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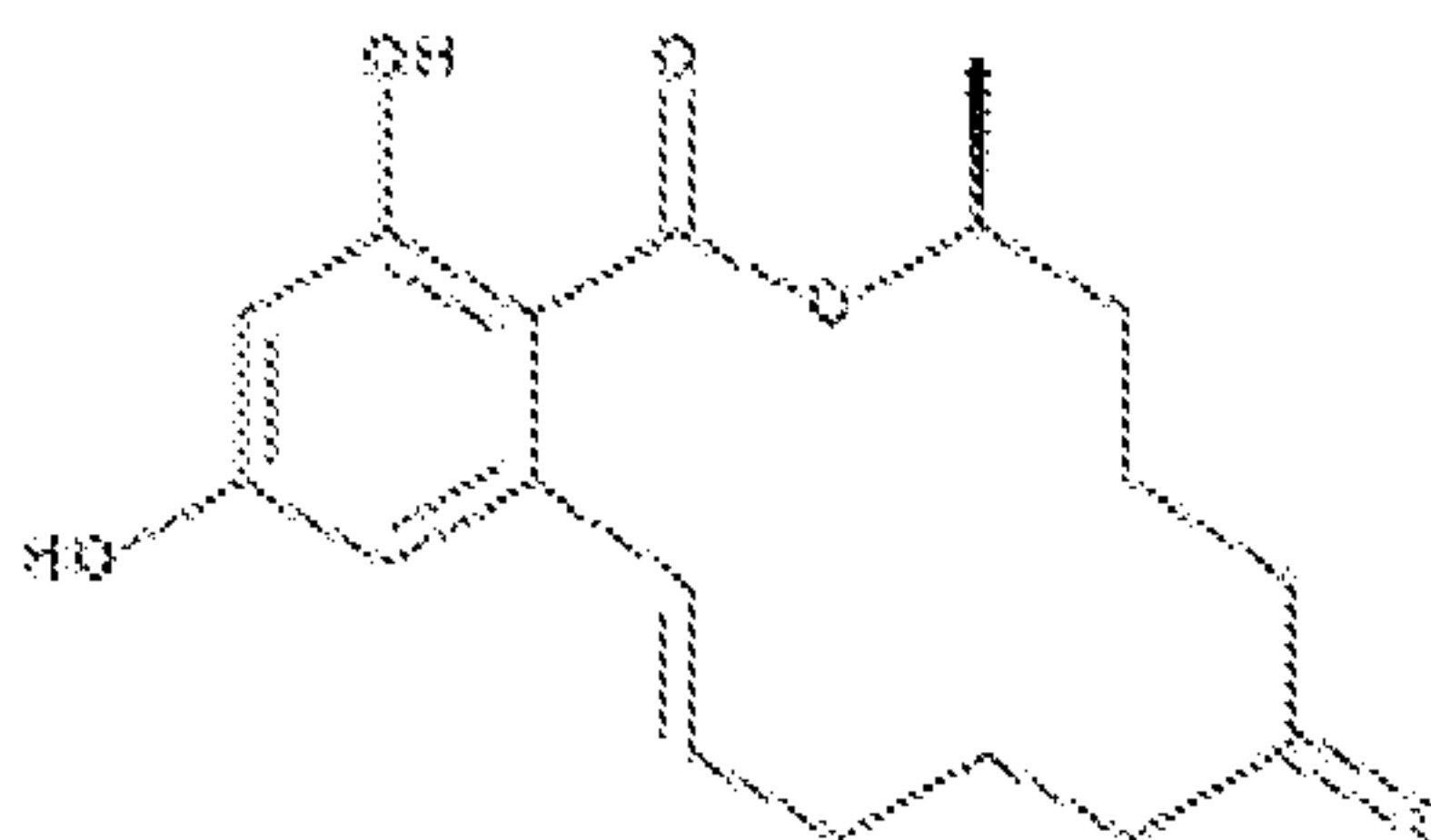
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The present invention relates to a process for hydrolytic cleavage of zearalenone and/or at least one zearalenone derivative, with a polypeptide as well as an additive containing such a polypeptide as well as a use of such a polypeptide

5 Mycotoxins are secondary metabolites produced by filamentary fungi. An important representative of mycotoxins is zearalenone (ZEN), which was previously known as F-2 toxin, which is produced by a variety of *Fusarium* fungi and can be found throughout the world. These fungi infest cultivated plants, among others, such as various types of grain, wherein the fungal infestation usually occurs before the harvest when the growth
10 of the fungi and/or the mycotoxin production may take place before storage or may even take place after harvest, either prior to storage or under improper storage conditions. The FAO has estimated that 25 % of agrarian products throughout the world are contaminated with mycotoxins, thus resulting in substantial economic losses. In an international study concluded recently, a total of 23,781 samples were analyzed from
15 January 2009 to December 2011, 81 % of them testing positive for at least one mycotoxin and 45 % testing positive for ZEN. ZEN has been found in all regions of the world and in all types of grain and feed crops tested, such as corn, soy flour, wheat, wheat bran, DDGS (dried distillers grains with solubles) as well as in finished animal feed mixtures with an incidence of up to 100 %.

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ZEN is a nonsteroidal estrogenic macrocyclic lactone with the following structural formula, synthesized by way of the polyketide metabolic pathway:



and its name according to the IUPAC nomenclature is (2E,11S)-15,17-dihydroxy-11-
30 methyl-12-oxabicyclo[12.4.0]octadeca-1(18),2,14,16-tetraene-7,13-dione.

However, a variety of ZEN derivatives also occur in nature and may be formed by enzymatic or chemical modifications of ZEN. Examples include glycosidic ZEN conjugates or those containing sulfate, formed by fungi, plants or a mammalian metabolism as well as ZEN metabolites formed in the human or animal organism, among others. ZEN derivatives are understood below to be ZEN conjugates or ZEN metabolites

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that occur naturally or are synthesized by chemical or biochemical synthesis but in particular α -zearalenol (α -ZEL; (2E,7R,11S)-7,15,17-trihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(18),2,14,16-tetraen-13-one), β -zearalenol (β -ZEL; (2E,7S,11S)-7,15,17-trihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(18),2,14,16-tetraen-13-one), α -zearalanol (α -ZAL; (7R,11S)-7,15,17-trihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(18),14,16-trien-13-one), β -zearalanol (β -ZAL; (7S,11S)-7,15,17-trihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(14),15,17-trien-13-one), zearalenone 14-sulfate (Z14S; [(2E,11S)-15-hydroxy-11-methyl-7,13-dioxo-12-oxabicyclo[12.4.0]octadeca-1(18),2,14,16-tetraen-17-yl] hydrogen sulfate), zearalenone-14-glycoside (Z14G; (2E,11S)-15-hydroxy-11-methyl-17-[(3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-12-oxabicyclo[12.4.0]octadeca-1(18)2,14,16-tetraene-7,13-dione) as well as zearalanone (ZAN; (11S)-15,17-dihydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(18),14,16-triene-7,13-dione).

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ZEN as well as ZEN derivatives, in particular α -ZEL, β -ZEL, Z14S, α -ZAL, β -ZAL, Z14G and ZAN can also be detected in processed foods and animal feed products, such as bread or beer because of their high chemical and physical stability.

20 ZEN binds to the estrogen receptor and can cause hormonal disruptions, being absorbed immediately after oral ingestion and converted by mammals into the two stereoisomeric metabolites α -ZEL and/or β -ZEL. For example, α -ZEL but also α -ZAL and/or ZAN have a much stronger estrogenic effect than ZEN. Meanwhile, conjugated ZEN derivatives have a lower estrogenic activity than ZEN but ZEN can be released
25 again from these ZEN derivatives in the digestive tract under some circumstances.

Although ZEN has a relatively low acute toxicity and has an oral LD₅₀ of up to 20,000 mg/kg body weight, subacute and/or subchronic toxic effects such as teratogenic, carcinogenic, estrogenic and immunosuppressant effects may occur in
30 animals or humans with prolonged exposure. Feed contaminated with ZEN leads to developmental disorders in mammalian animals, but pigs, in particular piglets, are extremely sensitive to ZEN. ZEN concentrations of more than 0.5 ppm in feed result in developmental disorders, and concentrations of more than 1.5 ppm in pigs, for example, can result in hyperestrogenicity, and concentrations of 12 ppm ZEN have been blamed
35 for spontaneous abortions in cattle. Since zearalenone is absorbed rapidly through the mucous membranes, in particular through the gastric mucosa as well as the oral

mucosa, immediate and quantitative deactivation is essential. ZEN can be detected in blood even 30 minutes after oral administration. In this case, the use of isolated enzymes offers some advantages with respect to microorganisms, such as a higher specific activity or a quicker effect. Because of the harmful effects of ZEN, the European Union
5 has binding upper limits for ZEN in foodstuffs as well as recommendations for upper limits for ZEN in animal feed products (EC No. 1881/2006).

The primary strategy for reducing ZEN contamination of foods and animal feed products is to restrict the growth of fungi, for example, by maintaining "good agricultural practice."
10 This includes, among other things, ensuring that the seed is free of pests and fungal infestation or that agricultural waste products are removed from the field promptly. In addition, fungal growth in the field can be reduced through the use of fungicides. After the harvest, the harvested material should be stored at a residual moisture level of less than 15 % and at a low temperature to prevent the growth of fungi. Likewise, material
15 contaminated by fungal infestation should be removed before further processing. Despite the list of measures, I. Rodrigues and K. Naehrer (2012) have reported that, even in regions with the highest agricultural standards, such as the United States and Central Europe in the years 2009 to 2011, 29 % and 39 % respectively, of the tested corn samples were contaminated with ZEN.

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Additional possibilities for removing ZEN from foodstuffs or animal feed products include adsorption and/or transformation of the mycotoxin. This requires that binding of the mycotoxin to the adsorbent must be strong and specific over a wide pH range and must remain stable in the gastrointestinal tract. Although some nonbiological adsorbents such
25 as activated carbon and silicates or synthetic polymers such as cholestyramine can be used efficiently for aflatoxins, their use for other mycotoxins is limited. The main disadvantage of adsorbents is the nonspecific binding of other molecules, which are in some cases essential for nutrition. Biological adsorbents such as yeast or yeast extracts have also been described in the literature but have a limitation similar to that of
30 nonbiological adsorbents.

Detoxification of ZEN by physical and chemical treatments is also limited. ZEN cannot be deactivated effectively by thermal treatment, but the ZEN content can be reduced by 83.9 % by extrusion and treatment with oxidizing agents, for example, for 16 hours at
35 80°C with 10 % hydrogen peroxide solution. Use of extrusion methods and oxidizing agents such as ozone or hydrogen peroxide in the production of foodstuffs and animal

feed products is limited because of the high cost, the loss of quality and in some cases the low efficacy and low specificity.

5 Biotransformation of ZEN by means of microorganisms such as *Trichosporon mycotoxinivorans*, *Gliocladium roseum* or *Bacillus subtilis* strains or enzymes isolated from them such as hydrolases or peroxidases has described, for example, by E. Vekiru et al. in Appl. and Environ. Microb., 2010, 76, 7, 2353-2359.

10 EP 0 938 575 B1 has described ZEN-degrading properties of bacteria of the genus *Rhodococcus* or *Nocardia*, in particular *R. globerulus*, *R. erythropolis* and *N. globerula*.

WO 02/076205 describes the ZEN-degrading effect of enzymes isolated from *Gliocladium roseum*, including α,β -hydrolase and zearalenone hydrolase 1 (ZHD1), which catalyze the degradation of ZEN by means of a catalytic triad.

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WO 2012/113827 discloses recombinant zonases, namely enzymes that degrade ZEN and remain stable in the gastrointestinal tract. These include microorganisms such as *Thermobifidia fusca*, *Streptomyces exfoliates*, *Acidovorans delafieldii* and *Streptomyces* sp. in particular.

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An α/β -hydrolase protein sequence can be gathered from the DATABASE UniProt, November 16th 2011, "SubName: Full=Alpha/beta hydrolase fold containing protein {ECO:0000313|EMBL:AEM80235.1}", found in EBI accession no. UNIPROT:G2P9U4, however no comment is made about the usage possibilities or properties thereof.

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Polypeptides or enzymes capable of hydrolyzing ZEN and/or at least one ZEN derivative may also be designated as zonases.

30 The terms used hereinafter are taken from the technical language and each is used in the traditional meanings, unless something to the contrary is indicated. Thus, for example, the term "polynucleotide" relates to all types of genetic material of all lengths and sequences 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 designation "polypeptide" includes proteins such as, for example, enzymes, antibodies as well as polypeptides with up to 500 amino acids, such as, for example, peptide inhibitors, domains of proteins or also short

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polypeptides with short sequence lengths, for example, less than 10 amino acids, such as receptors, ligands, peptide hormones, tags and the like. The designation "position" in a polynucleotide or polypeptide relates to a single specific base or amino acid in the sequence of the polynucleotide or of the polypeptide.

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The present invention is now aimed at making available a process with which it is possible to rapidly and reliably transform ZEN and/or at least one ZEN derivative.

To achieve this object, the present invention is characterized essentially in that
10 Zearalenon and/or at least one Zearalenon derivative is hydrolysed by the polypeptide, which is a hydrolase with an amino acid sequence selected from the group of sequence ID numbers 7, 8, 14 and 15, especially sequence ID No. 14 or a functional variant thereof, wherein there is a sequence identity of at least 70 % between the functional variant and at least one of the amino acid sequences.

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The term "sequence identity" according to the present invention relates to a percentage sequence identity. For amino acid sequences and nucleotide sequences, the sequence identity can be determined visually, but is preferably calculated by a computer program. The sequence comparison is also carried out within sequence segments, wherein the
20 segment is understood to be a continuous sequence of the reference sequence and preferably comprises a conserved region of the sequence.

In the present case, the sequence identity was determined with the help of the NCBI BLAST program (BLAST = Basic Logic Alignment Search Tool), in particular with
25 BLASTP for polypeptides and BLASTN for polynucleotides, which are made available on the homepage of the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>). It is thus possible to compare two or more sequences with one another according to the algorithm of Altschul et al., 1997 (Nucleic Acids Res., 25:3389-3402). For this purpose of this invention, the programs were used in the version
30 of 15 May 2013. The basic settings were used as the program settings, but in particular for the amino acid sequence comparison: "max target sequence" = 100; "expected threshold" = 10; "word size" = 3; "matrix" = BLOSUM62; "gap costs" = "existence: 11; extension: 1"; "computational adjustment" = "conditional compositional score matrix adjustment" as well as for the nucleotide sequence comparison word size: 11; expect
35 value: 10; gap costs: existence = 5, extension = 2; filter = low complexity activated; match/mismatch scores: 2-3; filter string: L; m.

The terms "functional polypeptide variant" or "functional variant" relate first to "allelic variants" of the polypeptide and to "functional fragments" of the polypeptide and secondly to "modification" of the polypeptide, wherein the enzymatic function is essentially unchanged. The term "allelic variant" relates to a polypeptide formed by naturally occurring mutation(s) in the nucleotide sequence and causing a change in the amino acid sequence, wherein the enzymatic function thereof is not affected. "Modifications" may be, for example, C- or N-terminal fusions with polypeptides or mutated polypeptides, wherein mutations can be obtained by substitution, insertion or deletion of at least one amino acid, in particular by site-directed mutagenesis, i.e., random mutagenesis, recombination and/or any other protein engineering method. The terms "substitution," "insertion" and "deletion" are used here in the common meanings in genetic engineering, with which those skilled in the art are familiar. The term "functional fragment" refers to a part or a subsequence of a polypeptide or a part and/or a subsequence of a functional variant thereof, wherein the enzymatic function is essentially retained. An enzymatic function is retained in particular when the enzymatic reaction mechanism remains unchanged, i.e., the mycotoxin is hydrolyzed in the same location, and the specific residual activity "functional variant" amounts to at least 5 %, preferably at least 10 %, especially at least 10 %, and in particular at least 50 %, based on the original polypeptide.

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Through the choice of such an amino acid sequence or a functional variant thereof, a surprisingly fast and complete hydrolysis of ZEN and/or at least one ZEN derivative has been detected.

25 As corresponds to a preferred further development of the invention, the hydrolytic cleavage is carried out using a polypeptide, which contains at least one conserved amino acid sequence segment or a functional variant thereof, wherein the functional variant of the amino acid sequence segment has a sequence identity of at least 70 %, preferably at least 84 %, more preferably at least 92 % and most preferably at least 98 %, and the at least one conserved amino acid sequence segment is selected from the group of amino acid sequences +24 to +50, +52 to +77, +79 to +87, +89 to +145, +150 to +171, +177 to +193, +223 to +228, +230 to +237, +239 to +247, +249 to +255, +257 to +261, +263 to +270, +272 to +279, +297 to +301, of the sequence having the sequence ID no. 1. Due to the presence of at least one such conserved amino acid sequence segment, it has been possible to make available a polypeptide which also has, in addition to the rapid and complete hydrolysis of ZEN and/or of at least one ZEN

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derivative, a particularly high activity value in comparison with ZEN degrading polypeptides known previously.

Equally good results have been achieved when according to a further functional development of the invention the functional variant has at least one amino acid modification selected from the group of substitution, deletion and insertion of one or more amino acids.

By further developing the invention in such a way that the polypeptide has a specific activity of at least 0.01 U/mg, preferably at least 0.1 U/mg, in particular at least 1 U/mg; and/or a K_M value of the hydrolytic cleavage of ZEN of at most 50 μM , preferably at most 3.5 μM , in particular at most 0.5 μM ; and/or a k_{cat} value of the hydrolytic cleavage of ZEN of at least 0.05 s^{-1} , preferably at least 0.6 s^{-1} , in particular at least 5 s^{-1} ; and/or a v_{max} value of the hydrolytic cleavage of ZEN of at least 0.00001 $\mu\text{M}^{-1} \text{s}^{-1}$, preferably at least 0.0001 $\mu\text{M}^{-1} \text{s}^{-1}$, in particular at least 0.001 $\mu\text{M}^{-1} \text{s}^{-1}$, then ZEN and/or ZEN derivatives can be hydrolyzed especially rapidly and completely, in particular being detoxified.

Furthermore the polypeptide may contain an amino acid sequence of sequence ID number 15 or a functional variant thereof, wherein the functional variant has at least 70 % sequence identity with the amino acid sequence, and the pH stability of the polypeptide at pH 5.0 amounts to at least 15 %, preferably 50 % and in particular preferably 90 %. It is possible in this way to ensure that the polypeptide zearalenone and/or at least one zearalenone derivative will be cleaved and/or detoxified even in an acidic medium, such as the mammalian stomach, for example. The pH stability of polypeptides is defined here as the percentage residual activity of the polypeptides at pH 5.0 in relation to the activity at the respective optimum pH.

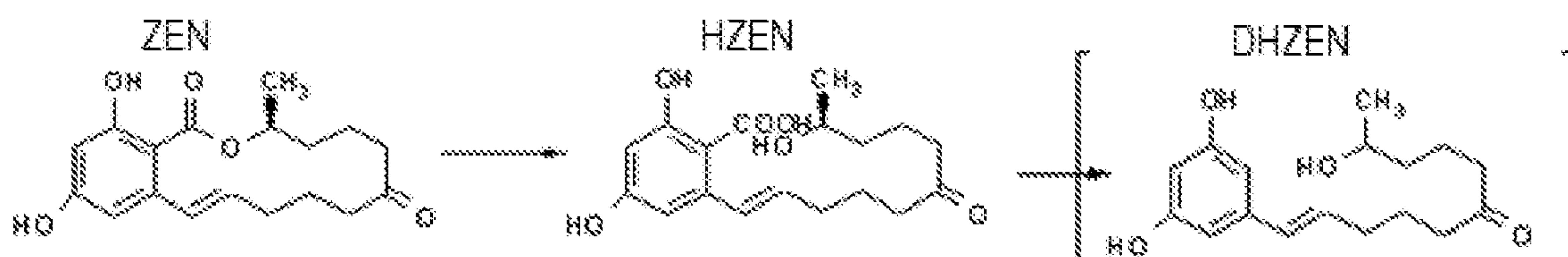
Furthermore the polypeptide may contain an amino acid sequence of sequence ID number 15 or a functional variant thereof, wherein the functional variant has at least 70 % sequence identity with the amino acid sequence, so that the polypeptide has the highest enzymatic activity in a temperature range between 30°C and 75°C, preferably between 38°C and 55°C, in particular preferably between 38°C and 52°C. This ensures that zearalenone and/or at least one zearalenone derivative is also hydrolyzed and/or detoxified by the polypeptide even at mesophilic temperatures, in particular at the body temperature of humans and farm animals. The temperature at which the polypeptide

has the highest enzymatic activity is defined as the optimum temperature of the polypeptide.

