

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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25. Mai 2020

PCT

WRITTEN OPINION OF THE
INTERNATIONAL PRELIMINARY
EXAMINING AUTHORITY

(PCT Rule 66)

25.07.2020

Date of mailing
(day/month/year)

25.05.2020

Applicant's or agent's file reference

ERB16135PCT

Schiweck Weinzierl Koch
VF: F:

REPLY DUE

within 2 month(s) from the above date of mailing

International application No.

PCT/EP2019/070434

International filing date (day/month/year)

30.07.2019

Priority date (day/month/year)

31.07.2018

International Patent Classification (IPC) or both national classification and IPC

INV. C12N15/10

Applicant

Erber Aktiengesellschaft

1. ☒ The written opinion established by the International Searching Authority:
☒ is ☐ is not
considered to be a written opinion of the International Preliminary Examining Authority.
2. This second opinion contains indications relating to the following items:
 - ☒ Box No. I Basis of the opinion
 - ☐ Box No. II Priority
 - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - ☐ Box No. IV Lack of unity of invention
 - ☒ Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - ☐ Box No. VI Certain documents cited
 - ☒ Box No. VII Certain defects in the international application
 - ☒ Box No. VIII Certain observations on the international application
3. The applicant is hereby **invited to reply** to this opinion.
When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(e).
How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 55.3 and 66.8.
Also: For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.6. For an additional opportunity to submit amendments, see Rule 66.4.
If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
4. The final date by which the international preliminary report on patentability (Chapter II of the PCT) must be established according to Rule 69.2 is: 30.11.2020

Name and mailing address of the international preliminary examining authority:



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**WRITTEN OPINION OF THE INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY**

International application No.
PCT/EP2019/070434

Box No. I Basis of the opinion

1. With regard to the **language** , this opinion has been established on the basis of:
- ☒ the international application in the language in which it was filed
 - ☐ a translation of the international application into ,
which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3(a) and 23.1(b)).
 - ☐ publication of the international application (under Rule 12.4(a))
 - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))
2. With regard to the elements of the international application, this opinion has been established on the basis of
(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"):

Description, Pages

1-48 as originally filed

Sequence listings, SEQ ID NO

1-11 as originally filed

Claims, Numbers

1-15 filed with the letter of 17-04-2020

Drawings, Sheets

1/11-11/11 as originally filed

- ☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
4. ☐ This opinion has been established as if (some of) the amendments listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):

**WRITTEN OPINION OF THE INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY**

International application No.
PCT/EP2019/070434

5. ☐ This opinion has been established:
- ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 66.1(d-bis)).
 - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 66.4bis).
6. ☐ Supplementary international search report(s) from Authority(ies) have been received and taken into account in drawing up this opinion (Rule 45bis.8(b) and (c)).

Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	No:	Claims	5, 7, 8, 10-15
Inventive step (IS)	No:	Claims	5, 7, 8, 10-15
Industrial applicability (IA)	No:	Claims	

2. Citations and explanations:

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
 - d. ☐ furnished to this Authority as an amendment under PCT Article 34 on :
 - ☐ in the form of an Annex C/ST.25 text file, and preferably identified as "Amended" at the first line of text.
 - ☐ on paper or in the form of an image file.
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

Reference is made to the following documents:

- D1 WO 2015/027258 A2 (ERBER AG [AT]) 5 March 2015 (2015-03-05); & DATABASE Geneseq [Online] 23 April 2015 (2015-04-23), "Streptomyces violaceusniger hydrolase protein, SEQ ID 2.", retrieved from EBI accession no. GSP:BBV28832 Database accession no. BBV28832
- D2 DATABASE UniProt [Online] 7 June 2017 (2017-06-07), "SubName: Full=Alpha/beta hydrolase {ECO:0000313|EMBL:OON79242.1};" , retrieved from EBI accession no. UNIPROT:A0A1V4A9G7 Database accession no. A0A1V4A9G7
- D3 DATABASE Geneseq [Online] 23 April 2015 (2015-04-23), "R. erythropolis Hydrolase mutant (Q296E/H298V/L302S/L307Q/F308S)." retrieved from EBI accession no. GSP:BBV29130 Database accession no. BBV29130

