302I	30 °C	100
	40 °C	104
	50 °C	91
	60 °C	60
	70 °C	18
SEQ ID No. 1 with R266S/A377V/E424K	30 °C	100
	40 °C	110
	50 °C	97
	60 °C	63
	70 °C	29
SEQ ID No. 1 with K293E/E424A/M469K	30 °C	100
	40 °C	98
	50 °C	81
	60 °C	50
	70 °C	< LOQ
SEQ ID No. 1 with F365I/A394P/R464I	30 °C	100
	40 °C	105
	50 °C	84
	60 °C	57

	70 °C	14
SEQ ID No. 1 with S418A/S430A/F462Y	30 °C	100
	40 °C	103
	50 °C	89
	60 °C	53
	70 °C	< LOD
SEQ ID No. 4	10 °C	33
	20 °C	58
	30 °C	100
	35 °C	109
	40 °C	110
	45 °C	114
	50 °C	148
	55 °C	113
	60 °C	94
	65 °C	83
	70 °C	46
SEQ ID No. 43	10 °C	31
	20 °C	56
	30 °C	100
	35 °C	113
	40 °C	120
	45 °C	126
	50 °C	157
	55 °C	168
	60 °C	134
	65 °C	102
	70 °C	98
SEQ ID No. 44	10 °C	33
	20 °C	59
	30 °C	100
	35 °C	110
	40 °C	124
	45 °C	131
	50 °C	162
	55 °C	174
	60 °C	126
	65 °C	99
	70 °C	95
SEQ ID No. 45	10 °C	41
	20 °C	68
	30 °C	100
	35 °C	115
	40 °C	136
	45 °C	141

	50 °C	164
	55 °C	177
	60 °C	137
	65 °C	121
	70 °C	100

Enzyme	Pelletizing temperature	Residual activity %
SEQ ID No. 1	75 °C	15
	80 °C	< LOQ
	85 °C	< LOQ
	90 °C	< LOQ
SEQ ID No. 4	75 °C	57
	80 °C	46
	85°C	37
	90 °C	14
SEQ ID No. 43	75 °C	78
	80 °C	73
	85 °C	58
	90 °C	31
SEQ ID No. 44	75 °C	70
	80 °C	59
	85 °C	48
	90 °C	25
SEQ ID No. 45	75 °C	72
	80 °C	68
	85 °C	48
	90 °C	30

Example 5: Determination of the pelletizing stability of fusarium toxin-degrading polypeptides

Selected polypeptide variants were cloned in *Pichia pastoris* in a bioreactor using standard methods under controlled aerobic conditions and extracellularly secreted. The clear supernatant was separated from the biomass, supplemented with a carrier substance (maltodextrin) and processed to a pelletizable powder using a spray-dryer. The fusarium toxin-degrading polypeptide variants present in power form were admixed to piglet rearing feed, each at the same concentration of 100 U/kg, and processed to feed pellets in a controlled process. During the pelletizing process, the feed was moistened by hot steaming and heated in individual batches at precisely defined temperatures (75 to 95 °C in 5°C steps). This preparation phase was followed by the pelletizing process proper. The residual activities of the fusarium toxin-degrading polypeptide variants

contained in the pellets were determined as described in Example 2, non-pelletized feed containing the respective fusarium toxin-degrading polypeptide variants serving as 100% controls. The enzyme activity remaining after the pelletizing process is therefore defined as residual activity. The values are indicated in Table 3.