Finally the polypeptide may have an amino acid sequence of sequence ID number 15
 5 or a functional variant thereof, wherein the functional variant has at least 70 % sequence identity with at least one of the amino acid sequences, and the polypeptide is thermally stable up to a temperature of 90°C, preferably 75°C and in particular preferably 60°C. This ensures that the polypeptide and its enzymatic function will remain essentially intact even under elevated temperature stress, such as that which may occur, for example,
 10 during shipping in a container or during pelletization of feed. The thermal stability of polypeptides is defined as the temperature at which, after 15 minutes of preliminary incubation, the polypeptide has a 50 % residual activity in comparison with the activity at the respective optimum temperature.

15 The polypeptide may be selected so that it has an α,β -hydrolase, which is suitable for oxygen-independent and cofactor-free hydrolytic cleavage of the ester group of zearalenone and/or of the ZEN derivatives, which has an amino acid triad that catalyzes the hydrolytic cleavage and consists of serine, an acidic amino acid selected from glutamic acid and aspartic acid, in particular aspartic acid and histidine, and the catalytic
 20 triad is, for example, S128, D264 and H303, wherein the positioning relative to the sequence ID no. 1 is shown.

Hydrolysis of ZEN and ZEN derivatives succeeds with any of the polypeptides of the sequence ID numbers 1 to 15 on the ester group of zearalenone or its derivatives
 25 according to the following reaction mechanism:



The hydrolysis of ZEN to form nontoxic hydrolyzed zearalenone (HZEN) and/or hydrolyzed ZEN derivatives takes place by means of polypeptides according to the invention, in particular α,β -hydrolases. The further decarboxylation of HZEN to decarboxylated hydrolyzed ZEN (DHZEN) and/or decarboxylated hydrolyzed ZEN
 30 derivatives is usually spontaneous.

In particular, by means of the aforementioned catalytic triad, it is possible to completely hydrolyze ZEN and ZEN derivatives, wherein the degradation reaction has a good pH stabilizing effect, in particular at a pH in the acidic range.

- 5 Furthermore it has been found that it is possible to achieve uniformly good results with a polypeptide that contains in a sequence segment consisting of three amino acids before the serine and three amino acids after the serine of the aforementioned catalytic triad, at least one polar amino acid selected from Y, Q, N, T, K, R, E, D and at least one nonpolar amino acid selected from F, M, L, I, V, A, G, P, and it is also possible to improve
10 at least one enzyme kinetic parameter.

In a preferred refinement of the invention, the process is carried out using a polypeptide, which has at least one mutation of the amino acid sequence with respect to the sequence ID no. 1 in at least one of the following positions: 22, 23, 25, 26, 27, 29, 31,
15 32, 35, 37, 42, 43, 46, 51, 53, 54, 57, 60, 69, 72, 73, 78, 80, 84, 88, 95, 97, 99, 114, 118, 119, 123, 132, 141, 146, 148, 149, 154, 163, 164, 165, 169, 170, 172, 176, 180, 182, 183, 190, 191, 194, 196, 197, 198, 201, 204, 205, 206, 207, 208, 209, 210, 212, 213, 214, 216, 217, 220, 221, 222, 229, 231, 233, 238, 240, 244, 245, 246, 248, 249, 251, 254, 256, 260, 262, 263, 266, 269, 271, 277, 280, 281, 282, 283, 284, 285, 286,
20 287, 292, 296, 298, 302, 307, 308, 309, 311, 314, 317, 319, 321, 323, 325 and 326.

According to one refinement of the invention, the process is carried out using a polypeptide, which has at least one mutation selected from the group comprising: D22A, S23Q, S23L, N25D, I26V, F27Y, F27H, S29P, R31A, F32Y, R35K, R35Q, V37A, V42I,
25 V43T, F46Y, S51E, S51D, D53G, N54M, N54R, L57V, L60I, S69G, P72E, V73A, A78S, N80H, F84Y, I88L, T95S, T97A, R99K, I114M, I118V, K119R, V123I, L132V, A141S, I146V, I146L, A148G, A149V, A154P, P163T, A164T, Y165C, Y165H, V169I, L170R, A172G, A176M, A176V, Y180F, D182T, F183Y, I190V, G191S, K194T, K194E, F196Y, V197C, V197R, E198R, E198S, K201D, K201G, P204S, P204A, A205S, K206P,
30 A207M, M208A, Q209R, L210A, L210S, Δ P212, T213V, P214A, E216T, E216G, A217I, N220H, L221M, K222R, K222Q, G229A, A231V, F233W, F233Y, F233H, A238G, H240N, H240S, D244E, R245Q, M246L, S248T, S248N, S248G, Q249R, K251N, I254V, I256L, A260M, T262D, T262G, I263T, E266D, E269H, E269N, L271V, L277E, E280A, E280L, H281R, H281Q, A282V, Q283R, D284L, D284R, I285L, I286M, R287E,
35 R287D, R292K, R292T, Q296A, Q296E, H298V, L302S, L307Q, F308S, D309A, A311P, A314V, L317F, S319Q, S319P, S319R, S321A, S321T, T323A, P325A, A326P

in the amino acid sequence with respect to sequence ID no. 1. With such a polypeptide, it is possible to completely hydrolyze ZEN within a short period of time, in particular to detoxify it, wherein the specific activity of the polypeptide amounts to at least 6.00 U/mg, preferably at least 7.00 U/mg, in particular at least 8.00 U/mg. The unit "U" or also "unit" is a measure of the absolute catalytic activity and is defined by the hydrolysis of 1 μ mol ZEN per minute at 32°C in 50 mM Tris-HCl buffer (pH 8.2), wherein "catalytic activity" is understood to refer to the enzymatic conversion of a substrate under defined reaction conditions, and "specific activity" is understood to refer to the ratio of the catalytic activity and the polypeptide mass concentration (mass per unit of volume).

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If, the polypeptide is embodied so that at least one of the following amino acid motifs with a sequence chosen from the group containing sequence ID numbers 32 to 69 is contained in the polypeptide, it is then possible to make available polypeptides having a specific activity of at least 7.00 U/mg, preferably at least 8.00 U/mg. It has surprisingly been found that when at least one of the following amino acid motifs with a sequence having the sequence ID numbers 51 to 58 is contained in it, the enzymatic activity of the polypeptide is increased further, for example, in comparison with a motif containing seven amino acids. An even higher specific activity is achieved when at least one of the following amino acid motifs having the sequence having the sequence ID numbers 59 to 69 is contained in it.

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Finally the polypeptide may contain at least one conservative amino acid substitution in at least one position, where the conservative amino acid substitution is selected from substitutions of G to A; or A to G, S; or V to I, L, A, T, S; or I to V, L, M; or L to I, M, V; or M to L, I, V; or P to A, S, N; or F to Y, W, H; or Y to F, W, H; or W to Y, F, H; or R to K, E, D; or K to R, E, D; or H to Q, N, S; or D to N, E, K, R, Q; or E to Q, D, K, R, N; or S to T, A; or T to S, V, A; or C to S, T, A; or N to D, Q, H, S; or Q to E, N, H, K, R, wherein the designation "conservative amino acid substitution" relates to the substitution of amino acids by other amino acids regarded by those skilled in the art as being conservative, i.e., having similar specific properties. Such specific properties include, for example, the size, polarity, hydrophobicity, charge or pKs value of the amino acid. A conservative mutation, for example, is understood to be a substitution of one acidic amino acid for another acidic amino acid, a basic amino acid for another basic amino acid or a polar amino acid for another polar amino acid.

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With such conservative amino acid substitutions, it is possible to produce functional polypeptide variants whose specific activity is approximately the same in comparison with the parental polypeptide but is preferably increased by at least 0.1 U/mg.

- 5 By so conducting the process that the polypeptide is used in an additive that cleaves a zearalenone and/or at least zearalenone derivative is available for animal feed products for pigs, poultry or aquaculture, for foodstuffs or DDGS (distillers dried grain and solubles), so that the biochemical conversion of ZEN and/or at least one ZEN derivative to hydrolyzed ZEN and/or hydrolyzed ZEN derivative is possible. This process can also
10 be used, for example, for stereoselective hydrolysis of ZEN and/or ZEN derivatives in industrial processes.

In a preferred refinement of the invention, the process is developed further so that the auxiliary substances are selected from at least one inert carrier as well as optionally
15 additional ingredients, such as vitamins and/or minerals and/or enzymes and/or additional components for detoxification of mycotoxins. Therefor, for example, in foodstuffs or animal feed products, it is possible to ensure that any amounts of ZEN and/or ZEN derivatives that might be present are reliably hydrolyzed, in particular being detoxified to the extent that they will have no harmful effect on the organism of the
20 subject consuming this foodstuff or animal feed product.

A polypeptide may also be present in an enzyme preparation, which additionally contains at least one enzyme in addition to at least one polypeptide, such that the enzyme takes part in the degradation of proteins, for example, such as proteases, or
25 plays a role in the metabolism of starch or fiber or fat or glycogen, such as, for example, amylase, cellulase or glucanases as well as, for example, hydrolases, lipolytic enzymes, mannosidase, oxidases, oxidoreductases, phytases, xylanases and/or combinations thereof.

30 Additional fields of use include enzyme preparations, which, in addition to at least one polypeptide, also contain at least one component for detoxification of mycotoxins, such as a mycotoxin-degrading enzyme, for example, aflatoxin oxidase, ergotamine hydrolases, ergotamine amidases, zearalenone esterases, zearalenone lactonases, ochratoxin amidases, fumonisin carboxyl esterases, fumonisin aminotransferases,
35 aminopolyol aminooxidases, deoxynivalenol epoxide hydrolases and/or at least one mycotoxin-degrading microorganism, such as *Bacillus subtilis* and/or at least one

mycotoxin-binding component, for example, microbial cell walls or inorganic materials such as bentonites.

According to one particularly preferred refinement of the invention, the process is
5 conducted so that at least one polypeptide is used in the additive in a concentration of
at most 10,000 U/g, preferably at most 1000 U/g, more preferably at most 100 U/g and
most preferably at most 10 U/g, so that it is possible to convert ZEN and/or ZEN
derivatives rapidly and in particular to do so already before they are absorbed by the
body of a subject, in particular a mammal consuming a contaminated foodstuff or animal
10 feed product, converting them into nontoxic or less toxic metabolites, in particular HZEN
and DHZEN.

According to a refinement of the invention, the additive is present in encapsulated or
coated form, wherein standard methods such as those described in WO 92/12645 can
15 be used for the encapsulation or coating. By encapsulation and/or coating, it is possible
to transport the polypeptide without any change, in particular without degradation or
damage, to its site of use, so that only after the protective shell has been dissolved in
the digestive tract of animals, for example, does the polypeptide begin to act so that an
even more targeted, rapid and complete degradation of ZEN and/or ZEN derivatives can
20 be achieved even in the acidic protease-rich and anaerobic medium. In addition, it is
also possible through encapsulation or coating to increase the thermal stability of the
polypeptides in the additive.

According to another preferred refinement, the method is carried out in such a way that
25 the polypeptide or the additive therein is mixed with a foodstuff or animal feed product
contaminated with zearalenone and/or with at least one zearalenone derivative; the
contaminated foodstuff or animal feed product is brought in contact with moisture and
the polypeptide or the additive hydrolyzes the zearalenone and/or at least one
zearalenone derivative contained in the contaminated foodstuff or animal feed product.
30 In the case of moist foodstuffs or animal feed products, such as mash or slurries, the
hydrolysis of the zearalenone and/or of at least one zearalenone derivative will take
place in the moist foodstuff or animal feed product before oral consumption. Due to this
method, it is possible to ensure that the harmful effects of zearalenone and zearalenone
derivatives on humans and animals will be largely eliminated. Moisture here is
35 understood to refer to the presence of water or aqueous liquids, which also include, for
example, saliva or other liquids present in the digestive tract. The digestive tract is

defined as the oral cavity, the pharynx (throat), the esophagus and the gastrointestinal tract or equivalents thereof, wherein there may be different designations for animals and/or individual components may not occur in the digestive tract of animals.

- 5 The method according to the invention may also be carried out in such a way that the foodstuff or animal feed product is pelletized before oral consumption.

According to one refinement of the invention, the method is carried out so that at least 70 %, preferably at least 80 % in particular at least 90 % of the zearalenone and/or at
10 least one zearalenone derivative is hydrolyzed. Therefore, subacute and/or chronic toxic effects such as teratogenic, carcinogenic, estrogenic and immunosuppressant effects in animals or humans, for example, can be suppressed.

The present invention is additionally aimed at use of an additive containing at least one
15 polypeptide having an amino acid sequence selected of the group consisting of sequence ID numbers 7, 8, 14 and 15 or a functional variant thereof, wherein the sequence identity between the functional variant and at least one of the amino acid sequences amounts to at least 70 % for hydrolytic cleavage of zearalenone and/or at least one zearalenone derivative in animal feed products, in particular for pigs, poultry
20 and agriculture, in foodstuffs or in distillers dried grain and solubles. Through the use of the additive according to the invention, it is possible to hydrolyze and/or detoxify the ZEN and/or ZEN derivatives contained in the foodstuff or animal feed product and/or distillers dried grain and solubles, wherein such a detoxification is possible even with polypeptide concentrations of approximately 1 U/g contaminated foodstuff or animal
25 feed product.

The invention is explained in greater detail below on the basis of exemplary embodiments as well as drawings, in which:

- 30 Figure 1, which is not part of the present invention shows the degradation of ZEN and the increase in the metabolites HZEN and DHZEN over time for the polypeptide having the sequence ID no. 1, wherein the polypeptide in Figure 1A has not been tagged, in Figure 1B the polypeptide has a C-terminal 6xHis tag, and in Figure 1C the polypeptide has an N-terminal 6xHis tag,
- 35 Figure 2, which is not part of the present invention shows the Michaelis-Menten kinetics of the polypeptide with sequence ID no. 1,

Figure 3 shows the degradation of ZEN and the increase in metabolites HZEN and DHZEN over time, due to purified polypeptides having the sequence ID numbers 1 (Figure 3A), 7 (Figure 3B) and 15 (Figure 3C), wherein all the sequences have a C-terminal 6xHis tag.

5

Example 1: Modification, cloning and expression of polynucleotides that code for polypeptides which are capable of hydrolytic cleavage of ZEN and/or at least one ZEN derivative

Amino acid substitutions, insertions or deletions were performed by mutation of the nucleotide sequences by means of PCR using the "quick change site-directed mutagenesis kits" (Stratagene) according to the instructions. As an alternative, complete nucleotide sequences were also ordered (GeneArt). The nucleotide sequences generated by means of PCR mutagenesis and/or ordered from GeneArt optionally also contained a C- or N-terminal 6xHis tag on an amino acid level and were integrated by means of standard methods into expression vectors for expression in *E. coli* or *P. pastoris*, transformed in *E. coli* or *P. pastoris* and expressed in *E. coli* and *P. pastoris* (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.

20

The designation "expression vector" relates to a DNA construct that is capable of expressing a gene *in vivo* or *in vitro*. In particular this is understood to refer to DNA constructs that are suitable for transferring the polypeptide coding nucleotide sequence into the host cell to integrate into the genome there or to be present freely in the extrachromosomal space and to express the polypeptide coding nucleotide sequence intracellularly and optionally also to remove the polypeptide from the cell.

25

The designation "host cell" refers to all cells containing either a nucleotide sequence to be expressed or an expression vector and being capable of synthesizing a polypeptide according to the invention. In particular this is understood to include 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*.

30

The soluble cell lysate in the case of *E. coli* and/or the culture supernatant in the case of *P. pastoris* was/were used for determination of the catalytic properties of the

35

polypeptides. To determine the K_M value, v_{max} , k_{cat} and the specific activity, the polypeptides were selectively enriched chromatographically by standard methods over nickel-Sepharose columns. The determination of the protein concentration was performed by means of standard methods, either being calculated by the BCA method (Pierce BCA Protein Assay KitProd #23225) or preferably photometrically with the specific extinction coefficients for the respective proteins that are available online with the ProtParam program at <http://web.expasy.org/protparam> (Gasteiger E. et al.; *Protein Identification and Analysis Tools on the ExPASy Server*, in John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press, 2005, pp. 571-607).

Example 2: Determination of the sequence identity and the conserved amino acid sequence segments

The determination of the percentage sequence identity based on the total polypeptide length of the polypeptides with eh amino acid sequences having the sequence ID numbers 1 to 15 relative to one another (Table 1) was performed with the help of the BLAST program (Basic Local Alignment Search Tool), in particular with BLASTP, which can be used at homepage of the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/>). It is thus possible to compare two or more sequences with one another according to the algorithm of Altschul et al., 1997 (Nucleic Acids Res. (1997), 25:3389-3402). The basic settings were used as the program settings in particular. However: "max target sequence" = 100; expected threshold" = 10; "word size" = 3; "matrix" = BLOSUM62; "gap costs" = "existence: 11; extension: 1"; "computational adjustment" = "conditional compositional score matrix adjustment."

To determine the conserved amino acid sequence segments, the polypeptides having sequence ID numbers 1 to 6, which have a sequence identity of at least 70 % with one another, were compared with the help of the COBALT software (J. S. Papadopoulos and R. Agarwala, 2007, COBALT: Constraint-Based Alignment Tool for Multiple Protein Sequences, Bioinformatics 23:1073-79) while using the standard parameters, in particular the parameters ("gap penalties": -11, -1; "end-gap penalties": -5, -1; "use RPS BLAST": on; "Blast E-value": 0.003; "find conserved columns and recompute": on; "use query clusters": on; "word size": 4; "may cluster distance": 0,8; "alphabet": regular; "homology conversation setting": 3 bits). The result of this analysis represents the conserved amino acids. The following ranges of at least five successive conserved amino acids were defined as the conserved amino acid sequence segments, namely with respect to the segment having the sequence ID no. 1, the segments A from position

+24 to position +50, B from position +52 to position +77, C from position +79 to position +87, D from position +89 to position +145, E from position +150 to position +171, F from position +177 to position +193, G from position +223 to position +228, H from position +230 to position +237, I from position +239 to position +247, J from position +249 to
5 position +255, K from position +257 to position +261, L from position +263 to position +270, M from position +272 to position +279, N from position +297 to position +301 and O from position +303 to position +313.

The determinations of the percentage sequence identity of the polypeptides to one
10 another and of the conserved amino acid sequence segments of the individual polypeptides relative to the conserved amino acid sequence segments of the sequence having the sequence ID no. 1 were exemplary formed as described above. The results are presented in Tables 1 and 2.

Table 1: Percentage sequence identity of the polypeptides to one another.

