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International Patent Application No. PCT/EP2019/070434

Title: "MEANS AND METHODS FOR CLEAVAGE OF ZEARELENONE"

Applicant: Erber Aktiengesellschaft

Our Ref.: ERB16135PCT

In Response to the Written Opinion of the International Preliminary Examining Authority pursuant to Rule 66 PCT dated 25 May 2020:

1. Filed documents/Requests

- 1.1. A new set of claims 1-15 is submitted herewith in a clean and marked-up form and it is requested to continue the examination proceedings based on these newly filed claims.
- 1.2. If further concerns about the patentability remain, the Examiner is kindly asked to issue a further Written Opinion pursuant to Rule 66 PCT.

2. Claim amendments

New claims correspond to the original claims which have been amended as follows:

- **New claims 1-3, 9** corresponding to the previous claims 1-3, 9 and are unamended;
- **New claims 4, 5, 6, 11-15** corresponding to the previous claims 4, 5, 6, 11-15 have been amended to delete references to "any one of the preceding claims" and instead have been further clarified to recite the actual numbers of the preceding claims. Support: previous claims 1-15;
- **New claim 7** corresponding to the previous claim 7 has been amended by adding a limiting structural feature "wherein the α/β -hydrolase has 95 % or more sequence identity to a

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sequence corresponding to positions 145 to 218 of SEQ ID NO: 1” to the claim language. Support: previous claims 1-7 and throughout the application as originally filed, e.g., paragraphs [0051, 0040, 0036, 0047-0050, 0054];

- **New claim 8** corresponding to the previous claim 8 has been amended by limiting the claimed subject matter to a hydrolase having at least **95%** sequence identity to a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1. Support: previous claims 1-8 and throughout the application as originally filed, e.g., paragraphs [0051, 0011, 0013];
- **New claim 10** corresponding to the previous claim 10 has been amended as follows: it has been limited to a hydrolase having at least **99%** sequence identity to a sequence corresponding to positions 161 to 235 of SEQ ID NO: 6. Support: previous claims 1-10 and throughout the application as originally filed, e.g., paragraphs [0050-0051].

3. Comments with respect to Patentability (Item V)

3.1. Novelty of the new claims

We respectfully submit that new claims 1-15 submitted herewith overcome the Examiner's objections raised in the IPEA of May 25, 2020 and requests that the arguments provided herewith be considered by the Examiner, e.g., in a **further Written Opinion pursuant to Rule 66 PCT**. In detail:

- In item 1.1 of the WO-IPEA the Examiner is of the opinion that since the structure of the hydrolase obtained by the method of claim 1 is undefined in claim 7 D2 and D3 sequences comprising G185R and V160A substitutions respectively are novelty-destroying for the claim 7. In item 1.2 of the WO-IPEA the Examiner is of the opinion that since D1 discloses the D3 sequence and its activity in hydrolysing zearalenone D1 is novelty-destroying for claims 7, 12-15.

In response we respectfully submit that the new claim 7 has been structurally limited to the α/β -hydrolase having 95 % or more sequence identity to a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1. Accordingly, the prior art sequences D2 (having 79.7% identity with the sequence 145 to 218 of SEQ ID NO: 1), D3 (having 61.3% identity with the sequence 145 to 218 of SEQ ID NO: 1) and D1 (disclosing D3) do not satisfy the new claim 7 requirement of the minimum sequence identity of 95% (corresponding fragments of D2, D3 sequences are aligned with the sequence 145 to 218 of SEQ ID NO: 1 below for the Examiner's convenience). Accordingly, new claims 7 and 12-15 are novel over D1/D2/D3 sequences.

- In item 1.3 of the WO-IPEA the Examiner is of the opinion that the fragment of SEQ ID NO: 3 of D1 corresponding to the polypeptide sequence 145 to 218 of SEQ ID NO: 1 of the pending invention has a 91% sequence identity and comprises the Q205K substitution and is therefore novelty-destroying for claims 8, 11-15.