Table 3: Pelletizing temperatures and residual activities of fusarium toxin-degrading polypeptides. <LOQ values are below detection limits (level of quantification: <0.15 U/l in the test mixture)

Enzyme	Pelletizing temperature	Residual activity %
SEQ ID No. 1	75 °C	15
	80 °C	< LOQ
	85 °C	< LOQ
	90 °C	< LOQ
SEQ ID No. 4	75 °C	57
	80 °C	46
	85°C	37
	90 °C	14
SEQ ID No. 43	75 °C	78
	80 °C	73
	85 °C	58
	90 °C	31
SEQ ID No. 44	75 °C	70
	80 °C	59
	85 °C	48
	90 °C	25
SEQ ID No. 45	75 °C	72
	80 °C	68
	85 °C	48
	90 °C	30

Claims:

1. Fusarium toxin-cleaving polypeptide variants of a fusarium toxin carboxylesterase of SEQ ID No. 46, characterized in that the polypeptide variants each possess an amino acid sequence truncated by 47 amino acids at the N-terminus, the amino acid sequences sharing at least 70%, preferably 80%, particularly preferably 100%, sequence identity, namely SEQ ID No. 1, with the amino acid sequence section 48-540 of SEQ ID No. 46, wherein the temperature stability (T(50%)) of the polypeptide of SEQ ID No. 46 is determined to be 42°C and that of the polypeptide variant of SEQ ID No. 1 is determined to be 45°, or modifications of SEQ ID No. 1 having a relative increase of T(50%) compared to the parental enzyme of SEQ ID No. 1, wherein on at least one position selected from the group consisting of 10, 33, 66, 107, 140, 144, 149, 151, 157, 199, 266, 267, 270, 272, 275, 280, 284, 286, 293, 302, 312, 329, 332, 360, 363, 364, 365, 367, 371, 372, 377, 389, 391, 394, 418, 419, 424, 427, 429, 430, 436, 440, 443, 447, 453, 455, 456, 457, 462, 463, 464, 465, 469, 473, 478, 487 and 490 an amino acid substitution is contained as modification, and that the amino acid substituents at positions 10 and 456 are selected from Q, E, N, H, K and R, at positions 33, 107, 293 and 332 from E, Q, D, K, R and N, at positions 66, 463 and 478 from D, E, K, N, Q and R, at positions 140 and 490 from P, A, S and N, at positions 144 and 367 from I, L, M and V, at positions 149, 270, 312, 329 and 372 from F, Y, W and H, at positions 151 and 453 from D, E, K and R, at positions 157 and 462 from F, H, W and Y, at positions 199, 302, 365 and 464 from I, L, M and V, at positions 266 and 455 and from A, S and T, at positions 267, 394 and 429 from A, N, P and S, at position 272 from H, N, Q and S, at position 275 from A, D, E, G, K, N, Q, R and S, at position 280 from A, D, E, K, N, P, Q, R and S, at position 284 from A, N, P, S, T and V, at position 286 from A, D, E, K, N, P, R and S, at positions 360, 377, 391, 419 and 427 from A, I, L S, T and V, at positions 363, 443 and 457 from A, S, T and V, at position 364 from H, I, L, M, N, Q, S and V, at position 371 from A, I, L, M, S, T and V, at position 389 from I, L, M and V, at positions 418, 430, 447 and 473 from A, G and S, at position 424 from A, D, E, G, K, R and S, at position 436 from A, G, S and T, at position 440 from A, G, S and T, at position 465 from A, G, H, N, Q, S and T, at position 469 from D, E, K and R and/or at position 487 from N, D, Q, H and S.

2. Polypeptide variants according to claim 1, characterized in that the amino acid substitutions are selected from the group consisting of 10Q, 33E, 66D, 107E, 140P, 144M, 149F, 151R, 157Y, 199I, 266S, 267P, 270F, 272H, 275E, 275A, 280D, 280P, 284T, 284P, 286P, 286R, 293E, 302I, 312F, 329F, 332E, 360V, 363T, 364H, 364L, 365I, 367H, 371V, 371M, 372F, 377V, 389L, 391V, 394P,

418A, 419V, 424A, 424K, 427V, 429P, 430A, 436A, 436S, 440G, 440S, 443T, 447A, 453R, 455S, 456Q, 457T, 462Y, 463D, 464I, 465H, 465S, 465G, 469K, 473A, 478D, 487N and 490P.