	SEQ ID No. 1	SEQ ID No. 2	SEQ ID No. 3	SEQ ID No. 4	SEQ ID No. 5	SEQ ID No. 6	SEQ ID No. 7
SEQ ID No. 1	-	70 %	71 %	71 %	71 %	71 %	64 %
SEQ ID No. 2	70 %	-	81 %	83 %	81 %	83 %	63 %
SEQ ID No. 3	71 %	81 %	-	95 %	99 %	92 %	60 %
SEQ ID No. 4	71 %	83 %	95 %		95 %	95 %	60 %
SEQ ID No. 5	71 %	81 %	99 %	95 %	-	93 %	60 %
SEQ ID No. 6	71 %	83 %	92 %	95 %	93 %	-	61 %
SEQ ID No. 7	64 %	63 %	60 %	60 %	60 %	61 %	-
SEQ ID No. 8	57 %	54 %	54 %	53 %	53 %	53 %	53 %
SEQ ID No. 9	50 %	50 %	53 %	53 %	53 %	55 %	51 %
SEQ ID No. 10	55 %	52 %	55 %	54 %	55 %	53 %	52 %
SEQ ID No. 11	53 %	51 %	53 %	51 %	51 %	52 %	54 %
SEQ ID No. 12	50 %	49 %	50 %	50 %	50 %	49 %	51 %
SEQ ID No. 13	55 %	49 %	51 %	51 %	51 %	52 %	54 %
SEQ ID No. 14	73 %	65 %	69 %	70 %	69 %	68 %	80 %
SEQ ID No. 15	79 %	68 %	71 %	71 %	71 %	72 %	63 %

	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. 1	57 %	50 %	55 %	53 %	50 %	55 %	73 %	79 %
SEQ ID No. 2	54 %	50 %	52 %	51 %	49 %	49 %	65 %	68 %
SEQ ID No. 3	54 %	53 %	55 %	53 %	50 %	51 %	69 %	71 %
SEQ ID No. 4	53 %	53 %	54 %	51 %	50 %	51 %	70 %	71 %
SEQ ID No. 5	53 %	53 %	55 %	51 %	50 %	51 %	69 %	71 %
SEQ ID No. 6	53 %	55 %	53 %	52 %	49 %	52 %	68 %	72 %
SEQ ID No. 7	53 %	51 %	52 %	54 %	51 %	54 %	80 %	63 %
SEQ ID No. 8	-	50 %	49 %	51 %	49 %	48 %	83 %	51 %
SEQ ID No. 9	50 %	-	51 %	52 %	69 %	51 %	67 %	51 %
SEQ ID No. 10	49 %	51 %	-	76 %	52 %	52 %	63 %	56 %
SEQ ID No. 11	41 %	50 %	76 %	-	52 %	51 %	58 %	52 %
SEQ ID No. 12	49 %	52 %	52 %	52 %	-	49 %	71 %	51 %
SEQ ID No. 13	48 %	51 %	52 %	51 %	49 %	-	54 %	53 %
SEQ ID No. 14	83 %	67 %	63 %	58 %	71 %	55 %	-	72 %
SEQ ID No. 15	51 %	51 %	56 %	52 %	51 %	53 %	72 %	-

Table 2: Percentage sequence identity of the conserved amino acid sequence segments A to O.

	Sequence identity relative to the sequence ID No. 1					
Polypeptide	Segment A	Segment B	Segment C	Segment D	Segment E	Segment F
SEQ ID No. 1	100 %	100 %	100 %	100 %	100 %	100 %
SEQ ID No. 2	59.6 %	76.9 %	88.9 %	87.7 %	77.3 %	76.5 %
SEQ ID No. 3	63.0 %	76.9 %	77.8 %	89.5 %	86.4 %	76.5 %
SEQ ID No. 4	63.0 %	80.8 %	77.8 %	91.2 %	86.4 %	76.5 %
SEQ ID No. 5	63.0 %	76.9 %	77.8 %	87.7 %	86.4 %	76.5 %
SEQ ID No. 6	63.0 %	80.8 %	77.8 %	91.2 %	86.4 %	76.5 %
SEQ ID No. 7	44.7 %	69.2 %	77.8 %	78.9 %	68.2 %	64.7 %
SEQ ID No. 8	40.7 %	50.0 %	66.7 %	82.5 %	59.1 %	64.7 %
SEQ ID No. 9	51.9 %	57.7 %	55.6 %	73.7 %	45.5 %	58.8 %
SEQ ID No. 10	44.4 %	61.5 %	77.8 %	75.4 %	47.8 %	76.5 %
SEQ ID No. 11	44.4 %	50.0 %	66.7 %	71.9 %	43.5 %	58.8 %
SEQ ID No. 12	51.9 %	53.8 %	55.6 %	71.9 %	50.0 %	58.8 %
SEQ ID No. 13	18.5 %	61.5 %	55.6 %	77.2 %	54.5 %	52.9 %
SEQ ID No. 14	55.6 %	69.2 %	77.8 %	84.2 %	54.5 %	52.9 %
SEQ ID No. 15	74.1 %	86.7 %	88.9 %	89.0 %	77.3 %	88.2 %

	Sequence identity relative to the SEQ ID No. 1					
Polypeptide	Segment G	Segment H	Segment I	Segment J	Segment K	Segment L
SEQ ID No. 1	100 %	100 %	100 %	100 %	100 %	100 %
SEQ ID No. 2	100 %	87.5 %	66.7 %	85.7 %	80.0 %	75.0 %
SEQ ID No. 3	100 %	87.5 %	77.8 %	57.1 %	80.0 %	75.0 %
SEQ ID No. 4	100 %	87.5 %	77.8 %	57.1 %	80.0 %	75.0 %
SEQ ID No. 5	100 %	87.5 %	77.8 %	57.1 %	80.0 %	75.0 %
SEQ ID No. 6	100 %	75.0 %	77.8 %	85.7 %	80.0 %	87.5 %

SEQ ID No. 7	100 %	87.5 %	66.7 %	71.4 %	100 %	50.0 %
SEQ ID No. 8	100 %	62.5 %	44.4 %	57.1 %	80.0 %	62.5 %
SEQ ID No. 9	100 %	12.5 %	44.4 %	42.9 %	60.0 %	62.5 %
SEQ ID No. 10	100 %	62.5 %	55.6 %	71.4 %	80.0 %	50.0 %
SEQ ID No. 11	100 %	50.0 %	55.6 %	57.1 %	80.0 %	50.0 %
SEQ ID No. 12	100 %	12.5 %	22.2 %	57.1 %	80.0 %	52.5 %
SEQ ID No. 13	100 %	50.0 %	44.4 %	57.1 %	80.0 %	75.0 %
SEQ ID No. 14	0 %	8.3 %	0 %	14.3 %	0 %	25.0 %
SEQ ID No. 15	100 %	87.5 %	100 %	85.7 %	100 %	75.0 %

	Sequence identity relative to the SEQ ID No. 1		
Polypeptide	Segment M	Segment N	Segment O
SEQ ID No. 1	100 %	100 %	100 %
SEQ ID No. 2	87.5 %	80.0 %	81.8 %
SEQ ID No. 3	87.0 %	80.0 %	81.8 %
SEQ ID No. 4	87.5 %	80.0 %	81.8 %
SEQ ID No. 5	87.5 %	80.0 %	81.8 %
SEQ ID No. 6	87.5 %	80.0 %	72.7 %
SEQ ID No. 7	75.0 %	40.0 %	36.4 %
SEQ ID No. 8	75.0 %	60.0 %	54.5 %
SEQ ID No. 9	62.5 %	40.0 %	54.5 %
SEQ ID No. 10	62.5 %	40.0 %	54.5 %
SEQ ID No. 11	75.0 %	40.0 %	54.5 %
SEQ ID No. 12	100 %	40.0 %	54.5 %
SEQ ID No. 13	50.0 %	40.0 %	63.6 %
SEQ ID No. 14	6.2 %	0 %	0 %
SEQ ID No. 15	87.5 %	80.0 %	63.6 %

Example 3: Hydrolysis of ZEN by polypeptides in cell lysates

To determine their ability to degrade ZEN into the nontoxic or less toxic metabolites

5 HZEN and DHZEN, the polypeptide with the sequence ID no. 1, coded by the nucleotide

sequence having the sequence ID no. 17 was synthesized as such and with a C-terminal and/or N-terminal 6xHis tag in *E. coli* as described in example 1. The polypeptides with the amino acid sequences having the sequence ID numbers 7, 8, 14 and 15 which were coded by the nucleotide sequences having the sequence ID numbers 18, 23, 24, 30 and 31 were labeled with 6xHis exclusively at the C-terminus. 100 mL portions of an *E. coli* culture having an optical density (OD 600 nm) of 2.0-2.5 were harvested by centrifugation at 4°C and resuspended in 20 mL Brunner mineral medium (DSMZ microorganisms medium number 462, 2012). The cell suspensions were lysed by treating three times with a French press at 20,000 psi. The resulting cell lysates were used in a 1:10, 1:100 or 1:1000 dilution prepared in Brunner mineral medium including 0.1 mg/mL BSA (bovine serum albumin). For the ZEN degradation experiments, 9.9 mL Brunner mineral medium was used, including 0.1 mg/mL BSA, 0.1 mL dilute cell lysate and 31 µL ZEN substrate stock solution. On the whole, the cell lysates were thus diluted 1:1000, 1:10,000 and/or 1:100,000. The ZEN substrate stock solution used was a 2.08 mM ZEN solution (40 vol % CAN + 60 vol % H₂O). To prepare this solution, ZEN in crystalline form (Biopure Standard from Romer Labs, article no. 001109, purity at least 98 %) was weighed and dissolved accordingly. Each degradation batch was carried out in 25 mL glass vials and incubated at 25°C and 100 rpm for a total of 120 hours with agitation. At the times 0, 0.5, 1, 2, 5, 24, 47, 72 and 120 h, a sample of 1 mL was taken each time, the polypeptides were heat inactivated for 10 minutes at 99°C and stored at -20°C. After thawing the sample, the insoluble constituents were separated by centrifugation. ZEN, HZEN and DHZEN were analyzed by means of LC/MS/MS. To do so, the metabolites were separated chromatographically on a Phenomenex Luna C18(2) column having the dimensions 250 mm × 3 mm and a particle size of 5 µm, using as the mobile phase an acetonitrile-water mixture with a formic acid concentration of 1 mL/L. The UV signal at 270 nm was recorded using electrospray ionization (ESI) as the ionizing source. ZEN, HZEN and DHZEN were quantified by means of QTrap/LC/MS/MS (triple quadrupole, Applied Biosystems) in the enhanced mode. After 24 hours at the latest, substantial amounts of ZEN could not be detected any more in any of the batches. Most of the ZEN, i.e., more than 80 %, was converted into HZEN or DHZEN.

Figure 1, which is not part of the present invention shows the degradation of ZEN over time and the increase in HZEN as well as DHZEN for a 1:10,000 diluted cell lysate solution as an example for untagged (Figure 1A) as well as for C-terminal 6xHis tagged (Figure 1B) and N-terminal 6xHis tagged (Figure 1C) polypeptide with the sequence ID

no. 1. It can be seen here clearly that 1) the reaction of ZEN takes place directly and completely because almost no ZEN could be detected any longer in the first sample (0 h), which was taken immediately after the start of the experiment, and 2) no mentionable losses of activity occurred as a result of attaching a tag, whether C-terminal or N-terminal.

Example 4: Hydrolysis of ZEN derivatives by polypeptides in cell lysates

To determine the capability of polypeptides to also transform ZEN derivatives, in addition to ZEN, into nontoxic and/or less toxic metabolites, the polypeptides having the sequence ID numbers 1, 7, 8, 14 and 15 were prepared as described in Example 3 with C-terminal His tag and the respective synthetic nucleotide sequences with the sequences having sequence ID numbers 17, 23, 24, 30 and 31 were used as the cell lysates in degradation.

The degradation experiments were performed as described in Example 3, where each polypeptide was tested with each ZEN derivative selected from the group comprised of α -ZEL, β -ZEL, α -ZAL, β -ZAL, Z14G, Z14S and ZAN. The cell lysates were used in a total dilution of 1:10,000. Instead of a 2.08 mM ZEN solution (40 vol % CAN + 60 vol % H₂O), equimolar, i.e., 2.08 mM solutions of the ZEN derivatives were used as the substrate stock solution. α -ZEL, β -ZEL, α -ZAL, β -ZAL and ZAN were obtained from Sigma and used as standards for the analysis. Z14G and Z14S were prepared in a purity of at least 90 % according to the methods such as those described by P. Krenn et al., 2007 (Mycotoxin Research, 23, 4, 180-184) and M. Sulyok et al., 2007 (Anal. Bioanal. Chem. 289, 1505-1523) and used as standards for the analysis. Another difference in comparison with Example 3 is that only one sample was taken, namely after 24 hours. The reduction in concentration of the ZEN derivatives during the degradation experiment was quantified by means of LC/MS/MS. α -ZEL, β -ZEL, Z14G and Z14S were measured by the method of M. Sulyok et al. (2010, Food Chemistry, 119, 408-416); α -ZAL, β -ZAL and ZAN were measured by the method of P. Songsermaskul et al. (2011, J. of Animal Physiol. and Animal Nutr., 97, 155-161). It was surprisingly found that only 0 to max. 13 % of the starting amounts of the ZEN derivatives was present after 24 hours of incubation in all the degradation experiments.

Example 5: Specific activity and enzyme kinetic parameters of the polypeptides as well as variants thereof

The specific activity of the polypeptides and variants thereof was determined photometrically, wherein all the polypeptides used had a C-terminal 6xHis tag. The preparation, enrichment and purification of the polypeptides and/or variants thereof were performed as described in example 1. Degradation of ZEN to HZEN was measured on the basis of the reduction in absorption at the wavelength of 315 nm. The molar extinction coefficients (ϵ) of ZEN and HZEN were determined experimentally and were found to amount to $0.0078895 \text{ L } \mu\text{mol}^{-1} \text{ cm}^{-1}$ and $0.0030857 \text{ L } \mu\text{mol}^{-1} \text{ cm}^{-1}$. The extinction coefficients have a strong dependence on pH and therefore the activity must always be measured precisely at the same pH and preferably also in the same matrix. The measurements were performed in a 50 mM Tris-HCl pH = 8.2 buffer solution in quartz cuvettes in a wavelength range of 200 to 2500 nm in a UV-VIS photometer (Hitachi U-2001) at 32°C.

A 2.08 mM ZEN solution (40 vol % ACN + 60 vol % H₂O) was used as the ZEN substrate stock solution. To prepare this solution, ZEN in crystalline form (Biopure Standard from Romer Labs, article no. 001109, purity at least 98 %) was weighed and dissolved accordingly. The ZEN substrate dilutions (0.79 μM , 1.57 μM , 2.36 μM , 3.14 μM , 4.71 μM , 6.28 μM , 7.85 μM , 9.42 μM , 10.99 μM , 12.56 μM , 14.13 μM , 15.71 μM , 17.28 μM and 18.85 μM) were prepared with 50 mM Tris-HCl pH = 8.2. The polypeptide solutions were diluted to a final concentration of approximately 70 ng/mL using 50 mM Tris-HCl buffer pH = 8.2. The ZEN substrate dilutions were preheated to 32°C in a water bath.

100 μL portions of the respective ZEN substrate dilution were mixed with 0.2 μL polypeptide solution, and the absorption was measured for 5 minutes, whereupon each combination of polypeptide solution and ZEN substrate dilution was measured at least twice.

Taking into account the extinction coefficients of ZEN and HZEN, the reaction rate was calculated for each substance concentration on the basis of the slope in the absorption over time.

The designations " K_M value" or "Michaelis-Menten constant" relate to a parameter for describing the enzymatic affinity of the units μM or mM, which are calculated with the help of the linear Hanes plots according to H. Bisswang (2002, Enzyme Kinetics, ISBN

3-527-30343-X, page 19), wherein the function "enzyme kinetics, single substrate" in the SigmaPlot 12.0 program is preferably used for this purpose. The designations "catalytic constant of the enzyme reaction" or " k_{cat} value" relate to a parameter for describing the conversion rate of a polypeptide and/or enzyme, which is given in s^{-1} and is preferably calculated with the help of the "enzyme kinetic, single substrate" function of the SigmaPlot 12.0 program. The "maximum enzyme rate" or " v_{max} value" is given in units of $\mu M/s$ or mM/s and is determined with the help of the linear Hanes plot by analogy with the K_M value, wherein the function "enzyme kinetic, single substrate" of the SigmaPlot 12.0 program is preferably used for this.

10

The specific activity was calculated by means of v_{max} and the enzyme concentration used according to the equation

$$\text{Specific activity (U/mg)} = \frac{v_{max} (\mu M/s) \times 60 (s/min)}{\text{enzyme concentration (mg/L)}}$$

wherein one unit is defined as hydrolysis of 1 μmol ZEN per minute at 32°C in 50 mM Tris-HCl buffer solution, pH = 8.2.

15

The raw data for determination of the enzyme parameters K_M , v_{max} , k_{cat} and the specific activity are given below for the polypeptide having the sequence ID no. 1. Table 3 shows the reaction rates at the respective ZEN substrate concentrations, while Figure 2 shows the respective Michaelis-Menton graphs and Table 4 shows the corresponding enzyme kinetic parameters. The enzyme solution that was used had a concentration of 68 ng/L.

20

Table 3: Reaction rates of the polypeptide with sequence ID no. 1 at different ZEN concentrations.

ZEN substrate dilution (μM)	Measurement 1 reaction rate (μM/s)	Measurement 2 reaction rate (μM/s)
0.79	0.0073	0.0071
1.57	0.0087	0.0082
2.36	0.0095	0.0080
3.14	0.0101	0.0073
4.71	0.0103	0.0087
6.28	0.0096	0.0088
7.85	0.0084	0.0088
9.42	0.0111	0.0087
10.99	0.0093	0.0081
12.56	0.0100	0.0086
14.13	0.0089	0.0101
15.71	0.0089	0.0090
17.28	0.0100	0.0074
18.85	0.0100	0.0085

Table 4: Enzyme kinetics parameters of the polypeptide having sequence ID no. 1.

	V _{max} (μM/s)		K _M (μM)		k _{cat} (s ⁻¹)		Specific activity (U/mg)	
Measurement	1	2	1	2	1	2	1	2
Value	0.00993	0.008756	0.2172	0.1898	5.44	4.79	8.76	7.73
Average	0.009343		0.2035		5.12		8.25	

5

The specific activities of the polypeptides tested are 8.25 U/mg for sequence ID no. 1, 3.83 U/mg for sequence ID no. 7, 2.57 U/mg for sequence ID no. 8, 3.33 U/mg for sequence ID no. 14 and 7.76 U/mg for sequence ID no. 15.

10 Example 6: Degradation of ZEN and ZEN derivatives in contaminated corn

To determine the capabilities of polypeptides to degrade naturally occurring ZEN and ZEN derivatives in a complex matrix and at a low pH, contaminated corn was mixed exemplary with different concentrations of one of the polypeptides having the sequence ID numbers 1 to 6 and the degradation of ZEN and ZEN derivatives was tracked.

15

The contaminated corn was ground and used in the degradation experiment wherein a batch would consist of 1 g ground contaminated corn, 8.9 mL 100 mM acetate buffer

pH 4.0 and 0.1 mL polypeptide solution. Enriched and purified polypeptide solutions were prepared as described in example 5, diluting them to a concentration of 10 mU/mL, 100 mU/mL and/or 1000 mU/mL. Thus in absolute amounts 1 mU (= 1 mU per gram corn), 10 mU (= 10 mU per gram corn) and/or 100 mU (= 100 mU per gram of corn) were used in the batch. Each degradation batch was carried out in 25 mL and incubated at 37°C and 100 rpm with agitation. Before adding the enzyme and/or after 1 hour of incubation, a sample of 1 mL was taken, the polypeptide was heat inactivated at 99°C for 10 minutes and the sample was stored at -20°C. After thawing the sample, the insoluble constituents were separated by centrifugation. Concentrations of ZEN and ZEN derivatives were measured by means of LC/MS/MS as described by M. Sulyok et al. (2007, Anal. Bioanal. Chem., 289, 1505-1523). The ZEN and ZEN derivative content in this corn was 238 ppb for ZEN, 15 ppb for α -ZEL, 23 ppb for β -ZEL, 32 ppb for Z14G and 81 ppb for Z14S. Table 5 shows the percentage reduction in the ZEN and ZEN derivative content in the degradation experiment.

Table 5: Reduction in ZEN and ZEN derivatives in percentage based on the starting content in the degradation experiment with different polypeptides and amounts of polypeptides.