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```
#=====
#
# Aligned_sequences: 2
# 1: SEQ1-145-218
# 2: D1-SEQ3
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 74
# Identity:      68/74 (91.9%)
# Similarity:    69/74 (93.2%)
# Gaps:          0/74 ( 0.0%)
# Score: 369.0
#
#
#=====
```

SEQ1-145-218	1 LVPAHGHSVRQGAGPVFELFRITYLGDQWSVGDWEGFCRAAGASASPMA ^R S	50
	.	
D1-SEQ3	1 LVPAHGHSVRQGAGPVFELFRITYLGDQWSVGDWEGFRSAADASASPMA ^R S	50
SEQ1-145-218	51 FVADGIPQHLEYPDPEWARVFYEG 74	
	. : .	
D1-SEQ3	51 FVADTIPOHLKEYDPEWARAFYEG 74	

- In item 1.4 of the WO-IPEA the Examiner acknowledges the novelty of claim 9. We agree with the Examiner.
- In item 1.5 of the WO-IPEA the Examiner seems to be of the opinion that the fragment of SEQ ID NO: 1 of D1 comprising I190V substitution and corresponding to the polypeptide sequence 161 to 235 of SEQ ID NO: 6 of the pending invention has a 98% sequence identity and is therefore novelty-destroying for claim 10.

In response we respectfully submit that the new claim 10 has been structurally limited to the α/β -hydrolase having 99 % or more sequence identity to a sequence corresponding to positions 161 to 235 of SEQ ID NO: 6. Accordingly, the corresponding fragment of the SEQ ID NO: 1 (I190V) of D1 does not satisfy the new claim 10 requirement of the minimum sequence identity of 99%. Accordingly, the new claim 10 is novel over the corresponding polypeptide of SEQ ID NO: 1 of D1 having I190V.

3.2 Inventive step of the new claims

- In items 2-2.3 of the WO-IPEA the Examiner objects to the inventive step of claims 7-15. The Examiner considers D1 as the closest prior art and seems to acknowledge that the solution suggested in claims 7-11, i.e. variants with increased temperature stability (supported by Figures 3A and 3B of the pending invention), allowing for pelletization of feed at elevated temperature. However, the Examiner seems to object that since the claimed variants are not limited to the variants with increased temperature stability it is therefore not credible that the claimed invention is achievable over the whole area claimed.

In response we respectfully disagree with the Examiner and submit that the **burden of proof lies initially with the Examiner** and that such an inventive step objection based on credibility over the whole breadth of claims objection **must be reasoned, substantiated**, and based on

the **balance of probabilities** (cf. Guidelines for Search and Examination at the EPO as PCT Authority: 11. "Arguments and evidence submitted by the applicant" https://www.epo.org/law-practice/legal-texts/html/guidelinespct/e/g_vii_11.htm; cf. EPO Guidelines for Examination: G-IV, 7.5.3 "Burden of proof"; C-III, 4.1.1 "Reasoned objections"; F-III, 4. "Burden of proof as regards the possibility of performing and repeating the invention") which has not been done in the present case, where the credibility of the invention over the whole breadth of claims is simply questioned without substantiating serious doubts. This view is also supported by the EPO's established case law, e.g.,

T 0019/90 (Onco-Mouse), item 3.3 of the Reasons (with emphasis added):

*"However, the mere fact that a claim is broad is not in itself a ground for considering the application as not complying with the requirement for sufficient disclosure under Article 83 EPC. **Only if there are serious doubts, substantiated by verifiable facts, may an application be objected to for lack of sufficient disclosure.**"*

T 0247/11 (Roasted cocoa beans/NESTEC), item 23 of the Reasons (with emphasis added):

*"As shown by the examples in the patent, the use of roasted cocoa beans and chocolate as starting material solve this problem. The arguments put forward by the appellant to substantiate its objection that the technical problem is not solved over the whole breadth of the claims are essentially the same as those put forward under Article 83 EPC (cf. point XIII supra), and have been dealt with by the board under this article (cf. points 13 to 16 supra). **In the absence of cogent arguments to the contrary, the technical problem is thus considered to be solved over the whole scope of the claims.**"*

T 2101/09 (Human Delta3 - Notch/MILLENNIUM), item 13 et seq. of the Reasons (with emphasis added):