3. Polypeptide variants according to any one of claim 1 or 2, characterized in that they comprise an amino acid substitution on at least one position selected from the group consisting of 66, 199, 302, 377, 394, 424, 430 and 463.

4. Polypeptide variants according to any one of claim 1, 2 or 3, characterized in that the amino acid substitution is selected from the group consisting of 66D, 199I, 302I, 377V, 394P, 424A, 430A and 463D.

5. Polypeptide variants according to claim 1, characterized in that the polypeptide variants comprise an amino acid substitution, on at least two, in particular three, positions of the amino acid sequence, selected from the group consisting of 10, 33, 66, 107, 140, 144, 149, 151, 157, 199, 266, 267, 270, 272, 275, 280, 284, 286, 293, 302, 312, 329, 332, 360, 363, 364, 365, 367, 371, 372, 377, 389, 391, 394, 418, 419, 424, 427, 429, 430, 436, 440, 443, 447, 453, 455, 456, 457, 462, 463, 464, 465, 469, 473, 478, 487 and 490, , whereat the amino acid substitution is selected from the group consisting of 10Q, 66D, 144M, 151R, 199I, 266S, 267P, 272H, 275E, 275A, 280D, 284T, 286P, 286R, 293E, 302I, 360V, 363T, 364H, 364L, 365I, 367H, 371V, 371M, 372F, 377V, 389L, 391V, 394P, 418A, 419V, 424A, 424K, 427V, 429P, 430A, 436A, 436S, 440G, 440S, 443T, 447A, 453R, 455S, 456Q, 457T, 462Y, 463D, 464I, 465H, 465S, 465G, 469K, 473A, 478D, 487N and 490P.

6. Polypeptide variants according to claim 1 or 2, characterized in that the amino acid sequence of the polypeptide variants comprises combinations of several amino acid substitutions, whereat the combinations of the positions being selected from the group consisting of 66/199/302/394/424/430, 66/199/302/377/394/424/430, 66/199/302/377/394/424/430/463, 66/144/199/302/360/372/377/394/424/430/443/463, 199/302/377/394/424/430/463, 66/199/302/377/394, 66/199/302/364/377/394/424/430/463, 66/199/302/377/394/424/430/463/465, 66/199/302/377/394/424/430/440/463, 66/199/302/377/394/424/430/447/463, 66/199/302/377/394/418/424/430/463, 66/199/302/377/394/424/436/430/463, 66/199/302/364/377/394/424/430/463, 66/199/302/377/394/424/430/463/490, 66/199/302/377/394/424/430/463/469, 66/199/302/377/389/394/424/430/463, 66/199/302/377/394/424/430/463/465, 66/199/302/377/394/424/430/463/464,

66/199/302/377/394/424/430/463/465, 66/199/302/377/394/424/430/440/463,
66/199/302/377/394/424/430/457/463, 66/199/302/377/394/424/430/436/463,
66/199/302/363/371/377/394/424/430/463, 66/199/302/377/394/424/430/447/453/463,
66/199/302/377/394/424/430/456/462/463, 66/199/302/377/394/419/424/427/430/463,
66/199/302/365/377/394/424/430/463/487 and 66/199/302/371/377/394/424/430/463/487.

7. Polypeptide variants according to claim 6, characterized in that the amino acid sequences of the polypeptide variants are selected from the group consisting of SEQ ID Nos. 2 to 29.