Polypeptide	Amount in the batch	ZEN	α -ZEL	β -ZEL	Z14G	Z14S
SEQ ID No. 1	0.1 mU	83 %	≥ 80 %	70 %	78 %	80 %
	1 mU	96 %	≥ 80 %	76 %	≥ 80 %	92 %
	10 mU	97 %	≥ 80 %	≥ 85 %	≥ 80 %	94 %
SEQ ID No. 2	0.1 mU	87 %	≥ 80 %	73 %	≥ 80 %	84 %
	1 mU	97 %	≥ 80 %	78 %	≥ 80 %	90 %
	10 mU	99 %	≥ 80 %	≥ 85 %	≥ 80 %	96 %
SEQ ID No. 3	0.1 mU	79 %	79 %	67 %	73 %	75 %
	1 mU	85 %	≥ 80 %	72 %	79 %	82 %
	10 mU	92 %	≥ 80 %	78 %	≥ 80 %	88 %
SEQ ID No. 4	0.1 mU	82 %	78 %	65 %	76 %	80 %
	1 mU	89 %	≥ 80 %	73 %	≥ 80 %	86 %
	10 mU	93 %	≥ 80 %	82 %	≥ 80 %	91 %
SEQ	0.1 mU	79 %	76 %	66 %	78 %	80 %

ID No. 5	1 mU	83 %	≥80 %	73 %	≥80 %	81 %
	10 mU	91 %	≥80 %	79 %	≥80 %	86 %
SEQ ID No. 6	0.1 mU	93 %	≥80 %	75 %	≥80 %	90 %
	1 mU	95 %	≥80 %	82 %	≥80 %	92 %
	10 mU	98 %	≥80 %	≥85 %	≥80 %	96 %

Example 7: Additives containing polypeptide for hydrolytic cleavage of ZEN and/or ZEN derivatives

5 To prepare additives for hydrolytic cleavage of ZEN, fermentation supernatants of polypeptides expressed by *P. pastoris* and having the sequence ID numbers 1, 2, 6 and 13 were exemplary purified by microfiltration and ultrafiltration (exclusion limit: 10 kDa) under standard conditions and concentrated up to a dry substance concentration of approximately 9 % by weight. Following that, these polypeptide-containing solutions

10 were also processed further to form dry powders under standard conditions in a spray dryer (Mini B290 from Büchi). These four powders were subsequently designated as Z1, Z2, Z6 and Z13. Z1, Z2, Z6 or Z13 were additionally mixed with bentonite having an average grain size of approximately 1 µm in a ratio of 1 % by weight of additives Z1, Z2, Z6 and/or Z13 and 99 % by weight bentonite in an overhead agitator. The resulting

15 additives are designated as additives Z1.B, Z2.B, Z6.B and Z13.B. In addition, Z1, Z2, Z6 and Z13 were mixed with bentonite and a vitamin trace element concentrate in a ratio of 0.1 % by weight additive Z1, Z2, Z6 or Z13, 0.9 % by weight vitamin trace elements concentrate and 99 % by weight bentonite in an overhead agitator. The resulting additives were designated as additive Z1.BVS, Z2.BVS, Z6.BVS and Z13.BVS. 100 g of

20 the additives Z1.BVS, Z2.BVS, Z6.BVS and Z13.BVS contained 200 mg iron sulfate, 50 mg copper sulfate, 130 mg zinc oxide, 130 mg manganese oxide, 2.55 mg calcium carbonate, 160 mg vitamin E, 6.5 mg vitamin K3, 6.5 mg vitamin B1, 14 mg vitamin B2, 15 mg vitamin B6, 0.15 mg vitamin B12, 150 mg nicotinic acid, 30 mg pantothenic acid and 5.3 mg folic acid.

25

The additives were extracted for 30 minutes in a 50 mM Tris-HCl buffer pH = 8.2 and diluted further in the same buffer so that the final concentration of polypeptide was approximately 70 ng/mL.

Following that, the zearalenone-degrading effect of these solutions was determined as described in Example 5. The corresponding activities were 8.230 U/g for Z1, 9.310 U/g for Z2, 9.214 U/g for Z6, 83 U/g for Z1.B, 92 U/g for Z2.B, 90 U/g for Z2.C, 8 U/g for Z1.BVS, 9 U/g for Z2.BVS, 9 U/g for Z6.BVS and 6U/g for Z13.BVS.

5

The ability to degrade ZEN derivatives α -ZEL, β -ZEL, α -ZAL, β -ZAL, Z14G, Z14S and ZAN by the additives Z1, Z2, Z6, Z13, Z1.B, Z2.B, Z6.B, Z13.B, Z1.BVS, Z2.BVS, Z6.BVS and Z13.BVS was tested as described in Example 4, but instead of 100 μ L of a cell lysate, 100 μ L of a polypeptide solution with a polypeptide concentration of approximately 70 ng/mL was used. After incubating for 6 hours, only max. 15 % of the starting amount was present as unhydrolyzed ZEN derivative.

10

Example 8: Optimum temperature

To determine the temperature optimum of the polypeptides having SEQ ID numbers 1, 7 and 15, they were cloned with a C-terminal 6xHis tag as described in example 1, expressed in *E. coli* and purified. In preliminary experiments, the concentration at which a complete conversion of ZEN could be ensured under the experimental conditions was determined (Teorell-Stenhagen buffer (Teorell and Stenhagen, A universal buffer for the pH range of 2.0 to 12.0. Biochem Ztschrft, 1938, 299:416-419), pH 7.5 with 0.1 mg/mL BSA at 30°C) after an experimental time of 3 hours. The preparations were used in the concentrations thus determined in the degradation batches for determining the optimum temperature. The experiments were carried out in a PCR Cycler (Eppendorf) using the temperature gradient function at 20°C $\pm$ 10°C, at 40°C $\pm$ 10°C and, if necessary, at 60°C $\pm$ 10°C (10 temperatures in the respective range; temperatures predefined by the PCR cycler). For the batches Teorell-Stenhagen buffer was mixed with the corresponding enzyme concentration and 0.1 mg/mL BSA plus 5 ppm ZEN at the respective optimum pH. Batches with 0.1 mg/mL BSA and 5 ppm ZEN without addition of an enzyme were used as negative controls. After 0 h, 0.5 h, 1 h, 2 h and 3 h incubation time, a sample was taken per incubation temperature, heat inactivated for 10 minutes at 99°C and stored at -20°C. After thawing, the samples were transferred to HPLC vials. ZEN, HZEN and DHZEN were analyzed by HPLC-DAD. To do so the metabolites were separated chromatographically on a Zorbax SB-Aq C18 column with the dimensions 4.6 mm $\times$ 150 mm and a particle size of 5 μ m. A methanol-water mixture with 5 mM ammonium acetate was used as the mobile phase. The UV signal at 274 nm was recorded. The metabolites were quantified by including entrained standard series. The optimum temperatures were determined on the basis of the slopes determined for the

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degradation curves, where the optimum temperature was defined as the temperature at which the slope was the greatest. Table 6 shows the optimum temperatures.

Table 6: Optimum temperatures of the polypeptides.

SEQ ID	SEQ ID	SEQ ID
No. 1	No. 7	No. 15
38°C	31°C	41°C

5

Example 9: Thermal stability

To determine the thermal stability of polypeptides with the SEQ ID numbers 1, 7 and 15, they were cloned with a C-terminal 6xHis tag as described in Example 1, expressed in *E. coli* and purified. They were then incubated in the PCR cycler with a gradient function at the respective optimum temperature $\pm 10^{\circ}\text{C}$. After 0 min, 15 min, 30 min and 60 min, one sample was taken per batch and per temperature. These pre-incubated samples were then used in a degradation experiment in the Teorell-Stenhagen buffer at the respective optimum pH with 0.1 mg/mL BSA and 5 ppm ZEN. In preliminary experiments, the concentration at which a complete reaction of ZEN could be ensured after an experimental duration of 3 hours under the experimental conditions (Teorell-Stenhagen buffer, pH 7.5 with 0.1 mg/mL BSA at 30°C) was determined for each polypeptide. The respective enzyme concentration thereby determined was used in the batches. The degradation batches were incubated at 30°C. Sampling was performed after 0 h, 0.5 h, 1 h, 2 h and 3 h incubation time. Next, the polypeptides were heat-inactivated for 10 minutes at 99°C and the samples were stored at -20°C. After thawing the samples were transferred to HPLC vials and analyzed by HPLC-DAD, as described in Example 8.

Thermal stability is defined as the temperature at which the polypeptides have a 50 % residual activity in comparison with the optimum temperature after 15 minutes of pre-incubation. As a measure of the activity, the slope in the degradation curves is used. The temperature stabilities are shown in Table 7.

Table 7: Temperature stability of the polypeptides (50 % residual activity after pre-incubation for 15 minutes).

SEQ ID	SEQ ID	SEQ ID
No. 1	No. 7	No. 15
38°C	28°C	49°C

Example 10: Optimum pH

To determine the optimum pH of the polypeptides having the SEQ ID numbers 1, 7 and 15, they were cloned with a C-terminal 6xHis tag as described in Example 1, expressed in *E. coli* and purified. In preliminary experiments, the concentration at which a complete conversion of ZEN could be ensured after an experimental duration of 3 hours under the experimental conditions was determined for each polypeptide (Teorell-Stenhagen buffer, pH 7.5 with 0.1 mg/mL BSA at 30°C). The respective enzyme concentration was used in the batches. The degradation batches were carried out in Teorell-Stenhagen buffer at pH levels of 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 11.0 and 12.0. For the degradation batches with 0.1 mg/mL BSA and 5 ppm ZEN, incubation was done at 30°C. Batches in Teorell-Stenhagen buffer were used as the negative controls at pH 3.0, pH 7.0 and pH 12.0 with 0.1 mg/mL BSA and 5 ppm ZEN. Sampling was performed after an incubation time of 0 h, 0.5 h, 1 h, 2 h and 3 h. Next the polypeptides were heat-inactivated for 10 minutes at 99°C and the samples were stored at -20°C. After thawing, the samples were transferred to HPLC vials and analyzed by HPLC-DAD as described in Example 8. The optimum pH was determined on the basis of the slopes found for the degradation curves, wherein the pH at which the slope was the greatest was defined as the optimum pH. Table 8 shows the optimum pH levels.

Table 8: Optimum pH of the polypeptides.

SEQ ID No.	SEQ ID No. 7	SEQ ID No. 15
1		
8.2	7.5 - 8.5	7.5

Example 11: pH stability at pH 5.0

To determine the pH stability, the polypeptides from Example 10 were incubated for one hour at 25°C in Teorell-Stenhagen buffer at pH 5.0 and at the respective optimum pH. These pre-incubated samples were used in a degradation experiment in the same concentrations of the respective polypeptide as those used to determine the optimum

pH in 100 mM Tris-HCl buffer at the respective optimum pH with 0.1 mg/mL BSA and 5 pm ZEN in the batch. The batches were incubated at the respective optimum temperature. Sampling was performed after 0 h, 0.5 h, 1 h, 2 h and 3 h incubation time. Next the polypeptides were heat inactivated for 10 minutes at 99°C and the samples were stored at -20°C. After thawing, the samples were transferred to HPLC vials and analyzed by means of HPLC-DAD as described in Example 8. The pH stability is defined as the percentage residual activity of the polypeptides at pH 5.0 relative to the activity at the respective optimum pH. The pH stabilities for 5.0 are shown in Table 9.

Table 9: pH stability of the polypeptides at pH 5.0.

SEQ ID	SEQ ID	SEQ ID
No. 1	No. 7	No. 15
3 %	100 %	19 %

Example 12: ZEN degradation experiment

The degradation of ZEN to HZEN and DHZEN was performed as an example for the polypeptides with sequence ID numbers 1, 7 and 15. The degradation batches were carried in Teorell-Stenhagen buffer pH 7.5 with 0.1 mg/mL BSA and 5 ppm ZEN. The degradation batches were incubated at 30°C. Sampling was performed after 0 h, 0.5 h, 1 h, 2 h and 3 h incubation time. Next the polypeptides were heat-inactivated for 10 minutes at 99°C and the samples were stored at -20°C. After thawing, the samples were transferred to HPLC vials and analyzed by HPLC-DAD, as described in Example 8. The polypeptide concentration was selected so that complete degradation was achieved after approximately 3 hours. Figure 3 shows the degradation kinetics, where the y axis shows the concentration of ZEN, HZEN and DHZEN in micromoles per liter ($\mu\text{mol/L}$) and the x axis shows the incubation time in hours (h).

* μM denotes micromolar and corresponds to the unit $\mu\text{mol/L}$

SEKVENSLISTE

[0082]

5 <110> Erber Aktiengesellschaft

 <120> Polypeptide for hydrolytic cleavage of Zearalenone and / or
Zearalenone Derivatives

 <130> P05341PCT

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Pro	Glu	Arg	Gln	Val	Asp	Leu	Gly	Glu	Val	Val	Met	Asn	Phe	Ala	Glu	35	40	45	
Ala	Gly	Ser	Pro	Asp	Asn	Pro	Ala	Leu	Leu	Leu	Leu	Pro	Glu	Gln	Thr	50	55	60	
Gly	Ser	Trp	Trp	Ser	Tyr	Glu	Pro	Val	Met	Gly	Leu	Leu	Ala	Glu	Asn	65	70	75	80
Phe	His	Val	Phe	Ala	Val	Asp	Ile	Arg	Gly	Gln	Gly	Arg	Ser	Thr	Trp	85	90	95	
Thr	Pro	Arg	Arg	Tyr	Ser	Leu	Asp	Asn	Phe	Gly	Asn	Asp	Leu	Val	Arg	100	105	110	
Phe	Ile	Ala	Leu	Val	Ile	Lys	Arg	Pro	Val	Val	Val	Ala	Gly	Asn	Ser	115	120	125	
Ser	Gly	Gly	Leu	Leu	Ala	Ala	Trp	Leu	Ser	Ala	Tyr	Ala	Met	Pro	Gly	130	135	140	
Gln	Ile	Arg	Ala	Ala	Leu	Cys	Glu	Asp	Ala	Pro	Phe	Phe	Ala	Ser	Glu	145	150	155	160

33

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Trp Lys Gly Phe Val Glu Ala Ala Lys Ala Ser Pro Ala Lys Ala Met
195 200 205

Gln Leu Phe Pro Thr Pro Asp Glu Ala Pro Gln Asn Leu Lys Glu Tyr
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Asp Pro Glu Trp Gly Arg Ala Phe Phe Glu Gly Thr Val Ala Leu His
225 230 235 240

Cys Pro His Asp Arg Met Leu Ser Gln Val Lys Thr Pro Ile Leu Ile
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Thr His His Ala Arg Thr Ile Asp Pro Glu Thr Gly Glu Leu Leu Gly
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Ala Leu Ser Asp Leu Gln Ala Glu His Ala Gln Asp Ile Ile Arg Ser
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Ala Gly Val Arg Val Asp Tyr Gln Ser His Pro Asp Ala Leu His Met
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Ile Ala Leu Val Val Lys Arg Pro Val Ile Val Ala Gly Asn Ser Ser
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Val Thr Thr Cys Gly His Ser Ile Arg Gln Ala Ala Gly Pro Met Phe
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Thr Gly Tyr Cys Arg Ala Ala Asp Ala Ser Ser Ser Pro Met Ala Arg
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His Met Arg Asp Ile Asp Pro Asp Thr Gly His Leu Val Gly Ala Leu
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Ser Asp Glu Gln Ala Ala Arg Ala Arg Leu Leu Met Glu Ser Ala Gly
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260 265 270

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Phe Ile Ala Leu Val Val Lys Arg Pro Val Val Val Ala Gly Asn Ser
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Arg Ser Phe Val Ala Asp Gly Ile Pro Gln His Leu Lys Glu Tyr Asp
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Pro His Glu Arg Met Leu Gly Arg Val Asn Thr Pro Val Leu Leu Thr
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 Thr Pro Lys Arg Tyr Ser Leu Asp Asn Phe Gly Asn Asp Leu Val Arg
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 Phe Ile Ala Leu Val Val Lys Arg Pro Val Val Val Ala Gly Asn Ser
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 Ser Gly Gly Val Leu Ala Ala Trp Leu Ser Ala Tyr Ser Met Pro Gly
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 Leu Val Pro Ala His Gly His Ser Val Arg Gln Gly Ala Gly Pro Val
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 Phe Glu Leu Phe Arg Thr Tyr Leu Gly Asp Gln Trp Ser Val Gly Asp
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Trp Glu Gly Phe Cys Arg Ala Ala Gly Ala Ser Ala Ser Pro Met Ala
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195 200 205

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Pro His Glu Arg Met Leu Gly Gln Val Lys Thr Pro Val Leu Leu Thr
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His His Met Arg Gly Ile Asp Pro Glu Thr Gly Asn Leu Leu Gly Ala
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Leu Leu Ala Glu Asp Tyr His Val Phe Ala Val Asp Met Arg Gly Gln
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Gly Arg Ser Thr Trp Thr Pro Gly Arg Tyr Ser Leu Asp Asn Phe Gly
65 70 75 80

42

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Asn Asp Leu Val Arg Phe Ile Asp Leu Val Ile Gly Arg Thr Val Ile
      85                      90                      95

Val Ser Gly Asn Ser Ser Gly Gly Val Val Ala Ala Trp Leu Ala Ala
      100                      105                      110

Phe Ser Leu Pro Gly Gln Val Arg Ala Ala Leu Ala Glu Asp Ala Pro
      115                      120                      125

Phe Phe Ala Ser Glu Leu Asp Pro Lys Val Gly His Thr Ile Arg Gln
      130                      135                      140

Ala Ala Gly His Ile Phe Val Asn Trp Arg Asp Tyr Leu Gly Asp Gln
      145                      150                      155                      160

Trp Ser Val Gly Asp Tyr Ala Gly Phe Leu Lys Ala Met Lys Ser Ser
      165                      170                      175

Glu Val Pro Met Leu Arg Gln Val Pro Leu Pro Glu Thr Ala Pro Gln
      180                      185                      190

Asn Leu Leu Glu Tyr Asp Pro Glu Trp Ala Arg Ala Phe Tyr Glu Gly
      195                      200                      205

Thr Val Ala Gln Thr Cys Pro His Asp Tyr Met Leu Ser Gln Val Lys
      210                      215                      220

Val Pro Met Leu Val Thr His His Ala Arg Met Ile Asp Glu Ala Thr
      225                      230                      235                      240

Ser Gly Leu Val Gly Ala Met Ser Asp Leu Gln Val Gln Lys Ala Ala
      245                      250                      255

Glu Ile Ile Arg Gly Thr Gly Val Gln Val Asp Val Val Asp Leu Pro
      260                      265                      270

Glu Ala Pro His Ile Leu His Gln Leu Ala Pro Lys Glu Tyr Val Glu
      275                      280                      285

Ile Leu Asn Asn Trp Val Glu Lys Leu Pro Pro Val
      290                      295                      300

```

<210> 8

< 211> 307

< 212> PRT

< 213> Hirschia baltica

<400> 8

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Met  Ile  Gln  Asn  Asn  Lys  Thr  Ala  Pro  Tyr  Lys  Tyr  Lys  Glu  Lys  Leu
1      5      10      15

Val  Asp  Leu  Gly  Glu  Ile  Lys  Met  Asn  Tyr  Ile  Val  Ala  Gly  Ala  Asp
      20      25      30

Val  Ser  Pro  Ala  Leu  Leu  Leu  Ile  Pro  Gly  Gln  Thr  Glu  Ser  Trp  Trp
      35      40      45

Gly  Phe  Glu  Ala  Ala  Ile  Glu  Lys  Leu  Glu  Ser  Asn  Phe  Gln  Val  Phe
      50      55      60

Ala  Ile  Asp  Leu  Arg  Gly  Gln  Gly  Lys  Ser  Thr  Gln  Thr  Pro  Gly  Arg
65      70      75      80

Tyr  Ser  Leu  Asn  Leu  Met  Gly  Asn  Asp  Leu  Val  Arg  Phe  Ile  Ser  Leu
      85      90      95

Val  Ile  Lys  Arg  Pro  Val  Ile  Val  Ser  Gly  Asn  Ser  Ser  Gly  Gly  Leu
      100     105     110

Leu  Ala  Ala  Trp  Leu  Ser  Ala  Tyr  Ala  Met  Pro  Asn  Gln  Ile  Arg  Ala
      115     120     125

Ile  His  Cys  Glu  Asp  Ala  Pro  Phe  Phe  Thr  Ala  Glu  Lys  Ala  Pro  Leu
      130     135     140

Tyr  Gly  His  Ala  Ile  Gln  Gln  Ala  Ala  Gly  Pro  Ile  Phe  Ser  Leu  Met
145     150     155     160

Ser  Lys  Phe  Leu  Gly  Asp  Gln  Trp  Ser  Ile  Asn  Asn  Trp  Glu  Gly  Leu
      165     170     175

Lys  Ala  Ala  Gln  Ala  Lys  Asp  Thr  His  Pro  Ala  Asn  Lys  Met  Ile  Ser
      180     185     190

Gln  Val  Glu  Gln  Pro  Pro  Gln  His  Leu  Lys  Glu  Tyr  Asp  Pro  Glu  Trp
      195     200     205

Gly  Arg  Ala  Phe  Ile  Glu  Gly  Lys  Phe  Asn  Leu  Asn  Ser  Pro  His  His
210     215     220

Thr  Leu  Leu  Ser  Asp  Ile  Lys  Thr  Pro  Met  Leu  Tyr  Thr  His  His  Met
225     230     235     240

Arg  Phe  Glu  Asp  Pro  Gln  Thr  Gly  Leu  Leu  Ile  Gly  Ala  Thr  Ser  Asp