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Novelty

- 1.1 The Examining Division maintains the view that the hydrolase of claim 7, defined as a product by process, lacks novelty in the sense of Article 33(2) PCT. Claim 1 relates to the process leading to said hydrolase. It comprises a method with two steps: 1.) taking a parental hydrolase which comprises at least a fragment of Seq ID No:1 (or 80% identical) and 2.) substituting in said enzyme at least one amino acid in a defined region. The "comprising" and the "at least" allows for additional modifications. The structure of the hydrolase obtained by the method of claim 1 is undefined. The *Streptomyces tsukubensis* hydrolases of D2 which comprises a G185R substitution when aligned with Seq ID No:1 as well as the *Rhodococcus erythropolis* hydrolase mutant of D3 which comprises a V160A substitution when aligned with Seq ID No:1 are therefore considered novelty destroying for claim 7.

- 1.2 The *Rhodococcus erythropolis* hydrolase mutant which is shown in D3 is derived from the patent application D1, Table 5: ZH1-N/O-002. In D1 the mutated enzyme was analysed for its activity in hydrolyzing zearalenone (Table 5). Therefore, D1 is novelty destroying for claims 7, 12-15 in the sense of Article 33(2) PCT.
- 1.3 D1 also discloses the *Streptomyces coelicoflavus* alpha/beta hydrolase encoded by Seq ID No:3 which comprises a cap domain having 91% identity with residues 145-218 of Seq ID No:1. Compared to Seq ID No:1 the *Streptomyces coelicoflavus* alpha/beta hydrolase also has a Q205K substitution. Example 6, Table 7 reveals the ZEN degrading activity for said enzyme. D1 therefore is also novelty destroying for claims 8, 11-15.
- 1.4 Amended claim 9 now fulfil the requirements of novelty.
- 1.5 Claim 10 lacks novelty in light of D1. D1 relates to the use of alpha/beta hydrolases for hydrolytic cleavage of zearalenone. D1 discloses in claim 9 the *Rhodococcus erythropolis* hydrolase mutant I190V. This protein (Databank entry GSP:BBV28956) comprises a cap domain having 98% identity with residues 161-235 of Seq ID No:6 with a substitution at the position corresponding to residue 190 in Seq ID No:6.

2 Inventive step

- 2.1 D1 can be considered as closest prior art. The document discloses ZEN degradation with several wild-type alpha/beta hydrolases as well as variants thereof. The problem to be solved in light of D1 is the provision of means and methods for the production of improved alpha/beta hydrolase capable of detoxifying ZEN and suited for use as food or feed additive.
- 2.2 The solution suggested in claims 7-11 are alpha/beta hydrolases with increased temperature stability (Figures 3A and 3B) allowing for pelletization of feed at elevated temperature.

Claims 7-11 and subject-matter dependent claims 12-15, however, are not limited to these specific variants. In order to acknowledge that the problem underlying the claimed subject matter has been solved it has to be credible that the effect for which inventive step is acknowledged (increased temperature stability) is achieved by substantially all claimed embodiments. This is presently not the case.

- 2.3 Also, even if the experimental setup to produce these alpha/beta hydrolase was not based on a random mutagenetic approach but involved careful consideration of the enzyme structure : function relation, this rational is not relevant for the products claimed in claims 7-11. The claimed variants per se have to fulfil the requirements of inventive step.

Re Item VII

Certain defects in the international application

The wording of claim 1 implies the causative effect between a mutation leading to a more negative hydropathy index and the increased enzyme stability. Such an effect has not been rendered plausible by the present application.

Re Item VIII

Certain observations on the international application

3 Clarity

Claims 5 and 11 seem to lack conciseness (Article 6 PCT) due to their references to preceding claims. Since the scope of said claims is unclear, novelty can presently not be acknowledged.

Claim 5, which refers to a hydrolase which comprises the cap domain of Seq ID No:1 with substitutions e.g. F183Y or V197C. The residues in Seq ID No:1 however are A183 and A197.

The same applies to claim 11 which refers to a hydrolase which comprises the cap domain of Seq ID No:6 with substitutions e.g. P190H. The residue in Seq ID No:6 however is I190.