*"13. **It is common practice in the field of biotechnology that claims in patents/patent applications related to a novel and inventive (nucleic acid, amino acid) sequence are not required to be limited to a very specific sequence but may also embrace molecules having a certain degree of homology and/or identity to this specific sequence. The degree of homology/identity accepted depends on the relevant prior art and the particular circumstances of each individual case.** This practice allows patentees/applicants to protect their inventions against arbitrary modifications of the specific sequences. **The reference to at least 95% identity in claims 1 and 5 is in line with this practice.** Moreover, the wording of these claims ("... whose ... sequence is at least 95% identical to the ... sequence..."; underlined by the board) requires a degree of identity over the full-length of the sequence and not over short, arbitrary fragments thereof [...]"*

*14. **In some cases, it might be necessary to further limit the scope of a claim referring to such group of molecules either by increasing the degree of identity and/or homology or by requiring the indication of further technical properties, such a specific activity, effect, etc.** None of these additional limitations is present in claims*

1 and 5. In particular, there is no limitation to polypeptides - or to nucleic acid molecules encoding them - that specifically bind to a Notch polypeptide.

16. In view of the close structural (at least 95%) identity of the group of molecules of claims 1 and 5 and their low degree of identity to the closest structurally related molecules, i.e. other human delta proteins (cf. page 8, Table 1 of the patent; Maximum 54% identity), there are no serious doubts (let alone substantiated by verifiable facts; cf. T 19/90, supra) that they can be used in at least one of the several biological activities contemplated in paragraph [0056] of the patent-in-suit.

Notwithstanding the above, the scope of the new claims has been narrowed down and therefore we respectfully submit that the new claims are credibly inventive over the whole area claimed. In detail:

- claims 7-12 are **limited to closely related polypeptide sequences with a very high degree of structural identity**, i.e., at least **95%** (new claims 7-8), at least **99%** (new claim 10) or at least **80%** (claim 9);
- claims 7-12 all recite the functional limitation of being α/β -hydrolases; as disclosed in paragraphs [0028]-[0030] of the application as filed, it was surprisingly found, that an α/β -hydrolase comprising a mutation in a specific region, namely the cap-domain and more particularly the CAP-loop, makes these hydrolases specifically stable. The reason for this presumably is that this region is particularly flexible, which flexibility might be negative for the stability of the hydrolases. Therefore, there is not a random mutagenesis taking place in the present invention. Rather, only a specific region, which has been shown to be particularly important for the stability of the hydrolases is mutated. Therefore, what is done here is not random, but highly specific mutagenesis in a very specific region of the hydrolases particularly susceptible for the stability of the hydrolases.
- representative examples of the claimed variants/properties/activities (i.e., increased stability) within the claimed scope are provided in the application as originally filed, e.g., example 3 [00172 et seq., Figure 2] and example 5 [00190 et seq., Figure 3].

Based on the above, we respectfully submit that there are no serious doubts (let alone substantiated by verifiable facts) that the claimed invention is inventive and enabled over the whole breadth of new claims and that the claimed subject matter is inventive enabled over the whole breadth of new claims. Based on the above, new claims are inventive.

4. Comments with respect to Certain defects in the international application (Item VII)

- In item VII of the WO-IPEA the Examiner objects to plausibility of the claim 1 reciting both hydropathy index and increased enzyme stability.

In response we respectfully disagree with the Examiner and submit that claim 1 is **limited to closely related polypeptide sequences with a very high degree of structural identity of at**