```

245 250 255

Phe Gln Ala Ser Lys Ile Lys Glu Ile Ala Leu Lys Thr Gly Asn Ser
260 265 270

Phe Glu Leu Ile Asp Ala Pro Asp Ala Phe His Ser Met His Glu Ala
275 280 285

Asp Pro Gln Arg Phe Val Asp Ile Leu Thr Ser Trp Ile Glu Arg Leu
290 295 300

Asn Leu Gln
305

<210> 9
< 211> 321
< 212> PRT
< 213> Nocardia brasiliensis

<400> 9
Met Gly Ile Ser Glu Ala Ala Asp Arg Ala Asp Thr Phe Val Ala His
1 5 10 15

Lys Phe Glu Glu Gln Leu Val Asp Leu Gly Glu Ile Arg Met Asn Tyr
20 25 30

Val Ala Ala Gly Asp Pro Thr Ser Pro Ala Leu Leu Leu Ile Pro Ala
35 40 45

Gln Gly Glu Ser Trp Trp Gly Tyr Glu Asn Ala Ile Thr Leu Leu Ala
50 55 60

Asn Asp Phe Arg Val Phe Ala Ile Asp Leu Arg Gly Gln Gly Arg Ser
65 70 75 80

Thr Trp Thr Pro Gly Arg Tyr Asn Leu Asn Thr Trp Gly Asn Asp Val
85 90 95

Glu Arg Phe Ile Asp Leu Val Ile Gly Arg Pro Thr Leu Val Ala Gly
100 105 110

Asn Ser Ser Gly Gly Val Ile Ala Ala Trp Leu Ala Ala Tyr Ala Lys
115 120 125

Pro Gly Gln Ile Arg Gly Ala Met Leu Glu Asp Pro Pro Leu Phe Ala
130 135 140

Ser Gln Ala Ala Pro Pro Tyr Gly Pro Gly Ile Met Gln Thr Leu Gly

5

45

145					150								155								160
Pro	Ile	Phe	Val	Leu	Trp	Ala	Lys	Trp	Leu	Gly	Pro	Gln	Trp	Ser	Val						
				165					170								175				
Gly	Asp	Trp	Asp	Gly	Met	Val	Ala	Ala	Ala	Pro	Arg	Glu	Leu	Pro	Glu						
				180					185								190				
Phe	Leu	His	Pro	Gly	Ile	Ala	Phe	Leu	Phe	Gly	Asp	Gly	Thr	Gly	Glu						
				195					200								205				
Gly	Ala	Ala	Ala	Thr	Pro	Pro	Gln	His	Leu	Lys	Glu	Tyr	Asp	Pro	Glu						
				210					215								220				
Trp	Ala	Gln	Ala	Trp	Ala	Thr	Asp	Val	Ala	Asn	Ala	Gly	Cys	Asp	His						
225					230								235								
Ala	Thr	Met	Leu	Ala	Gln	Asn	Arg	Val	Pro	Val	Leu	Leu	Thr	His	His						
				245					250								255				
Phe	His	Leu	Thr	Asp	Pro	Asp	Thr	Gly	Gln	Leu	Met	Gly	Ala	Met	Thr						
				260					265								270				
Asp	Ile	Gln	Ala	Gln	Gln	Ala	Arg	Arg	Leu	Leu	Ala	Ala	Thr	Gly	Gln						
				275					280								285				
Pro	Val	Thr	Phe	Thr	Ala	Leu	Asp	Ala	Pro	His	Thr	Met	His	Asp	Pro						
				290					295								300				
Glu	Pro	Glu	Arg	Tyr	Phe	Glu	Val	Leu	Thr	Glu	Trp	Ala	Ser	Ala	Leu						
305					310								315								

Asp

 $\langle 210 \rangle$ 10

< 211> 319

< 212> PRT

< 213> *Mycobacterium vaccae*

<400> 10

Met Gly Arg Tyr Ala Gly Val Phe Gly Pro His Ala Pro Glu Ser Thr
1 5 10 15

Tyr Val Gly His Ala Tyr Pro Glu Gln Leu Phe Asp Thr Gly Glu Val
20 25 30

Arg Leu Asn Tyr Ala Val Ala Gly Asp Ala Ser Ala Ser Pro Leu Leu

5

46

35					40					45					
Leu	Ile	Pro	Gly	Gln	Thr	Glu	Ser	Trp	Trp	Gly	Tyr	Glu	Pro	Ala	Met
50						55					60				
Gly	Leu	Leu	Ala	Glu	His	Phe	His	Val	His	Ala	Val	Asp	Leu	Arg	Gly
65					70					75					80
Gln	Gly	Arg	Ser	Thr	Arg	Thr	Pro	Arg	Arg	Tyr	Thr	Leu	Asp	Asn	Ile
				85					90					95	
Gly	Asn	Asp	Leu	Val	Arg	Phe	Leu	Asp	Gly	Val	Ile	Gly	Arg	Pro	Ala
			100					105					110		
Phe	Val	Ser	Gly	Leu	Ser	Ser	Gly	Gly	Leu	Leu	Ser	Ala	Trp	Leu	Ser
		115					120					125			
Ala	Phe	Ala	Glu	Pro	Gly	Gln	Val	Leu	Ala	Ala	Cys	Tyr	Glu	Asp	Pro
130						135					140				
Pro	Phe	Phe	Ser	Ser	Glu	Leu	Asp	Pro	Val	Ile	Gly	Pro	Gly	Leu	Met
145					150					155					160
Ser	Thr	Val	Gly	Pro	Leu	Phe	Ala	Leu	Tyr	Val	Lys	Tyr	Leu	Gly	Asp
				165					170					175	
Gln	Trp	Ser	Ile	Gly	Asp	Trp	Asp	Gly	Phe	Val	Ala	Gly	Ala	Pro	Gln
			180					185					190		
Glu	Leu	Ala	Gly	Trp	Gln	Ala	His	Val	Ala	Leu	Ala	Gly	Gly	Thr	Ala
		195					200					205			
Glu	Pro	Pro	Gln	His	Leu	Lys	Glu	Tyr	Asp	Pro	Glu	Trp	Gly	Arg	Ala
	210					215					220				
Phe	Val	Gly	Gly	Thr	Phe	Thr	Thr	Gly	Cys	Pro	His	Gln	Val	Met	Leu
225					230					235					240
Ser	Gln	Val	Lys	Val	Pro	Val	Leu	Phe	Thr	His	His	Phe	Arg	Met	Leu
				245					250					255	
Asp	Asp	Glu	Ser	Gly	Ser	Leu	Ile	Gly	Ala	Ala	Thr	Asp	Asp	Gln	Ala
			260					265					270		
Ala	Arg	Val	Val	Glu	Leu	Val	Glu	Asn	Ser	Gly	Ala	Pro	Leu	Thr	Tyr
		275					280					285			
Arg	Ser	Phe	Pro	Met	Met	Gly	His	Ser	Met	His	Ala	Gln	Asp	Pro	Ala
	290					295					300				
Leu	Phe	Ala	Gly	Thr	Leu	Val	Asp	Trp	Phe	Thr	Ala	Ala	Arg	Ser	
305					310					315					

<210> 11

< 211> 319

< 212> PRT

< 213> Mycobacterium gilvum

<400> 11

Met	Gly	Arg	Tyr	Ala	Gly	Val	Phe	Gly	Pro	His	Ala	Pro	Glu	Ala	Thr
1				5				10					15		

Tyr	Val	Glu	His	Gly	Tyr	Pro	Glu	Arg	Leu	Phe	Asp	Thr	Gly	Glu	Val
			20					25					30		

Gln	Leu	Asn	Tyr	Val	Val	Ala	Gly	Asp	Ala	Ala	Ala	Pro	Pro	Leu	Leu
		35					40					45			

Leu	Ile	Pro	Gly	Gln	Ser	Glu	Ser	Trp	Trp	Gly	Tyr	Glu	Ala	Ala	Ile
	50					55					60				

Pro	Leu	Leu	Ala	Arg	His	Phe	His	Val	His	Ala	Val	Asp	Leu	Arg	Gly
65					70					75					80

Gln	Gly	Arg	Ser	Thr	Arg	Thr	Pro	Gly	Arg	Tyr	Thr	Leu	Asp	Asn	Val
				85					90					95	

Gly	Asn	Asp	Leu	Val	Arg	Phe	Leu	Asp	Gly	Val	Ile	Gly	Arg	Pro	Ala
			100					105					110		

Phe	Val	Ser	Gly	Leu	Ser	Ser	Gly	Gly	Leu	Ala	Ser	Ala	Trp	Leu	Ser
		115					120					125			

Ala	Phe	Ala	Lys	Pro	Gly	Gln	Val	Val	Ala	Ala	Cys	Trp	Glu	Asp	Pro
	130					135					140				

Pro	Phe	Phe	Ser	Ser	Glu	Thr	Ala	Pro	Ile	Val	Gly	Pro	Pro	Ile	Thr
145					150					155					160

Asp	Ser	Ile	Gly	Pro	Leu	Phe	Gly	Met	Trp	Ala	Arg	Tyr	Leu	Gly	Asp
				165					170					175	

Gln	Trp	Ser	Val	Gly	Asp	Trp	Asp	Gly	Phe	Val	Ala	Ala	Val	Pro	Thr
			180					185					190		

Glu Leu Ala Asp Trp Gln Ala His Val Ala Leu Val Val Gly Thr Ala
195 200 205

Asp Pro Pro Gln Asn Leu Arg Glu Tyr Asp Pro Glu Trp Gly Lys Ala
210 215 220

Phe Ile Thr Gly Thr Phe Ala Ala Ser Cys Pro His His Val Met Leu
225 230 235 240

Ser Lys Val Lys Val Pro Val Leu Tyr Thr His His Phe Arg Met Ile
245 250 255

Asp Glu Gly Ser Gly Gly Leu Ile Gly Ala Cys Ser Asp Ile Gln Ala
260 265 270

Gly Arg Val Thr Gln Leu Ala Lys Ser Gly Gly Arg Ser Val Thr Tyr
275 280 285

Arg Ser Phe Pro Met Met Ala His Ser Met His Gly Gln Asp Pro Ala
290 295 300

Leu Phe Ser Glu Thr Leu Val Glu Trp Phe Ser Arg Phe Thr Gly
305 310 315

<210> 12

< 211> 322

< 212> PRT

< 213> *Gordonia effusa*

<400> 12

Met Pro Lys Ser Glu Ala Ala Asp Arg Ala Asp Ser Phe Val Ser His
1 5 10 15

Asp Phe Lys Glu Asn Ile Val Asp Leu Gly Glu Ile Arg Met Asn Tyr
20 25 30

Val Val Gln Gly Asn Lys Lys Ser Pro Ala Leu Leu Leu Ile Pro Ala
35 40 45

Gln Gly Glu Ser Trp Trp Gly Tyr Glu Ala Ala Ile Pro Leu Leu Ala
50 55 60

Lys His Phe Gln Val Phe Ala Ile Asp Leu Arg Gly Gln Gly Arg Thr
65 70 75 80

Thr Trp Thr Pro Gly Arg Tyr Thr Leu Asp Ile Phe Gly Asn Asp Val
85 90 95

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Val Arg Phe Ile Asp Leu Val Ile Gly Arg Glu Thr Leu Ile Ala Gly
      100                      105                      110

Asn Ser Ser Gly Gly Leu Ile Gly Ala Trp Leu Ala Ala Phe Ala Lys
      115                      120                      125

Pro Gly Gln Val Arg Ala Val Met Leu Glu Asp Pro Pro Leu Phe Ala
      130                      135                      140

Ser Glu Ile Arg Pro Pro Tyr Gly Pro Gly Ile Trp Gln Gly Leu Gly
      145                      150                      155                      160

Pro Met Phe Ala Ala Trp Ala Lys Trp Leu Gly Pro Gln Trp Ser Ile
      165                      170                      175

Gly Asp Trp Asp Gly Met Val Lys Ala Leu Pro Asp Glu Leu Pro Glu
      180                      185                      190

Asp Leu Leu Pro Gly Ile Gly Phe Met Leu Gly Asp Gly Glu Ser Asp
      195                      200                      205

Gly Ala Ala Pro Thr Pro Pro Gln His Leu Lys Glu Tyr Asp Pro Glu
      210                      215                      220

Trp Gly Ala Ser Trp Ala Ser Gly Phe Ala Asn Thr Gly Cys Glu His
      225                      230                      235                      240

Glu Ala Val Ile Ser Gln Val Arg Val Pro Val Leu Leu Thr His His
      245                      250                      255

Phe Arg Gln Ile Asn Glu Glu Thr Gly His Leu Met Gly Ala Leu Ser
      260                      265                      270

Asp Leu Gln Ala Ala Gln Val Arg His Ile Ile Glu Glu Val Ala Gly
      275                      280                      285

Gln Glu Val Thr Tyr Val Ser Leu Asp Ala Pro His Thr Met His Glu
      290                      295                      300

Pro Gln Pro Glu Arg Tyr Thr Asp Val Leu Leu Asp Trp Val Lys Lys
      305                      310                      315                      320

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Leu Gly

<210> 13

<211> 328

<212> PRT

<213> Togninia minima

<400> 13

50

Met	Asn	Tyr	Ala	Thr	Ala	Gly	Ser	Ser	Asp	Lys	Pro	Ala	Leu	Leu	Leu			
1				5					10				15					
Val	Pro	Gly	Gln	Ser	Glu	Ser	Trp	Trp	Gly	Tyr	Glu	Met	Ala	Met	Trp			
			20					25					30					
Leu	Leu	Lys	Asp	Asp	Tyr	Gln	Val	Phe	Ala	Val	Asp	Met	Arg	Gly	Gln			
		35					40					45						
Gly	Gln	Ser	Thr	Trp	Thr	Pro	Gly	Arg	Tyr	Ser	Leu	Asp	Thr	Phe	Gly			
	50					55					60							
Asn	Asp	Leu	Val	Lys	Phe	Ile	Asp	Ile	Val	Ile	Lys	Arg	Pro	Val	Val			
65					70					75					80			
Val	Ser	Gly	Leu	Ser	Ser	Gly	Gly	Val	Val	Ser	Ala	Trp	Leu	Ser	Ala			
				85					90					95				
Phe	Ala	Lys	Pro	Gly	Gln	Ile	Arg	Ala	Ala	Val	Tyr	Glu	Asp	Pro	Pro			
			100					105					110					
Leu	Phe	Ala	Ser	Gln	Ser	Lys	Pro	Ala	Ile	Gly	Gln	Ser	Val	Met	Gln			
		115					120					125						
Thr	Val	Ala	Gly	Pro	Phe	Phe	Asn	Leu	Trp	Tyr	Lys	Trp	Leu	Gly	Ala			
	130					135					140							
Gln	Trp	Thr	Ile	Gly	Asp	Gln	Ala	Gly	Met	Val	Ala	Ala	Met	Pro	Lys			
145					150					155					160			
Glu	Ile	Pro	Ala	Trp	Ile	Leu	Gln	Tyr	Leu	Gly	Asn	Thr	Thr	Ser	Gly			
				165					170					175				
Pro	Thr	Gly	Leu	Asp	Leu	Thr	Leu	Asn	Glu	Tyr	Asp	Pro	Glu	Trp	Gly			
			180					185					190					
His	Gly	Phe	Val	Ser	Gly	Thr	Val	Asp	Ala	Thr	Cys	Asp	His	Glu	Ala			
		195					200					205						
Met	Leu	Thr	His	Val	Lys	Val	Pro	Val	Leu	Phe	Thr	His	His	Ser	Arg			
	210					215					220							
Ala	Ile	Asp	Pro	Tyr	Thr	Gly	Asn	Leu	Ile	Gly	Ser	Val	Ser	Asp	Thr			
225					230					235					240			

51

Gln Val Ser Tyr Ala Gln Gly Leu Ile Thr Thr Asn Gly Asn Gln Ser
245 250 255

Phe Thr Leu Lys Asn Phe Pro Leu Ala Ser His Asp Met His Asn Ser
260 265 270

Asp Pro Ala Thr Tyr Val Ser Ala Ile Thr Thr Trp Met Ala Ser Leu
275 280 285

Gly Ile Gly Ser Ala Val Ile Pro Gly Pro Val Lys Val Ala Ser Ala
290 295 300

Ser Ala Gln Val Ser Ala Ala Ser Thr Ala Pro Pro Ser Cys Thr Ser
305 310 315 320

Thr Ser Ala Pro Ser Thr Gly His
325

<210> 14

< 211> 280

< 212> PRT

< 213> Actinosynnema mirum

<400> 14

Met Thr Val Val Asp Pro Pro Ala Pro Arg Asp Phe Pro Glu Leu Leu
1 5 10 15

Val Asp Leu Gly Glu Val Val Leu Asn His Ala Glu Ala Gly Ser Pro
20 25 30

Asp Arg Pro Ala Leu Val Pro Val Pro Glu Gln Gly Gly Ser Trp Trp
35 40 45

Ser Tyr Glu Arg Val Met Pro Leu Pro Ala Arg Asp Phe His Val Phe
50 55 60

Ala Val Asp Leu Arg Gly Arg Gly Arg Ser Thr Arg Thr Pro Arg Arg
65 70 75 80

Tyr Ser Leu Asp Asp Phe Gly Asn Asp Leu Val Arg Phe Leu Ala Leu
85 90 95

Val Val Arg Arg Pro Ala Val Val Ala Gly Asn Ser Ser Gly Gly Val
100 105 110

Leu Ala Ala Trp Ser Ser Ala Tyr Ala Met Pro Gly Gln Val Arg Ala
115 120 125

52

Val Leu Leu Glu Asp Pro Pro Leu Phe Ser Ser Glu Leu Thr Pro Val
 130 135 140

Cys Gly Pro Gly Val Arg Gln Ala Ala Gly Pro Leu Phe Glu Leu Leu
 145 150 155 160

Ser Thr His Leu Gly Asp Gln Trp Gly Gly Gly Arg Pro Gly Arg Val
 165 170 175

His Gly Gly Val Pro Arg Leu Gly Leu Ala Ala Ala Ala Val Arg
 180 185 190

Val Ala Arg Arg Ala Ala Ala Thr Asp Ala Arg Gly Arg Pro Gly Ala
 195 200 205

Ala Arg Gly Arg Pro Ala Gly Val Gly Gly Ala Ala Arg Arg Gly Arg
 210 215 220

Gly Gly Arg Glu Arg Thr Gly Thr Thr Thr Val Leu Ser Gly Leu Thr
 225 230 235 240

Gly Ser Arg Thr Ser Gly Thr Gly Arg Cys Arg Lys Pro Phe Arg Leu
 245 250 255

Arg Gln Trp Trp Ala Gly Gly Ala Arg Gly Pro Pro Pro Pro Arg Gln
 260 265 270

Ile Arg Ala Asp Val Arg Thr Arg
 275 280

<210> 15

< 211> 326

< 212> PRT

< 213> Kutzneria albida

<400> 15

Met Ser Val Pro Val Thr Pro Ser Ala Arg Asn Val Phe Val Pro His
 1 5 10 15

Ala Phe Pro Glu Lys Gln Ile Asp Leu Gly Glu Val Val Leu Asn Tyr
 20 25 30

Ala Glu Ala Gly Thr Pro Asp Lys Pro Ala Leu Leu Leu Leu Pro Glu
 35 40 45

Gln Thr Gly Ser Trp Trp Ser Tyr Glu Pro Ala Met Gly Leu Leu Ala
 50 55 60

53

Glu 65	His	Phe	His	Val	Phe 70	Ala	Val	Asp	Leu	Arg 75	Gly	Gln	Gly	Arg	Ser 80
Thr	Trp	Thr	Pro	Gly 85	Arg	Tyr	Ser	Leu	Asp 90	Asn	Phe	Gly	Asn	Asp 95	Leu
Val	Arg	Phe	Ile 100	Ala	Leu	Ala	Ile	Arg 105	Arg	Pro	Val	Val	Val 110	Ala	Gly
Cys	Ser	Ser 115	Gly	Gly	Val	Leu	Ala 120	Ala	Trp	Leu	Ser	Ala 125	Tyr	Ala	Leu
Pro	Gly 130	Gln	Ile	Arg	Gly	Ala 135	Leu	Cys	Glu	Asp	Ala 140	Pro	Leu	Phe	Ala
Ser 145	Glu	Leu	Thr	Pro	Ala 150	His	Gly	His	Gly	Val 155	Arg	Gln	Gly	Ala	Gly 160
Pro	Val	Phe	Glu	Leu 165	Tyr	Arg	Asp	Tyr	Leu 170	Gly	Asp	Gln	Trp	Ser 175	Val
Gly	Asp	Trp	Ala 180	Gly	Leu	Val	Ala	Ala 185	Ala	Gln	Ala	Ser	Pro 190	Ala	Lys
Met	Met	Ser 195	Leu	Phe	Lys	Met	Pro 200	Gly	Glu	Pro	Pro	Gln 205	Asn	Leu	Arg
Glu 210	Tyr	Asp	Pro	Glu	Trp	Ala 215	Arg	Val	Phe	Phe	Glu 220	Gly	Thr	Val	Gly
Leu 225	His	Cys	Pro	His	Asp 230	Arg	Met	Leu	Ser	Gln 235	Val	Lys	Thr	Pro	Val 240
Leu	Ile	Thr	His	His 245	Ala	Arg	Thr	Thr	Asp 250	Pro	Glu	Thr	Gly	Glu 255	Phe
Leu	Gly	Ala	Leu 260	Ser	Glu	Leu	Gln	Ala 265	Glu	Arg	Ala	Gln	Ala 270	Ile	Ile
Arg	Ala	Ala 275	Gly	Val	Pro	Val	Asp 280	Tyr	Gln	Ser	Phe	Pro 285	Asp	Ala	Ala
His 290	Ala	Met	His	Thr	Thr	Glu 295	Pro	Ala	Arg	Tyr	Ala 300	Ala	Val	Leu	Thr
Ala 305	Trp	Ala	Ala	Lys	Leu 310	Pro	Pro	Val	Ala	Asp 315	Thr	Ser	Pro	Ser	Ala 320
Ala	Ala	Ser	Ala	His	Val 325										