8. Polypeptide variants according to claim 1 or 2, characterized in that the amino acid sequence of the polypeptide variants comprises combinations of several amino acid substitutions, the combinations of the positions being selected from the group consisting of
66/99/302/364/377/389/394/419/424/427/430/447/463/465/469,
66/199/302/377/389/394/419/424/427/430/447/463/465/469,
66/199/302/363/364/371/377/389/394/419/424/427/430/447/463/464/465/469,
66/199/302/363/371/377/389/394/419/424/427/430/447/463/464/465/469,
66/199/302/364/367/371/377/389/394/418/419/424/427/430/436/440/447/463/464/465/469/490,
66/199/302/367/371/377/389/394/418/419/424/427/430/436/440/447/463/464/465/469/490,
66/199/302/363/367/371/377/394/424/430/463/490,
66/199/302/377/394/418/419/424/427/430/436/440/447/463,
66/199/302/377/389/394/424/430/457/463/464/465/469,
66/199/302/363/371/377/389/394/419/424/427/430/440/447/457/463/464/469/490,
66/199/302/377/394/424/430/463/447/490/469/465,
66/199/302/377/394/424/430/463/490/469/465/419/427/371/487,
66/199/302/371/377/394/419/424/427/430/447/453/463/465/469/487/490,
66/199/302/367/371/377/389/394/418/419/424/427/429/430/436/440/447/457/463/464/465/469/490
,66/199/302/371/377/389/394/419/424/427/430/436/447/453/456/462/463/465/469/490/487
and
66/199/302/367/371/377/389/394/418/419/424/427/429/430/436/440/447/453/456/457/462/463/464
/465/469/487/490.

9. Polypeptide variants according to claim 8, characterized in that the amino acid sequences of the polypeptide variants are selected from the group consisting of SEQ ID Nos. 30 to 45.

10. Isolated polynucleotides, characterized in that the polynucleotides comprises nucleotide sequences encoding a polypeptide variant according to any one of claims 1 to 9.

11. Fusarium toxin-cleaving additive, characterized in that said additive comprises at least one polypeptide variant according to any one of claims 1 to 9 and optionally at least one supplement material.

12. Additive according to claim 11, characterized in that the supplement material is selected from the group consisting of inert carriers, vitamins, minerals, phytogenic substances, enzymes and other components for detoxifying mycotoxins, such as mycotoxin-degrading enzymes, in particular aflatoxin oxidases, ergotamine hydrolases, ergotamine amidases, zearalenone esterases, zearalenone lactonases, zearalenone hydrolases, ochratoxin amidases, fumonisin aminotransferases, aminopolyol aminoxidases, deoxynivalenol epoxide hydrolases, deoxynivalenol dehydrogenases, deoxynivalenol oxidases, trichothecene dehydrogenases, trichothecene oxidases; and mycotoxin-degrading microorganisms; and mycotoxin-binding substances, for instance microbial cell walls or inorganic materials such as bentonite.

13. Use of the additive according to any one of claim 11 or 12 for cleaving at least one fusarium toxin in, in particular pelletized, food or feed products, in particular for pigs, poultry, cattle, horses, fishes or aquaculture.

14. Use of the additive according to any one of claim 11 or 12 for cleaving at least one fusarium toxin in a process, in particular at temperatures of at least 50°C, preferably at least 70°C, for the production or processing of food or feed products.

15. Polypeptide variants according to any one of claims 1 to 9 for use in a preparation for the prophylaxis and/or treatment of fusarium toxin mycotoxicoses.

16. Method for hydrolytically cleaving at least one fusarium toxin, characterized in that at least one fusarium toxin is contacted with at least one polypeptide variant according to any one of claims 1 to 9, or at least one additive according to any one of claim 11 or 12, and that the mixture of the polypeptide variant and the fusarium toxin is subjected to a temperature treatment at at least 50°C, preferably at least 70°C, and that the mixture is contacted with an amount of moisture sufficient for hydrolytic cleavage either during or after the temperature treatment.

17. Method according to claim 16, characterized in that the polypeptide variant, or the additive, is mixed with a feed or food product contaminated with at least one fusarium toxin, and the temperature treatment is optionally performed during a pelletizing process.

18. Method according to any one of claim 16 or 17, characterized in that the polypeptide variant is added at a concentration range from 5 U to 500 U, preferably from 10 U to 300 U, and more preferably from 15 U to 100 U, per kilogram of feed or food product.