least 80%, wherein representative examples of the claimed methods/variants with claimed improved properties/activities (i.e., increased stability) within the claimed scope are provided in the application as originally filed, e.g., example 3 [00172 et seq., Figure 2] and example 5 [00190 et seq., Figure 3]. Accordingly, there are no serious doubts (let alone substantiated by verifiable facts) that the claimed invention plausibly enabled/claimed over the whole breadth of the new claims, e.g., as supported by **T 0578/06**, items 12 et seq. of the Reasons (with emphasis added):

*[...] **"The board notes that neither in its decision nor during the prosecution of the application has the examining division produced arguments which could discredit the plausibility of the claimed invention. Also the board sees no reasons to doubt the usefulness of somatostatin to attain the claimed effect."***

*13. The board notes that the **EPC requires no experimental proof for patentability and considers that the disclosure of experimental data or results in the application as filed and/or post-published evidence is not always required to establish that the claimed subject-matter solves the objective technical problem. This is in particular true in the absence of any formulated substantiated doubt as is the case here.***

Based on the above we respectfully submit that the new claims including their respective technical effects are plausible.

5. Comments with respect to Certain observations on the international application (Item VIII)

- In item VIII of the WO-IPEA the Examiner objects to clarity of claim 5, 11 based on the use of the "preceding" claim dependency language as well as to the recitation of F183Y/V197C substitution/s with reference to SEQ ID NO: 1 and P190H substitution with reference to SEQ ID NO 6.

In response we respectfully submit that new claims are clear and concise and the Examiner's objections have been rendered moot. In detail: references to "any one of the preceding claims" have been deleted and the actual numbers of the preceding claims have been recited in the new claims. Furthermore, new claim 5 depending from the method of claims 1-4, wherein claim 1 referring to positions corresponding to positions in SEQ ID NO: 6 comprising F183 and V197, therefore F183Y/V197C substitutions are possible in said positions. New claim 11 depending from hydrolases of claims 7-10, wherein claim 9 referring to positions corresponding to positions SEQ ID NO: 5 comprising P190, therefore P190H substitution is possible in said position. Based on the above, references to F183Y/V197C/P190H are instantly clear to a person skilled in the art and the new claims are clear and concise.

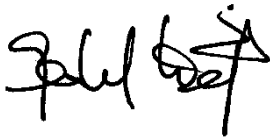
6. Conclusion

- We trust the above will address any concerns the International Preliminary Examining authority may have had over patentability of the claims of this application. We look forward

to receipt of an International Preliminary Examination Report (IPRP) (Chapter II) which is positive for all claims.

- The Examiner is also kindly invited to contact us by telephone or by email should clarification be required on any matter.

With kind regards

A handwritten signature in black ink, appearing to read 'Gerhard Weinzierl', written over a horizontal line.

Dr. Gerhard Weinzierl
European Patent Attorney

Encl.:

- New claims 1-15 (clean and marked copy)

New Claims (clean)

1. A method for increasing the stability of an α/β -hydrolase, which α/β -hydrolase comprises a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1 or a sequence having 80 % or more sequence identity to a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1, comprising

substituting at least one amino acid

- at a position corresponding to position 160 to 205 of SEQ ID NO: 1, or
- at a position corresponding to position 159 to 204 of SEQ ID NO: 2, or
- at a position corresponding to position 160 to 205 of SEQ ID NO: 3, 4, or 5, or
- at a position corresponding to position 176 to 222 of SEQ ID NO: 6,

wherein the amino acid(s) are substituted with an amino acid, which has a more negative hydropathy index than the substituted amino acid, wherein the hydropathy index is determined by the Kyte and Doolittle hydropathy index,

thereby obtaining an α/β -hydrolase with increased stability compared to the α/β -hydrolase before substituting said amino acid(s).

2. The method of claim 1, wherein the method comprises substituting at least one amino acid