<210> 16

<211> 987

<212> DNA

<213> Rhodococcus erythropolis

<220>

< 221> misc_feature

< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 1

<400> 16

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atggccgaag aaggaactag gtccgaagca gcggatgctg ccacacaagc gagacagcta      60
cccgattcgc ggaacatctt tgtctcgcac cgatttccgg aaaggcaggt cgatctcggg      120
gaagtgggtga tgaacttcgc ggaggcgggc tctccggaca acccggcact gtcctctctc      180
cccgagcaga ccgggtcgtg gtggagttac gagccagtga tgggtcttct ggcagagaac      240
tttcatgtct ttgccgtcga tatccgtggg caaggctcga gtacctggac gccacggcga      300
tacagcctgg acaacttcgg caatgatctg gtgcgtttca tcgctctggg catcaagcgc      360
cctgtcgtcg tggcagggaa ctctcggggg gggctgctgg ccgcctgggt ctccggcgtac      420
gcgatgcccc gccagatccg tgcagcattg tgtgaggacg caccgttctt tgcgtcggag      480
ttggtccccg catacgggtca ctcggttctg caggcggcgg gtccggcatt cgagttgtac      540
cgggacttcc tcggggacca gtgggtcgatt ggggactgga aagggttcgt tgaggcagcc      600
aaagcgtcgc cggcaaaggc tatgcaatta tttccgacct cggatgaggg gccgcagaat      660
ctcaaggaat acgacctcga atggggggcg gcattcttcg aagggactgt ggcactgcac      720
tgcccacacg acaggatgct ctgcgaagtc aagacaccaa ttctcatcac tcaccacgcg      780
cggacgatcg accccgagac gggcgagctg ttgggcgcgc tctccgacct tcaggcagag      840
catgcgcagg acatcattcg gtctgcgggc gttcgggtgg actatcagtc gcaccccgac      900
gcgcttcaca tgatgcatct gttcgatccc gctcgttacg cggagatctt gacatcctgg      960
tccgcaacac tgcctgcgaa cgactag                                     987

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<210> 17

<211> 987

<212> DNA

<213> Artificial sequence

<220>

<223> ORF was codon optimized and thus difference from natural occuring DNA sequence.

<400> 17

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atggcagaag aaggcaccgg tagcgaagca gcagatgcag caaccaggg acgtcagctg      60

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ccg gatagcc gtaacatttt tgtagccat cgttttccgg aacgtcaggt tgatctgggt 120
gaagttgtta tgaattttgc agaagcaggt agtccggata atccggcatt actgctgctg 180
ccggaacaga ccggtagttg gtggtcttat gaaccggtta tgggtctgct ggcagaaaac 240
tttcatgttt ttgcagttga tttcgtgggt cagggtcgta gcacctggac accgcgtcgt 300
tatagcctgg ataattttgg taatgatctg gtgcgtttta ttgccctgggt tattaaacgt 360
ccggttggtg ttgcaggtaa tagcagcgggt ggcctgctgg ctgcatgggt gagcgcctat 420
gcaatgcctg gtcagattcg tgcagcactg tgtgaagatg caccgttttt tgcaagcgaa 480
ctggttcctg cctatggcca tagcgttctg caggcagcag gtccggcatt tgaactgtat 540
cgtgattttc tgggtgatca gtggtcaatt ggtgattgga aaggttttgt tgaagcagca 600
aaagcaagtc cggctaaagc aatgcagctg tttccgacac cggatgaagc accgcagaat 660
ctgaaagaat atgatccgga atggggctgt gcattttttg aaggcaccgt tgcactgcat 720
tgtccgcatg atcgtatgct gagccagggt aaaaccccga ttctgattac ccatcatgca 780
cgtaccatcg atccggaaac cggatgaactg ctgggtgcac tgagtgatct gcaggccgaa 840
catgcacagg atattattcg tagtgccgggt gttcgtgttg attatcagag ccatcctgat 900
gcactgcaca tgatgcacct gtttgatccg gcacgttatg cagaaattct gaccagttgg 960
agcgcaaccc tgccctgcaa tgattaa 987

5

<210> 18
< 211> 927
< 212> DNA
< 213> Artificial sequence

10

<220>
< 223> ORF was codon optimized and thus differce from natural occuring DNA sequence.

<220>
< 221> misc_feature
< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 2

<400> 18
atggcagatc cggcacagcg tgatgtttat gttccgcatg catatccgga aaaacaggca 60
gatctgggtg aaattaccat gaattatgcc gaagccgggtg aacctgatat gcctgcagtt 120
ctgctgattc cggaacagac cggtagttgg tggggttatg aagaagcaat gggctctgctg 180
gcagaaaact ttcatgttta tgcagttgat ctgcgtggtc agggtcgtag cagctgggca 240
ccgaaacggt atagcctgga taattttgggt aatgatctgg tgcgttttat tgccctgggtt 300
gttaaacgtc cggttattgt tgcaggtaat agcagcgggtg gtgttctggc agcatggctg 360
agcgcatata gcatgcctgg tcaggttcgt ggtgcactgt gtgaagatgc accgtttttt 420

```

gcaagcgaac tggttaccac ctgtggtcat agcattcgtc aggcagcagg tccgatgttt      480
gaactgtttc gtacctatct gggcgatcag tggtcagttg gtgattggac cggctattgt      540
cgtgcagcag atgcaagcag cagcccgatg gcacgttatt ttgttgcaga tgaaattccg      600
cagcacatgc gtgaatatga tccggaatgg gcacgtgcat tttgggaagg caccgttgca      660
ctgcattgtc cgcataaaca gctgctgacc cagggttaaaa caccggtgct gctgacacat      720
cacatgcgcg atattgatcc tgataccggt catctggttg gtgccctgag tgatgaacag      780
gcagcccggtg cacgtctgct gatggaaagt gccggtgtta aagttgatta tgcaagcgtt      840
ccggatgcac tgcacatgat gcaccagttt gatccgcctc gttatgttga aatctttacc      900
cagtgggcag caaccctggc agcataa                                          927

```

<210> 19

< 211> 930

5 < 212> DNA

< 213> Artificial sequence

<220>

< 223> ORF was codon optimized and thus differ from natural occurring DNA sequence.

10

<220>

< 221> misc_feature

< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 3

<400> 19

```

atggttacca gtccggcact gcgtgatgtt catgttccgc atgcatatcc ggaacagcag      60
gttgatctgg gtgaaattac catgaattat gccgaagccg gtgatccggg tcgtccggca      120
gttctgctga tcccgggaaca gaccggtagt tgggtggtctt atgaagaagc aatgggtctg      180
ctggcagaac attttcatgt ttatgcagtt gatctgcgtg gtcagggtcg tagcagctgg      240
accccgaaac gttatagcct ggataatttt ggtaatgatc tgggtgcgttt tattgccctg      300
gttggttcgtc gtccgggttggt tgttgcaggt aatagcagcg gtggtgttct ggcagcatgg      360
ctgagcgcac atagcatgcc tggtcagatt cgtggtgtgc tgtgtgaaga tccgcctttt      420
tttgcaagcg aactggttcc ggcacatggt catagcggtc gtcaggggtgc aggtccgggt      480
tttgaactgt ttcgtaacct tctgggcgat cagtggtcag ttggtgattg ggaagggttt      540
cgtagcgcag cagatgcaag cgcaagcccg atggcacgta gctttgttgc agataccatt      600
ccgcagcatc tgaaagaata tgatccggaa tgggcacgtg cattttatga aggcaccggt      660
ggtctgaatt gtccgcatga acgtatgctg aatcgtgtta atacaccggt gctgctgacc      720
catcacatgc gtggcaccga tccggaaacc ggtaatctgc tgggtgcact gagtgatgaa      780
caggcagcac aggtgcgtcg tctgatggaa agtgccggtg ttaaagttga ttatgaaagc      840
gttccggatg caagccacat gatgcaccag agcgatccgg cacgttatgc agaaattctg      900

```

15

accccggtga ccgcagcact ggcaccgtaa

930

<210> 20
 < 211> 930
 < 212> DNA
 < 213> Artificial sequence

5 <220>
 < 223> ORF was codon optimized and thus differce from natural
 occuring DNA sequence.

10 <220>
 < 221> misc_feature
 < 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 4

<400> 20
 atggttacca gtccggcact gcgtgatggt catgttccgc atgcatatcc ggaacagcag 60
 gttgatctgg gtgaaattac catgaattat gccgaagccg gtgatcctga tcgtccggca 120
 gttctgctga tcccgggaaca gaccggtagt tgggtggcat atgaagaagc aatgggtctg 180
 ctggcagaac attttcatgt ttatgcagtt gatctgcgtg gtcagggctcg tagcagctgg 240
 accccgaaac gttatagcct ggataatttt ggtaatgatc tgggtgcgttt tattgccctg 300
 gttgttaaac gtccggttgt tgttgcaggt aatagcagcg gtggtgttct ggcagcatgg 360
 ctgagcgcac atagcatgcc tggtcagctg cgtggtgtgc tgtgtgaaga tccgcctttt 420
 tttgcaagcg aactggttcc ggcacatggt catagcggtc gtcaggggtgc aggtccgggt 480
 tttgaactgt ttcgtaccta tctgggcgat cagtggtcag ttagcgattg ggaagggttt 540
 tgtcgtgcag ccggtgcaag cgcaagcccg atggcacgta gctttgttgc agatgggtatt 600
 ccgcagcatc tgaaagaata tgatccggaa tgggcacgtg catttcatga aggcaccgtt 660
 ggtctgaatt gtccgcatga acgtatgctg ggtcgtgtta atacaccggt gctgctgacc 720
 catcatatgc gtggcaccga tccggaaacc ggtaatctgc tgggtgcact gagtgatgaa 780
 caggcagcac aggcacgtct gctgatggaa agtgccggtg ttcgtgttga ttatgaaagc 840
 gttccggatg caagccatat gatgcaccag agcgatccgg cacgttatgc agaaatcttt 900
 acccggtggg cagcagccct ggcaccgtaa 930

15 <210> 21
 < 211> 930
 < 212> DNA
 < 213> Artificial sequence

<220>
 < 223> ORF was codon optimized and thus differce from natural
 occuring DNA sequence.

20 <220>
 < 221> misc_feature
 < 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 5

<400> 21

atggttacca	gtccggcact	gcgtgatggt	catgttccgc	atgcatatcc	ggaacagcag	60
gttgatctgg	gtgaaattac	catgaattat	gccgaagccg	gtgatccggg	tcgtccggca	120
gttctgctga	tcccgggaaca	gaccggtagt	tgggtggtcct	atgaagaagc	aatgggtctg	180
ctggcagaac	attttcatgt	ttatgcagtt	gatctgcgtg	gtcagggtcg	tagcagctgg	240
accccgaaac	gttatagcct	ggataatttt	ggtaatgata	tgggtgcgttt	tatggcactg	300
gttggttcgtc	gtccgggttgt	tgttgcaggt	aatagcagcg	gtggtgttct	ggcagcatgg	360
ctgagcgcac	atagcatgcc	tggtcagatt	cgtggtgtgc	tgtgtgaaga	tccgcctttt	420
tttgcaagcg	aactggttcc	ggcacatggg	catagcggtc	gtcaggggtgc	aggtccgggt	480
tttgaactgt	ttcgtaccta	tctgggcgat	cagtggtcag	ttggtgattg	ggaagggttt	540
cgtagcgcag	ccggtgcaag	cgcaagcccg	atggcacgta	gctttgttgc	agataccatt	600
ccgcagcatc	tgaaagaata	tgatccggaa	tgggcacgtg	cattttatga	aggcaccgtt	660
ggtctgaatt	gtccgcatga	acgtatgctg	aatcgtgtta	atacaccggg	gctgctgacc	720
catcacatgc	gtggcaccga	tccggaaacc	ggtaatctgc	tgggtgcact	gagtgatgaa	780
caggcagcac	aggcacgtcg	tctgatggaa	agtgccgggt	ttaaagttga	ttatgaaagc	840
gttccggatg	caagccacat	gatgcaccag	agcgatccgg	caogttatgc	agaaattctg	900
accccggtggg	cagcagccct	ggcaccgtaa				930

<210> 22
< 211> 930
< 212> DNA
< 213> Artificial sequence

<220>
< 223> ORF was codon optimized and thus difference from natural
occurring DNA sequence.

<220>
< 221> misc_feature
< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO : 6

atggttacca	gtccggcact	gcgtgatggt	catgttccgc	atgcatatcc	ggaacagcag	60
gttgatctgg	gtgaaattac	catgaattat	gccgaagccg	gtgatccctga	tcgtccggca	120
gttctgctga	tcccgggaaca	gaccggtagt	tgggtggtcct	atgaagaagc	aatgggtctg	180
ctgagcgaac	attttcatgt	ttatgcagtt	gatctgcgtg	gtcagggtcg	tagcagctgg	240
accccgaaac	gttatagcct	ggataatttt	ggtaatgata	tgggtgcgttt	tattgccctg	300
gttggttaa	gtccgggttgt	tgttgcaggt	aatagcagcg	gtggtgttct	ggcagcatgg	360

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ctgagcgcac atagcatgcc tggtcagctg cgtggtgtgc tgtgtgaaga tccgcctttt 420
tttgcaagcg aactggttcc ggcacatggc catagcgttc gtcaggggtgc aggtccgggt 480
tttgaactgt ttcgtacctt tctgggcatg cagtggtcag ttgggtgattg ggaagggttt 540
tgtcgtgcag ccggtgcaag cgcaagcccg atggcacgta gctttgttgc agatgggtatt 600
ccgcagcatc tgcaagaata tgatccggaa tgggcacgtg ttttttatga aggcaccgtt 660
gggtctgagct gtccgcatga acgtatgctg ggtcagggtta aaacaccggg gctgctgacc 720
catcacatgc gtgggtatga tccggaaacc ggtaatctgc tgggtgcact gagtgatgaa 780
caggccctgc gtgcacgtcg tctgatggat agtgccgggtg ttaccgttga ttatgaaagc 840
gttccggatg caagccacat gatgcaccag agcgcaccgg cacgttatgt tgaaatcttt 900
accggttggg cagcagccct ggcaccgtaa 930

```

<210> 23

< 211> 903

5 < 212> DNA

< 213> Artificial sequence

<220>

< 223> ORF was codon optimized and thus differ from natural occurring DNA sequence.

10

<220>

< 221> misc_feature

< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO : 7

<400> 23