 - at a position corresponding to position 185 to 191 of SEQ ID NO: 1, and/or
 - at a position corresponding to position 184 to 190 of SEQ ID NO: 2 and/or
 - at a position corresponding to position 185 to 191 of SEQ ID NO: 3, 4 or 5 and/or
 - at a position corresponding to position 201 to 208 of SEQ ID NO: 6.

3. The method of claim 1 or 2, wherein the amino acid(s) are substituted with an amino acid selected from R, K, N, Q, D, E, H, P, Y, W, S, T, G, A, M, C, F, L or V, preferably the amino acid is selected from R, K, N, Q, D, E, H, P, Y, W, S, T or G, more preferably R, K, N, Q, D, E, H or P.
4. The method of claims 1-3, wherein the amino acid(s) are substituted with an amino acid selected from R, D, H, G, N or P, preferably R, D, H, G or N, more preferably the amino acid is selected from R or N.
5. The method of claims 1-4, wherein the amino acid substitution is selected from one or more of V→A, G→R, G→S, A→P, A→R, A→D, A→H, A→N, A→G, S→D, P→H, M→D, G→E, I→A, I→V, H→N, Q→K, F→Y and/or V→C, preferably the amino acid substitution is selected from one or more of V160A, G185R, G185S, A186P, A186R, A188D, A188H, A188N, A188G, A188R, S189D, P190H, M191D, G199E, I200A, I200V, H203N, Q205K, F183Y and/or V197C, more preferably the amino acid substitution is selected from G185R, A186R, A188R, A188D, A188H, A188N and/or M191D.
6. The method of claims 1-5, wherein the increased stability is a decrease in GRAVY value, an increase in pH stability and/or an increase in temperature stability.
7. An α/β -hydrolase obtainable by the method of any one of claims 4, 5 or 6, wherein the α/β -hydrolase has 95 % or more sequence identity to a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1.
8. An α/β -hydrolase having a polypeptide sequence comprising a sequence

corresponding to positions 145 to 218 of SEQ ID NO: 1 or a sequence having more than 95 % sequence identity to a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1,

wherein the polypeptide sequence comprises at least one amino acid substitution

- at a position corresponding to position 160 to 205 of SEQ ID NO: 1, or
- at a position corresponding to position 159 to 204 of SEQ ID NO: 2, or
- at a position corresponding to position 160 to 205 of SEQ ID NO: 3, 4 or 5, or
- at a position corresponding to position 176 to 222 of SEQ ID NO: 6,

wherein the α/β -hydrolase has a more negative GRAVY value of at least 0.6 % compared to the GRAVY value of an α/β -hydrolase having a polypeptide sequence of SEQ ID NO: 1.

9. An α/β -hydrolase having a polypeptide sequence comprising a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1 or a sequence having more than 80 % sequence identity to a sequence corresponding to positions 145 to 218 of SEQ ID NO: 1,

wherein the polypeptide sequence comprises at least one amino acid substitution

- at a position corresponding to position 185 to 191 of SEQ ID NO: 1, or
- at a position corresponding to position 184 to 190 of SEQ ID NO: 2 or
- at a position corresponding to a position 185 to 191 of SEQ ID NO: 3, 4 or 5, or
- at a position corresponding to a position of 201 to 208 of SEQ ID NO: 6,

wherein the amino acid substitution is selected from V→A, G→R, G→S, A→P, A→R, A→D, A→H, A→N, A→G, S→D, P→H, M→D, G→E, I→A, I→V, H→N and Q→K and/or

wherein the amino acid(s) are substituted with an amino acid selected from P, R, D, H, G or N, preferably the amino acid is selected from R, D, H, G or N, more preferably the amino acid is selected from R or N, wherein the α/β -hydrolase has an increased stability compared to a α/β -hydrolase before substituting said amino acid(s).

10. An α/β -hydrolase having a polypeptide sequence comprising a sequence corresponding to positions 161 to 235 of SEQ ID NO: 6 or a sequence having more than 99 % sequence identity to a sequence corresponding to positions 161 to 235 of SEQ ID NO: 6,

wherein the polypeptide sequence comprises at least one amino acid substitution

- at a position corresponding to position 176 to 222 of SEQ ID NO: 6,

wherein the α/β -hydrolase has a more negative GRAVY value of at least 0.6 % compared to the GRAVY value of an α/β -hydrolase having a polypeptide sequence of SEQ ID NO: 6.

11. The α/β -hydrolase of claims 7-10, wherein the α/β -hydrolase comprises the amino acid(s) substitutions

- G→R and A→N, preferably G185R and A188N;
- G→S and A→R, preferably G185S and A188R;