```

atgccgcacg attatgaaga aaaactgggt gatctgggag aaatcgatct gaattatgca 60
gaagcaggta gtccggataa accggcactg ctgctgattc cgagccagag cgaaagttgg 120
tggggctatg aagaagcaat gggctctgct gccgaagatt atcatgtttt tgcagttgat 180
atgcgtggtc agggtcgtag cacctggaca ccgggtcggt atagcctgga taatttttgt 240
aatgatctgg tgcgctttat cgatctgggt attggctgta ccgttattgt tagcggtaat 300
agcagcgggt gtgttggtgc agcatggctg gcagcattta gcctgcctgg tcaggttcgt 360
gcagcactgg cagaagatgc accgtttttt gcaagcgaac tggaccgaa agtgggtcat 420
accattcgtc aggcagcagg tcatattttt gttaactggc gtgattatct gggatgatcag 480
tggtcagttg gtgattatgc aggtttttct aaagcaatga aaagcagcga agttccgatg 540
ctgcgtcagg ttccgctgcc ggaaaccgca ccgcagaatc tgctggaata tgatccggaa 600
tgggcacgtg cattttatga aggcaccgtt gcacagacct gtccgcatga ttatatgctg 660
agccagggtta aagtgcctat gctggttacc catcatgcac gtatgattga tgaagcaacc 720
agcgggtctg ttgggtgcaat gagcgatctg caggttcaga aagcagcaga aattattcgt 780
ggcaccgggtg ttcaggttga tgttggtgat ctgccggaag caccgcatat tctgcatcag 840
ctggcaccga aagaatatgt ggaaattctg aataactggg tggaaaaact gcctccgggt 900
taa 903

```

15

<210> 24
 < 211> 924
 < 212> DNA
 < 213> Artificial sequence

5 <220>
 < 223> ORF was codon optimized and thus differce from natural
 occuring DNA sequence.

10 <220>
 < 221> misc_feature
 < 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 8

<400> 24
 atgatccaga acaataaaac cgcaccgtat aaatacaaag aaaaactggt tgatctgggc 60
 gaaatcaaaa tgaactatat tggtgccggt gcagatgtta gtccggcact gctgctgatt 120
 ccgggtcaga ccgaaagttg gtgggggtttt gaagcagcaa ttgagaaact ggaaagcaac 180
 tttcaggtgt ttgcaattga tctgcgtggt cagggtaaaa gcacccagac accgggtcgt 240
 tatagcctga atctgatggg taatgatctg gttcgtttta ttagcctggt tattaacgt 300
 ccggttattg ttagcggtaa tagcagcggg ggtctgctgg cagcatggct gagcgcctat 360
 gcaatgccga atcagattcg tgcaattcat tgtgaagatg caccgttttt taccgcagaa 420
 aaagcaccgc tgtatggtca tgcaattcag caggcagcag gtccgatttt tagcctgatg 480
 agcaaatttc tgggtgatca gtggtcaatt aacaattggg aaggtctgaa agcagcacag 540
 gcaaaagata cccatccggc aaacaaaatg attagccagg ttgaacagcc tccgcagcat 600
 ctgaaagaat atgatccgga atgggggtcgt gcatttattg aaggcaaatt taacctgaac 660
 agtccgcata ataccctgct gagcgcacatt aaaaccccga tgctgtatac ccatcacatg 720
 cgttttgaag atccgcagac aggtctgctg attggtgcaa ccagcgattt tcaggcaagc 780
 aaaatcaaag aaattgccct gaaaaccggc aatagcttcg aactgattga tgcaccggat 840
 gcatttcata gtatgcatga agccgatccg cagcgttttg ttgatattct gaccagctgg 900
 attgaacgtc tgaatctgca gtaa 924

15 <210> 25
 < 211> 966
 < 212> DNA
 < 213> Artificial sequence

<220>
 < 223> ORF was codon optimized and thus differce from natural
 occuring DNA sequence.

20 <220>
 < 221> misc_feature
 < 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 9

<400> 25

atgggtatta gcgaagcagc agatcgtgca gatacctttg ttgcacataa atttgaagaa 60
cagctgggtg atctgggtga aattcgtatg aattatggtg cagccggtga tccgaccagt 120
ccggcactgc tgctgattcc ggcacagggg gaaagttggg ggggttatga aaatgcaatt 180
accctgctgg caaatgattt tcgtgttttt gcaattgatc tgcgtgggtca gggtcgtagc 240
acctggacac cgggtcgtta taatctgaat acctggggta atgatgtgga acgctttatt 300
gatctggtta ttggtcgtcc gaccctgggt gcaggtaata gcagcgggtg tgttattgca 360
gcatggctgg cagcctatgc aaaaccgggt cagattcgtg gtgcaatgct ggaagatccg 420
cctctgtttg caagccaggg agcaccgcct tatgggtccg gtattatgca gaccctgggt 480
ccgatttttg ttctgtgggc aaaatggctg ggtccgcagt ggtcagttgg tgattgggat 540
ggtatgggtg cagcggcacc gcgtgaactg ccggaatttc tgcacccggg tatcgcattt 600
ctgtttgggt atggcaccgg tgaagggtgca gcagcaaccc ctccgcagca tctgaaagaa 660
tatgatccgg aatgggcaca ggcattgggc accgatgttg caaatgcagg ttgtgatcat 720
gcaaccatgc tggcacagaa tcgtgttccg gttctgctga cccatcattt tcatctgacc 780
gatccggata caggccagct gatgggtgca atgaccgata ttcaggcaca gcaggcacgt 840
cgtctgctgg cagcaaccgg tcagccgggt acctttaccg cactggatgc accgcatacc 900
atgcatgatc ctgaacctga acgttatatt gaagttctga ccgaatgggc aagtgcactg 960
gattaa 966

5 <210> 26
< 211> 960
< 212> DNA
< 213> Artificial sequence

<220>
< 223> ORF was codon optimized and thus differce from natural occuring DNA sequence.

10 <220>
< 221> misc_feature
< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 10

<400> 26
atgggtcgtt atgccggtgt ttttgggtccg catgcaccgg aaagcaccta tgttgggtcat 60
gcatatccgg aacaactggt tgataccggg gaagttcgtc tgaattatgc agttgccggg 120
gatgcaagcg caagtccgct gctgctgatt ccgggtcaga ccgaaagttg gtgggggttat 180

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gaaccggcaa tgggtctgct ggcagaacat tttcatgttc atgcagttga tctgcgtggt      240
cagggtcgta gcacccgtac accgcgtcgt tataccctgg ataatttgg taatgatctg      300
gtgcgttttc tggatggtgt tattggtcgt ccggcatttg ttagcggctct gagcagcggg      360
ggtctgctga gcgcatggct gagcgcttt gcagaaccgg gtcaggttct ggcagcatgt      420
tatgaagatc cgcctttttt tagcagcgaa ctggaccggg tgattgggcc gggctctgatg      480
agcaccgttg gtccgctgtt tgcactgtat gttaaataatc tgggtgatca gtggtcaatt      540
ggtgattggg atggttttgt tgcaggcgca ccgcaagaac tggcagggtg gcaggcacat      600
gttgactggg caggcggtac agcagaaccg cctcagcatc tgaaagaata tgatccggaa      660
tggggtcgtg catttggttg tggcaccttt accaccggtt gtccgcatca gggtatgctg      720
agccaggtta aagttccggg tctgtttacc catcattttc gtatgctgga tgatgaaagc      780
ggtagcctga ttggtgcagc aaccgatgat caggcagcac gtgttggtga actgggtgaa      840
aatagtggg caccgctgac ctatcgtagc tttccgatga tgggtcatag tatgcatgca      900
caagatccgg cactgtttgc aggcaccctg gttgattggt ttaccgcagc acgtagctaa      960

```

<210> 27

< 211> 960

< 212> DNA

< 213> Artificial sequence

<220>

< 223> ORF was codon optimized and thus differ from natural occurring DNA sequence.

<220>

< 221> misc_feature

< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 11

<400> 27

```

atgggtcgtt atgccggtgt ttttgggtccg catgcaccgg aagcaaccta tggtgaacat      60
gggttatccgg aacgtctgtt tgataccggg gaagtgcagc tgaattatgt tggtgccggg      120
gatgcagcag caccgcctct gctgctgatt ccgggtcaga gcgaaagttg gtgggggttat      180
gaagcagcaa ttccgctgct ggcacgtcat tttcatgttc atgcagttga tctgcgtggt      240
cagggtcgta gcacccgtac accgggtcgc tataccctgg ataatttgg taatgatctg      300
gtgcgttttc tggatggtgt tattggtcgt ccggcatttg ttagcggctct gagcagcggg      360
ggtctggcaa gcgcatggct gagcgcatth gcaaaaccgg gtcaggttgt tgcagcatgt      420
tgggaagatc cgcctttttt tagcagcgaa accgcaccga ttgttggtcc gcctattacc      480
gatagcattg gtccgctgtt tggatgtgg gcacgttatc tgggtgatca gtggtcagtt      540
ggtgattggg atggttttgt tgccgcagtt ccgaccgaac tggcagattg gcaggcacat      600
gttgactggg ttgttggcac cgcagatcct ccgcagaatc tgcgatgaata tgatccggaa      660

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```

tggggtaaag catttattac cggcaccttt gcagcaagct gtccgcatca tgttatgctg      720
agcaaagtta aagttccggt tctgtatacc catcactttc gcatgattga tgaaggtagt      780
ggtggtctga ttggtgcatg tagcgatatt caggcaggtc gtgttaccca gctggcaaaa      840
tcagggtggtc gtagcgttac ctatcgtagc tttccgatga tggcacatag catgcatggt      900
caagatccgg cactgttttag cgaaaccctg gttgaatggt ttagccgttt taccggttaa      960

```

<210> 28

< 211> 969

< 212> DNA

< 213> Artificial sequence

<220>

< 223> ORF was codon optimized and thus differce from natural occuring DNA sequence.

<220>

< 221> misc_feature

< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 12

<400> 28

```

atgccgaaaa gcgaagcagc agatcgtgca gatagctttg ttagccatga tttcaaagaa      60
aacattgtgg atctgggcga aatccgcatg aattatgttg ttcagggcaa caaaaaaagt      120
ccggcactgc tgctgattcc ggcacagggt gaaagttggt ggggttatga agcagcaatt      180
ccgctgctgg caaaacattt tcaggttttt gcaattgata tgcgtggtca gggtcgtacc      240
acctggacac cgggtcgtta taccctggat atttttggta atgatgtggt gcgctttatc      300
gatctggtta ttggtcgtga aaccctgatt gcaggtaata gcagcgggtgg tctgattggt      360
gcatggctgg cagcatttgc aaaaccgggt caggttcgtg cagttatgct ggaagatccg      420
cctctgtttg caagcgaaat tcgtccgcct tatgggtccg gtatttggca gggctctgggt      480
ccgatgtttg cagcatgggc aaaatggctg ggtccgcagt ggtcaattgg tgattgggat      540
ggtatggtta aagcactgcc ggatgaactg ccggaagatc tgctgcctgg tattggtttt      600
atgctgggtg atggtgaaag tgatggtgca gcaccgaccc ctccgcagca tctgaaagaa      660
tatgatccgg aatggggtgc aagctgggca agcggttttg ccaataccgg ttgtgaacat      720
gaagcagtta ttagccaggt gcgtgttccg gttctgctga cccatcattt tcgtcagatt      780
aatgaagaaa ccggtcatct gatgggtgca ctgagcgatc tgcaggcagc acaggttcgt      840
catatcattg aagaagttgc aggtcaagag gttacctatg ttagcctgga tgcaccgcat      900
accatgcatg aaccgcagcc ggaacgttat accgatgttc tgctggattg ggtaaaaaaa      960
ctgggttaa                                     969

```

<210> 29

< 211> 987

< 212> DNA

< 213> Artificial sequence

<220>
< 223> ORF was codon optimized and thus differce from natural occuring DNA sequence.

<220>
< 221> misc_feature
< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 13

<400> 29
atgaattatg caaccgcagg tagcagcgat aaaccggcac tgctgctggt tccgggtcag 60
agcgaaagtt ggtggggtta tgaaatggca atgtggctgc tgaaagatga ttatcaggtt 120
tttgagtttg atatgcgtgg tcaggggtcag agtacctgga caccgggtcg ttatagcctg 180
gatacctttg gtaatgatct ggtgaaattc atcgatatcg tgattaaacg tccggttggt 240
gttagcggtc tgagcagcgg tgggtgtgtg agcgcattgc tgagcgcatt tgcaaacct 300
ggtcagattc gtgcagcagt ttatgaagat ccgcctctgt ttgcaagcca gagcaaaccg 360
gcaattggtc agagtgttat gcagaccgtt gcagggtcgt tttttaacct gtggtataaa 420
tggctgggtg cacagtggac cattggtgat caggcaggta tggttgcagc aatgccgaaa 480
gaaattccgg catggattct gcagtatctg ggtaatacca ccagtgggtcc gaccgggtctg 540
gatctgacac tgaatgaata tgatccggaa tggggtcatt gttttgttag tggcaccgtt 600
gatgcaacct gtgatcatga agcaatgctg acccatgtta aagttccggt tctgtttacc 660
catcatagcc gtgcaattga tccgtatacc ggtaatctga ttggtagcgt tagcgatacc 720
caggtttagct atgcacaggg tctgattacc accaatggca atcagagctt taccctgaaa 780
aactttccgc tggcaagcca tgatatgcat aattctgata cggcaacctg tgtagcgcga 840
attaccacct ggatggcaag cctgggtatt ggtagtgcag ttattccggg tccgggttaa 900
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<220>
< 223> ORF was codon optimized and thus differce from natural occuring DNA sequence.

<220>
< 221> misc_feature
< 223> Artificial DNA sequence encodes polypeptid with SEQ ID NO: 14

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ccggaacagg gtggtagttg gtgggtcttat gaacgtgtta tgccgctgcc tgcacgcgat 180
tttcatgttt ttgcagttga tctgcgtggg cgtgggtcgt gcacccgtac accgcgtcgt 240
tatagcctgg atgattttgg taatgatctg gttcgttttc tggccctggg tggtcgccgt 300
ccggcagttg ttgcaggtaa tagcagcggg ggtgttctgg cagcatgggc aagcgcctat 360
gcaatgcctg gtcaggttcg tgcagttctg ctggaagatc cgcctctgtt tagcagcgaa 420
ctgacaccgg tttgtgggcc ggggtgttct caggcagcag gtccgctgtt tgaactgctg 480
agcaccatc tgggcatca gtgggggtgg ggtcgtccgg gtcgtgttca tgggtggcgtt 540
ccgctcttgg gtctggcagc cgcagcagca gttcgtgttg cacgtcgtgc agcagcaacc 600
gatgcacgtg gtcgccctgg tgcagcacgt ggacgtcctg ccggtgttgg tgggtgcagct 660
cgtcgcggtc gcggtggtcg tgaacgcacc ggtacaacca ccgttctgag cggctctgacc 720
ggtagccgta ccagcggcac cggtcgttgt cgtaaaccgt ttcgtctgcg tcagtgggtg 780
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taa 843

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ccggcattac tgctgctgcc ggaacagacc ggtagttggg ggtcttatga accggcaatg 180
ggctctgctgg cagaacattt tcatgttttt gcagttgatc tgcgtgggtca gggtcgtagc 240
acctggacac cgggtcgtta tagcctggat aattttggta atgatctggg gcgttttatt 300
gcactggcaa ttcgtcgtcc ggttggtgtt gcaggttgta gcagcgggtg tggtctggca 360
gcatggctga gcgcctatgc actgcctggg cagattcgtg gtgcactgtg tgaagatgca 420
ccgctgtttg caagcgaact gacaccggca catggtcatg gtgttcgtca ggggtgcaggt 480
ccggtttttg aactgtatcg tgattatctg ggcgatcagt ggtcagttgg tgattgggca 540
ggctctgggtg cagcagcaca ggcaagtccg gcaaaaatga tgagcctgtt taaaatgcct 600

	ggtgaaccgc ctcagaatct gcgtgaatat gatccggaat gggcacgtgt tttttttgaa	660
	ggcaccgttg gtctgcattg tccgcatgat cgtatgctga gccagggttaa aacaccggtt	720
	ctgattaccc atcatgcacg taccaccgat ccggaaaccg gtgaatttct gggtgactg	780
	agcgaactgc aggcagaacg tgcacaggcc attattcgtg cagccggtgt tccggttgat	840
	tatcagagct ttccggatgc agcacatgca atgcatacca cagaaccggc acgttatgca	900
	gcagttctga ccgcatgggc agcaaaactg cctccggttg cagataccag cccgtcagca	960
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75

	Val Arg Arg Pro Val Val Val Ala Gly Asn Ser Ser Gly Gly Val Leu 1 5 10 15
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	Ala Ala Trp Leu Ser 20
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Leu Val Gly Ala Leu
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Leu Leu Gly Ala Leu
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Leu Leu Gly Ala Leu
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Asp Pro Ala Arg Tyr
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Val Asp Tyr Ala Ser Val Pro Asp Ala Leu His Met Met His Gln Phe
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Asp Pro Pro Arg Tyr
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<400> 69
Val Asp Tyr Glu Ser Val Pro Asp Ala Ser His Met Met His Gln Ser
1 5 10 15

Ala Pro Ala Arg Tyr
20

30

PATENTKRAV

1. Fremgangsmåde till hydrolytisk spaltning af zearalenon og/eller i det mindste et zearalenonderivat ved hjælp af et hydrolytisk kløvningspolypeptid, **kendetegnet ved, at**
 - 5 zearalenonen og/eller i det mindste et zearalenonderivat hydrolyseres af polypeptidet, som er en hydrolase med en aminosyresekvens valgt fra gruppen af sekvenserne ID No. 7, 8, 14 og 15, især sekvensen ID No. 14 eller en funktionel variant deraf, hvor en sekvensidentitet på i det mindste 70% foreligger imellem den funktionelle variant og aminosyresekvensen.
- 10 2. Fremgangsmåde ifølge krav 1, **kendetegnet ved, at** polypeptidet har i det mindste et bevaret aminosyresekvenssegment eller en funktionel variant deraf, hvor den funktionelle variant af aminosyresekvenssegmentet har en sekvensidentitet på i det mindste 70%, fortrinsvis i det mindste 84%, mere foretrukket i det mindste 92% og mest foretrukket i det mindste 98% og i det mindste ét bevaret aminosyresekvenssegment er
 - 15 valgt fra gruppen af aminosyresekvenser +24 til +50, +52 til +77, +79 til +87, +89 til +145, +150 til +171, +177 til +193, +223 til +228, +230 til +237, +239 til +247, +249 til +255, +257 til +261, +263 til +270, +272 til +279, +297 til +301 af sekvensen, som har sekvens ID No. 1.
- 20 3. Fremgangsmåde ifølge krav 1 eller 2, **kendetegnet ved, at** polypeptidet har i det mindste én mutation af aminosyresekvensen i forhold til sekvens ID No. 1 i i det mindste én af følgende positioner valgt fra gruppen: 22, 23, 25, 26, 27, 29, 31, 32, 35, 37, 42, 43, 46, 51, 53, 54, 57, 60, 69, 72, 73, 78, 80, 84, 88, 95, 97, 99, 114, 118,
 - 25 119, 123, 132, 141, 146, 148, 149, 154, 163, 164, 165, 169, 170, 172, 176, 180, 182, 183, 190, 191, 194, 196, 197, 198, 201, 204, 205, 206, 207, 208, 209, 210, 212, 213, 214, 216, 217, 220, 221, 222, 229, 231, 233, 238, 240, 244, 245, 246, 248, 249, 251, 254, 256, 260, 262, 263, 266, 269, 271, 277, 280, 281, 282, 283, 284, 285, 286, 287, 292, 296, 298, 302, 307, 308, 309, 311, 314, 317, 319, 321,
 - 30 323, 325 og 326.
4. Fremgangsmåde ifølge ethvert af kravene 1, 2 eller 3, **kendetegnet ved, at** polypeptidet har i det mindste én mutation af aminosyresekvensen i forhold til sekvens ID No. 1, valgt fra gruppen D22A, S23Q, S23L, N25D, 126V, F27Y, F27H, S29P, R31A,
 - 35 F32Y, R35K, R35Q, V37A, V421, V43T, F46Y, S51E, S51D, D53G, N54M, N54R, L57V, L601, S69G, P72E, V73A, A78S, N80H, F84Y, 188L, T95S, T97A, R99K,

1114M, 1118V, K119R, V1231, L132V, A141S, 1146V, 1146L, A148G, A149V, A154P, P163T, A164T, Y165C, Y165H, V1691, L170R, A172G, A176M, A176V, Y180F, D182T, F183Y, 1190V, G191S, K194T, K194E, F196Y, V197C, V197R, E198R, E198S, K201D, K201G, P204S, P204A, A205S, K206P, A207M, M208A, 5 Q209R, L210A, L210S, LIP212, T213V, P214A, E216T, E216G, A2171, N220H, L221M, K222R, K222Q, G229A, A231V, F233W, F233Y, F233H, A238G, H240N, H240S, D244E, R245Q, M246L, S248T, S248N, S248G, Q249R, K251N, 1254V, 1256L, A260M, T262D, T262G, 1263T, E266D, E269H, E269N, L271V, L277E, E280A, E280L, H281R, H281Q, A282V, Q283R, D284L, D284R, 1285L, 1286M, 10 R287E, R287D, R292K, R292T, Q296A, Q296E, H298V, L302S, L307Q, F308S, D309A, A311P, A314V, L317F, S319Q, S319P, S319R, S321A, S321T, T323A, P325A, A326P.

5. Fremgangsmåde ifølge ethvert af kravene 1 til 4, **kendetegnet ved, at** polypeptidet 15 indeholder i det mindste ét af følgende aminosyremotiver valgt fra gruppen af sekvens ID No. 32 til 69.

6. Fremgangsmåde ifølge ethvert af kravene 1 til 4, **kendetegnet ved, at** polypeptidet anvendes i et zearalenon og/eller zearalenonderivatkløvende, adjuvant indeholdende 20 additiv til foder til svin, fjerkræ og aquakultur, i foder eller i tør mask.

7. Fremgangsmåde ifølge krav 5, **kendetegnet ved, at** adjuvanterne indeholdt i additivet vælges blandt i det mindste et inaktivt bærestof samt eventuelle yderligere ingredienser, såsom vitaminer og/eller mineraler og/eller enzymer og/eller andre kom- 25 ponenter til detoxificering af mycotoxiner.

8. Fremgangsmåde ifølge krav 6 eller 7, **kendetegnet ved, at** polypeptidet er indeholdt i additivet i en koncentration på maksimalt 10.000 U/g, fortrinsvis maksimalt 1000 U/g, mere foretrukket maksimalt 100 U/g og mest foretrukket maksimalt 10 U/g. 30

9. Fremgangsmåde ifølge krav 6, 7 eller 8, **kendetegnet ved, at** additivet foreligger i indkapslet eller belagt form.

10. Fremgangsmåde ifølge ethvert af kravene 1 til 9, **kendetegnet ved, at** polypeptidet 35 eller additivet blandes med en fødevare eller et dyrefoderprodukt, som er kontamineret med zearalenon og/eller med i det mindste et zearalenonderivat; at den kontaminede

fødevarer eller dyrefoderprodukt bringes i kontakt med fugtighed og at polypeptidet eller additivet hydrolyserer zearalenonen og/eller i det mindste ét zearalenonderivat, som foreligger i den kontaminerede fødevarer eller dyrefoderprodukt.