- G→R, A→R, A→H, S→D, P→H and M→D, preferably G185R, A186R, A188H, S189D, P190H and M191D;
- V→A, G→R, A→N, G→E, I→A, H→N and Q→K, preferably V160A, G185R, A188N, G199E, I200A, H203N and Q205K;
- V→A, G→S, A→R, G→E, I→A, H→N and Q→K, preferably V160A, G185S, A188R, G199E, I200A, H203N and Q205K;
- V→A, G→E, I→A, H→N and Q→K, preferably V160A, G199E, I200A,

H203N and Q205K

- V→A, G→R, A→R, A→H, G→E, I→V, H→N and Q→K, preferably V160A, G185R, A186R, A188H, G199E, I200V, H203N and Q205K;

- V→A, G→R, A→R, A→H, S→D, P→H, M→D, G→E, I→V, H→N and Q→K, preferably V160A, G185R, A186R, A188H, S189D, P190H, M191D, G199E, I200V, H203N and Q205K and/or

F→Y and V→C, preferably F183Y and V197C.

12. A use of an α/β -hydrolase of claims 7-11 for degrading zearalenone (ZEN).
13. A composition comprising an α/β -hydrolase of claims 7-11, preferably the composition is a food or feed additive or a food or feed product.
14. The α/β -hydrolase of claims 7-11 or the composition of claim 13 for use in the treatment or prophylaxis of a disease.
15. Kit comprising the α/β -hydrolase of claims 7-11 or 14 or the composition of claims 13-14.

REIVINDICACIONES

1. Un método para incrementar la estabilidad de una α/β -hidrolasa, comprendiendo la α/β -hidrolasa una secuencia que corresponde a las posiciones 145 a 218 de la SEQ ID NO: 1 o una secuencia que tiene 80 % o más de identidad de secuencia con una secuencia correspondiente a las posiciones 145 a 218 de SEQ ID NO: 1, que comprende
 - sustituir al menos un aminoácido
 - en una posición correspondiente a una posición 160 a 205 de SEQ ID NO: 1, o
 - en una posición correspondiente a una posición 159 a 204 de SEQ ID NO: 2, o
 - en una posición correspondiente a una posición 160 a 205 de SEQ ID NO: 3, 4, o 5, o
 - en una posición correspondiente a una posición 176 a 222 de SEQ ID NO: 6,
 - en donde el(los) aminoácido(s) está(n) sustituido(s) con un aminoácido, el cual tiene un índice de hidropatía más negativo que el aminoácido sustituido, en donde el índice de hidropatía está determinado mediante el índice de hidropatía de Kyte y Doolittle,
 - obteniendo de ese modo una α/β -hidrolasa con estabilidad incrementada comparada con la α/β -hidrolasa antes de sustituir dicho(s) aminoácido(s).
2. El método de la reivindicación 1, en donde el método comprende sustituir al menos un aminoácido
 - en una posición correspondiente a una posición 185 a 191 de SEQ ID NO: 1, y/o
 - en una posición correspondiente a una posición 184 a 190 de SEQ ID NO: 2 y/o
 - en una posición correspondiente a una posición 185 a 191 de SEQ ID NO: 3, 4 o 5 y/o
 - en una posición correspondiente a una posición 201 a 208 de SEQ ID NO: 6.
3. El método de la reivindicación 1 o 2, en donde el(los) aminoácido(s) está(n) sustituido(s) con un aminoácido seleccionado de R, K, N, Q, D, E, H, P, Y, W, S, T, G, A, M, C, F, L o V, preferiblemente el aminoácido está seleccionado de R, K, N, Q, D, E, H, P, Y, W, S, T o G, más preferiblemente R, K, N, Q, D, E, H o P.

4. El método de las reivindicaciones 1-3, en donde el(los) aminoácido(s) está(n) sustituido(s) con un aminoácido seleccionado de R, D, H, G, N o P, preferiblemente R, D, H, G o N, más preferiblemente el aminoácido está seleccionado de R o N.
5. El método de las reivindicaciones 1-4, en donde la sustitución de aminoácidos está seleccionada de una o más de V→A, G→R, G→S, A→P, A→R, A→D, A→H, A→N, A→G, S→D, P→H, M→D, G→E, I→A, I→V, H→N, Q→K, F→Y y/o V→C, preferiblemente la sustitución de aminoácidos está seleccionada de una o más de V160A, G185R, G185S, A186P, A186R, A188D, A188H, A188N, A188G, A188R, S189D, P190H, M191D, G199E, I200A, I200V, H203N, Q205K, F183Y y/o V197C, más preferiblemente la sustitución de aminoácidos está seleccionada de G185R, A186R, A188R, A188D, A188H, A188N y/o M191D.
6. El método de las reivindicaciones 1-5, en donde la estabilidad incrementada es un valor GRAVY disminuido, un incremento en la estabilidad frente a pH y/o un incremento en la estabilidad térmica.
7. Una α/β -hidrolasa obtenible por el método de una cualquiera de las reivindicaciones 4, 5 o 6, en donde la α/β -hidrolasa tiene 95 % o más de identidad de secuencia para una secuencia que corresponde a las posiciones 145 a 218 de SEQ ID NO: 1.