- 5 11. Fremgangsmåde ifølge ethvert af kravene 1 til 10, **kendetegnet ved, at** i det mindste 70%, fortrinsvis i det mindste 80%, især i det mindste 90% af zearalenonen og/eller det i det mindste ene zearalenonderivat hydrolyseres.

- 10 12. Anvendelse af i det mindste et polypeptid med en aminosyresekvens valgt fra gruppen af sekvenserne ID No. 7, 8, 14 og 15, især sekvensen ID No. 14 eller en funktionel variant deraf, hvorved en sekvensidentitet imellem den funktionelle variant og i det mindste én aminosyresekvens udgør i det mindste 70%, indeholdende additiver til hydrolytisk spaltning af zearalenon og/eller i det mindste et zearalenonderivat i dyrefoderprodukter, især til svin, fjerkræ og aquakultur, i fødevarer eller i tør mask.

Fig. 1

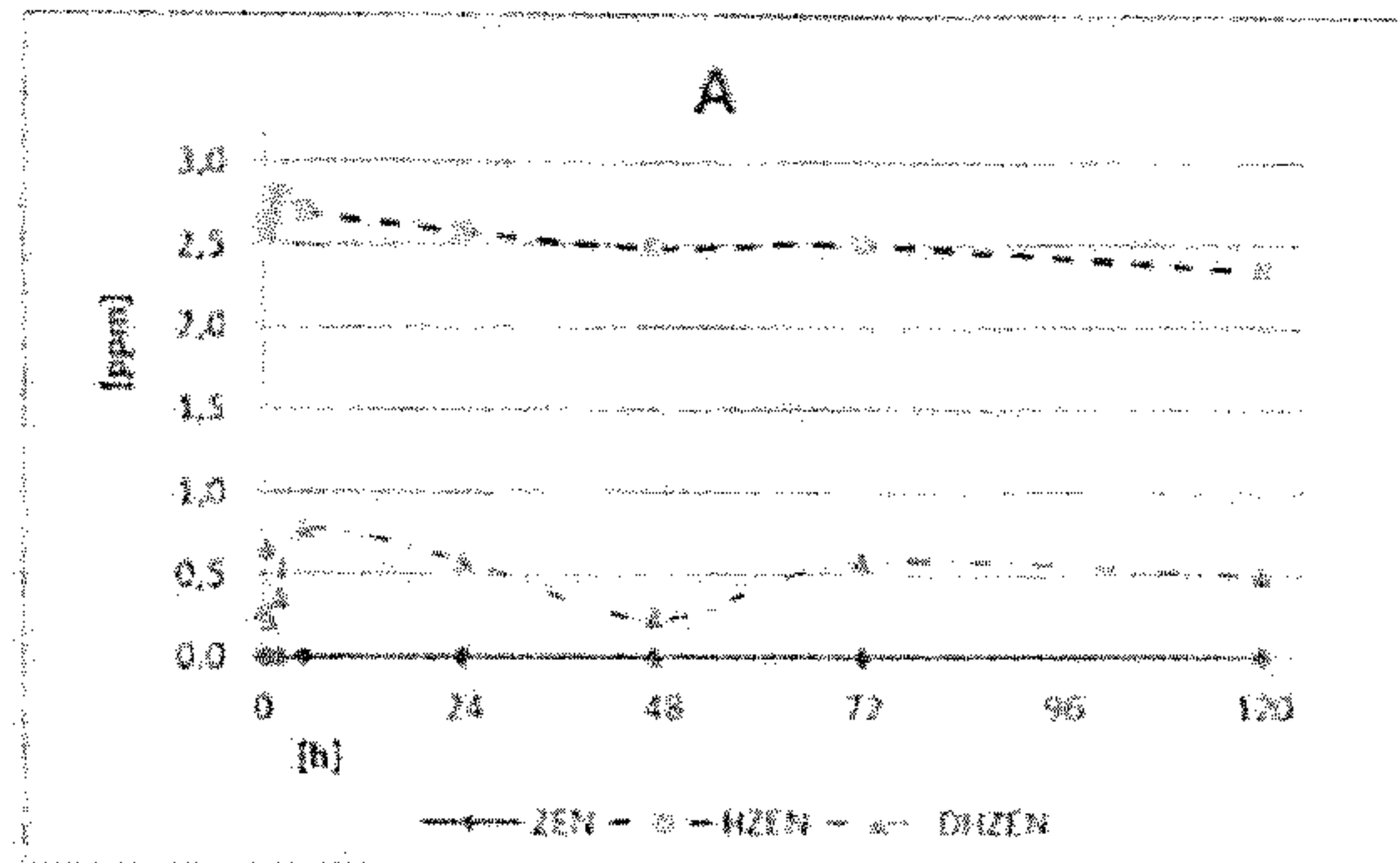


Fig. 1A

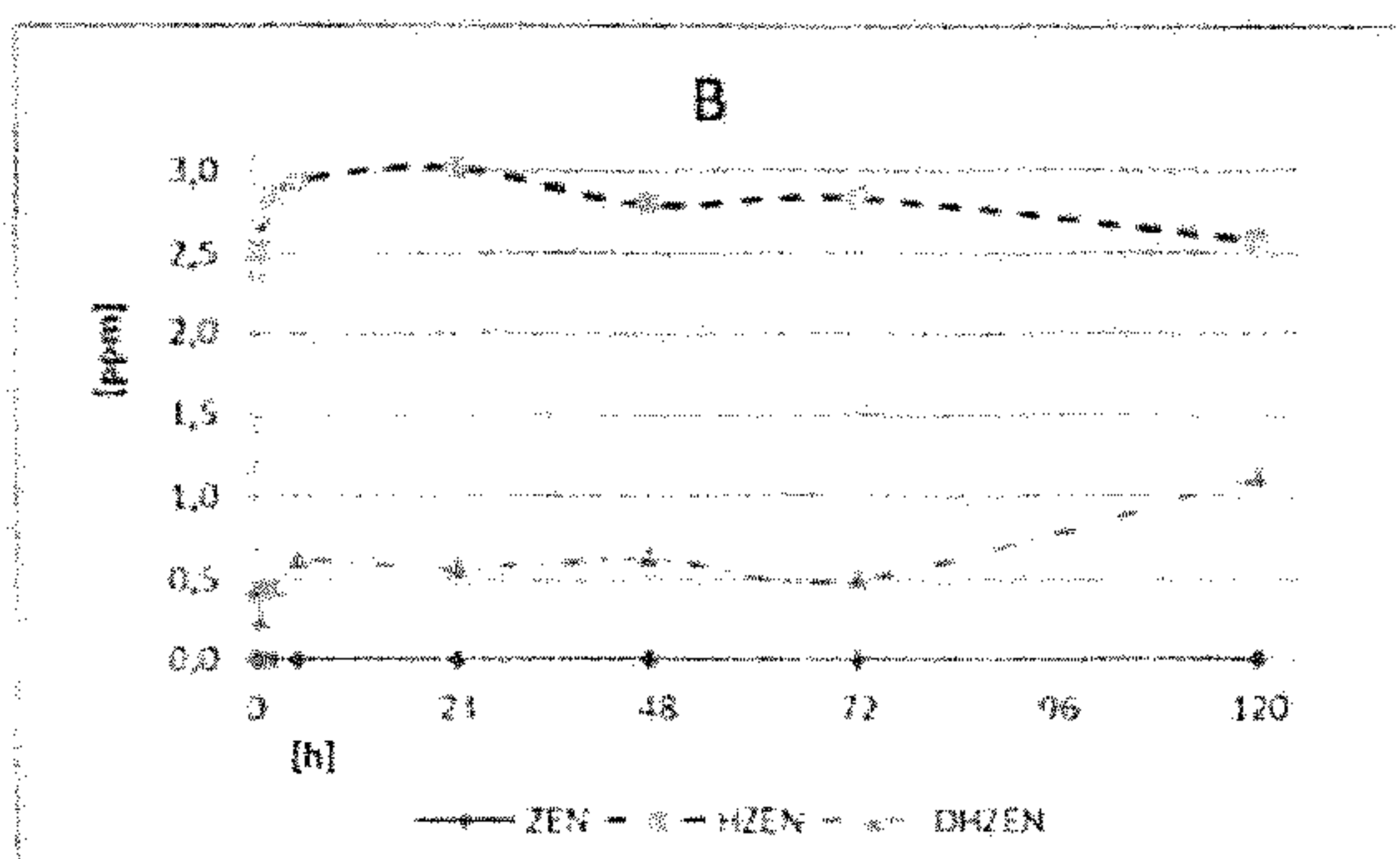


Fig. 1B

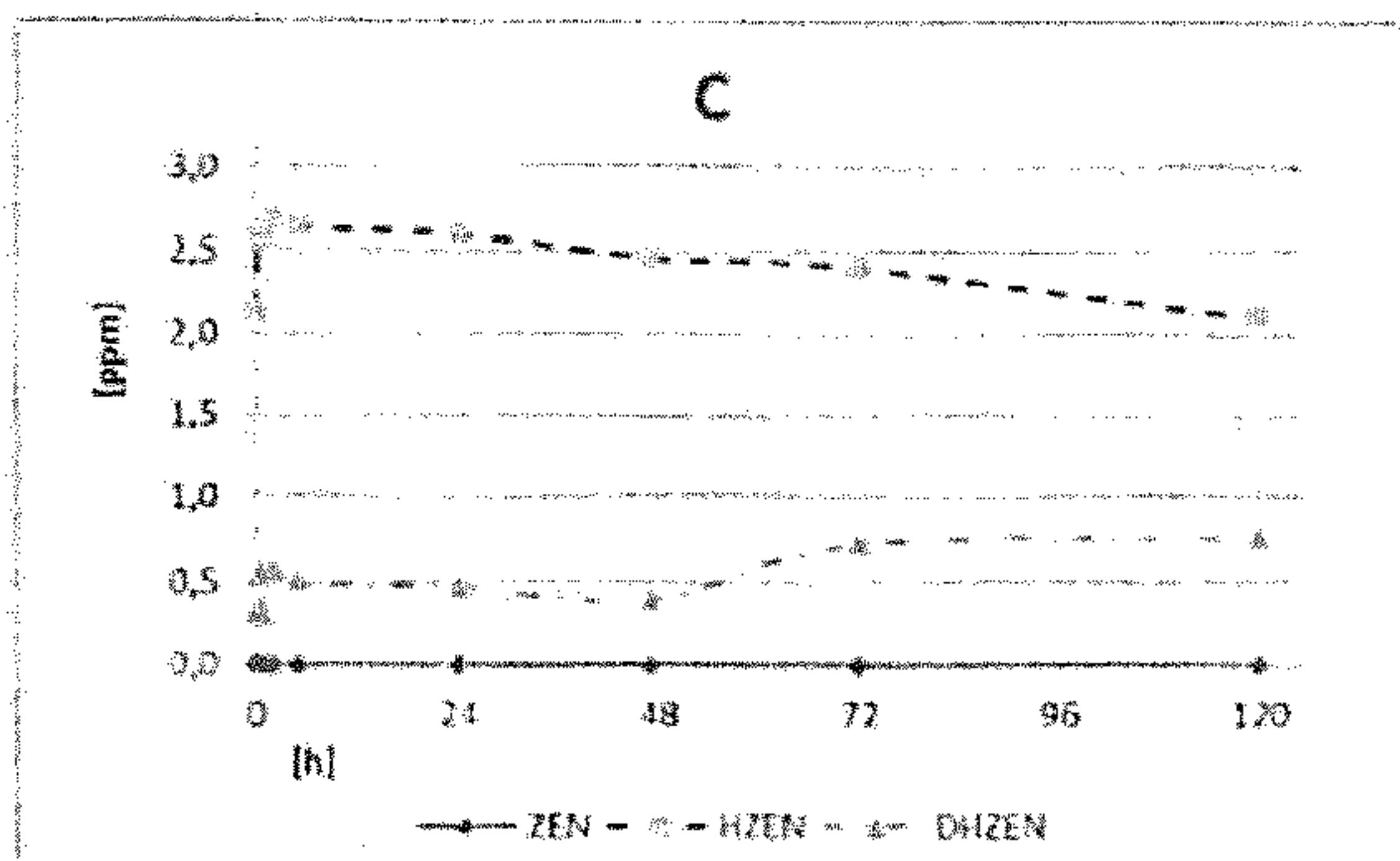


Fig. 1C

Fig. 2

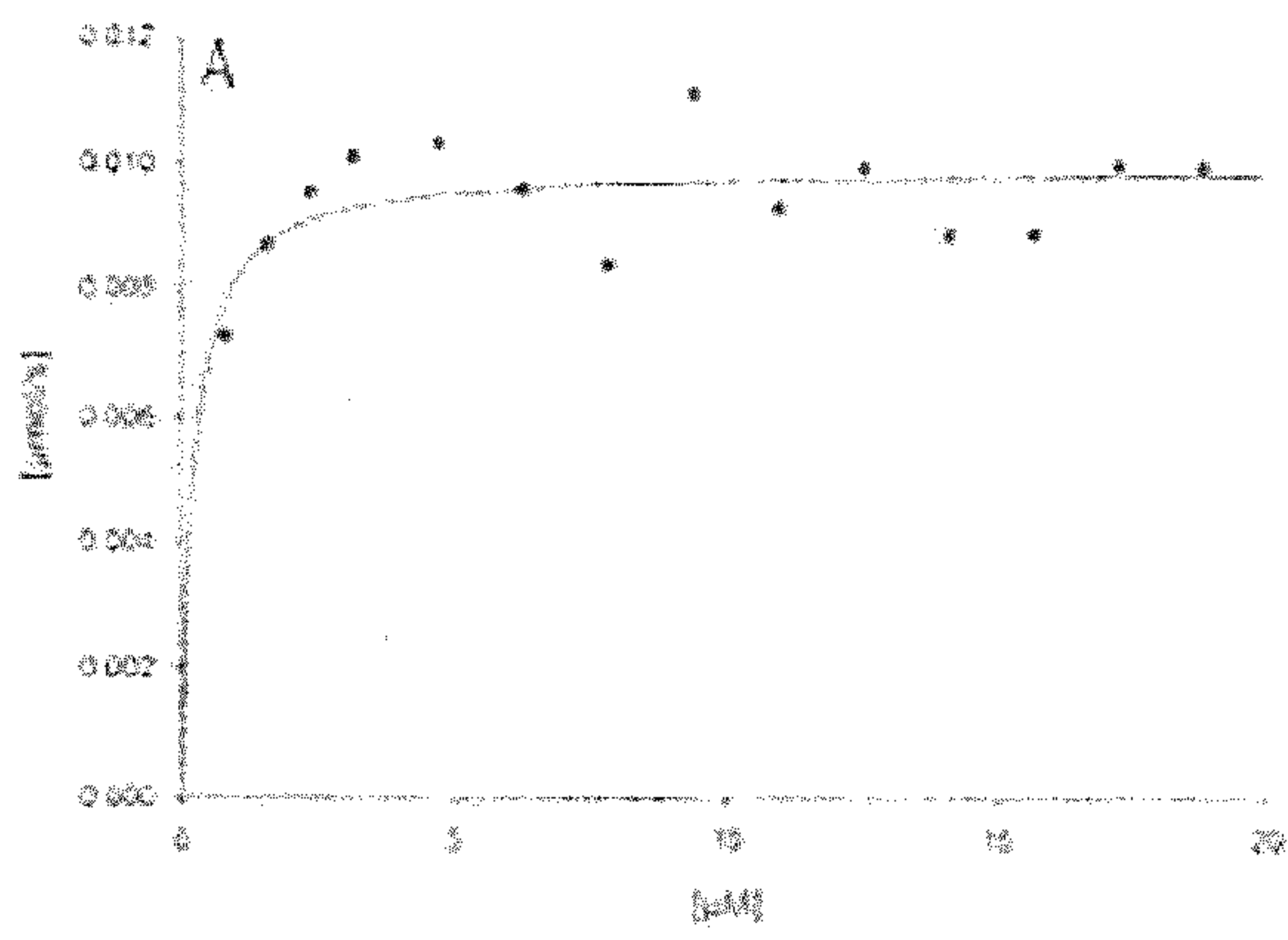


Fig. 2A

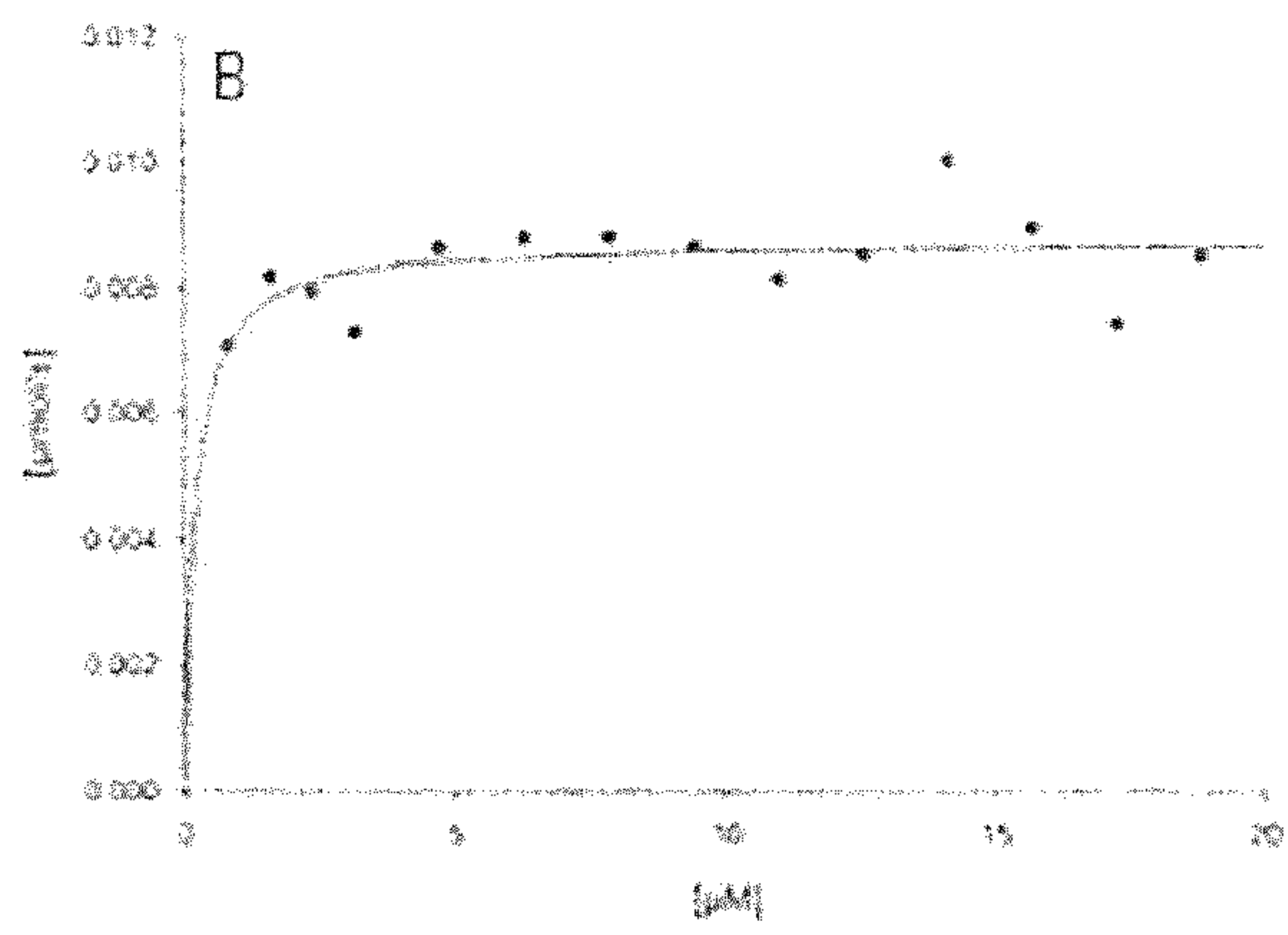


Fig. 2B