8. Una α/β -hidrolasa que tiene una secuencia polipeptídica que comprende una secuencia correspondiente a las posiciones 145 a 218 de SEQ ID NO: 1 o una secuencia que tiene más de 95% de identidad de secuencia con una secuencia correspondiente a las posiciones 145 a 218 de SEQ ID NO: 1,
 - en donde la secuencia polipeptídica comprende al menos una sustitución de aminoácidos
 - en una posición correspondiente a una posición 160 a 205 de SEQ ID NO: 1, o
 - en una posición correspondiente a una posición 159 a 204 de SEQ ID NO: 2, o
 - en una posición correspondiente a una posición 160 a 205 de SEQ ID NO: 3, 4 o 5, o
 - en una posición correspondiente a una posición 176 a 222 de SEQ ID NO: 6,

en donde la α/β -hidrolasa tiene un valor GRAVY más negativo de al menos 0,6% comparado con un valor GRAVY de una α/β -hidrolasa que tiene una secuencia polipeptídica de la SEQ ID NO: 1.

- 5 9. Una α/β -hidrolasa que tiene una secuencia polipeptídica que comprende una secuencia correspondiente a las posiciones 145 a 218 de SEQ ID NO: 1 o una secuencia que tiene más de 80 % de identidad de secuencia con una secuencia correspondiente a las posiciones 145 a 218 de SEQ ID NO: 1,

10 en donde la secuencia polipeptídica comprende al menos una sustitución de aminoácidos

- en una posición correspondiente a una posición 185 a 191 de SEQ ID NO: 1, o
- en una posición correspondiente a una posición 184 a 190 de SEQ ID NO: 2 o
- en una posición correspondiente a una posición 185 a 191 de SEQ ID NO: 3, 4 o 5,
- 15 o
- en una posición correspondiente a una posición de 201 a 208 de SEQ ID NO: 6,

en donde la sustitución de aminoácidos está seleccionada de V→A, G→R, G→S, A→P, A→R, A→D, A→H, A→N, A→G, S→D, P→H, M→D, G→E, I→A, I→V, H→N y Q→K y/o

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en donde el(los) aminoácido(s) está(n) sustituido(s) con un aminoácido seleccionado de P, R, D, H, G o N, preferiblemente el aminoácido está seleccionado de R, D, H, G o N, más preferiblemente el aminoácido está seleccionado de R o N, en donde la α/β -hidrolasa tiene una estabilidad incrementada comparada con una α/β -hidrolasa antes de sustituir dicho(s) aminoácido(s).

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10. Una α/β -hidrolasa que tiene una secuencia polipeptídica que comprende una secuencia que corresponde a las posiciones 161 a 235 de la SEQ ID NO: 6 o una secuencia que tiene más de 99% de identidad de secuencia correspondiente a las posiciones 161 a 235 de la SEQ ID NO: 6,
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en donde la secuencia polipeptídica comprende al menos una sustitución de aminoácidos

- 35 - en una posición correspondiente a una posición 176 a 222 de SEQ ID NO: 6,

en donde la α/β -hidrolasa tiene un valor GRAVY más negativo de al menos 0,6% comparado con un valor GRAVY de una α/β -hidrolasa que tiene una secuencia polipeptídica de la SEQ ID NO: 6.

- 5 11. La α/β -hidrolasa de las reivindicaciones 7-10, en donde la α/β -hidrolasa comprende las sustituciones de aminoácidos
 - G→R y A→N, preferiblemente G185R y A188N;
 - G→S y A→R, preferiblemente G185S y A188R;
 - G→R, A→R, A→H, S→D, P→H y M→D, preferiblemente G185R, A186R, A188H,
 - 10 S189D, P190H y M191D;
 - V→A, G→R, A→N, G→E, I→A, H→N y Q→K, preferiblemente V160A, G185R, A188N, G199E, I200A, H203N y Q205K;
 - V→A, G→S, A→R, G→E, I→A, H→N y Q→K, preferiblemente V160A, G185S, A188R, G199E, I200A, H203N y Q205K;
 - 15 - V→A, G→E, I→A, H→N y Q→K, preferiblemente V160A, G199E, I200A, H203N y Q205K
 - V→A, G→R, A→R, A→H, G→E, I→V, H→N y Q→K, preferiblemente V160A, G185R, A186R, A188H, G199E, I200V, H203N y Q205K;
 - V→A, G→R, A→R, A→H, S→D, P→H, M→D, G→E, I→V, H→N y Q→K,
 - 20 preferiblemente V160A, G185R, A186R, A188H, S189D, P190H, M191D, G199E, I200V, H203N y Q205K y/o
 - F→Y y V→C, preferiblemente F183Y y V197C.
- 25 12. Un uso de una α/β -hidrolasa de las reivindicaciones 7-11 para degradar zearalenona (ZEN).
13. Una composición que comprende una α/β -hidrolasa de las reivindicaciones 7-11, preferiblemente la composición es un o aditivo alimenticio o de alimentación animal o un producto alimenticio o de alimentación animal.
- 30 14. La α/β -hidrolasa o la composición de las reivindicaciones 7-11 o la composición de reivindicación 13 para uso en el tratamiento o profilaxis de una enfermedad.
15. Kit que comprende la α/β -hidrolasa de las reivindicaciones 7-11 o 14 o la composición de las reivindicaciones 13-14.
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