

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference CABO-016-EP-WO	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/EP2023/062397	International filing date (day/month/year) 10.05.2023	Priority date (day/month/year) 10.05.2022	
International Patent Classification (IPC) or national classification and IPC INV. C07K14/415			
Applicant Cabosse Naturals NV			
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of <u>6</u> sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ a total of <u>6</u> sheets, as follows: ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions). ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2). ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)). b. ☐ a separate electronic file containing a sequence listing (<i>sent to the International Bureau only</i>)			
4. This report contains indications relating to the following items: ☒ Box No. I Basis of the report ☐ 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 Article 35(2) 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			
Date of submission of the demand 08.03.2024		Date of completion of this report 24.07.2024	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0		Authorized officer Bilang, Jürg Telephone No. +49 89 2399-8707 	

- 1 The present application is concerned with an antimicrobial peptide derived from *Theobroma cacao* vicillin.

Re Item V

- 2 Reference is made to the following documents:

D1 WO 02/086125 A2

D2 WO 98/27805 A1

D3 JOHN P. MARCUS ET AL: "Peptide Fragments From Plant Vicilins Expressed in Escherichia Coli Exhibit Antimicrobial Activity In Vitro",
PLANT MOLECULAR BIOLOGY REPORTER.,
vol. 26, no. 2, April 2008, pages 75-87

- 3 D1 is concerned with artificial cocoa flavour. It discloses peptides derived from cocoa and their use for the manufacture of cocoa flavour (p. 5, l. 19-20). The peptide disclosed in D1 with SEQ ID NO: 2 is 100% identical to residues 2-100 of SEQ ID NO: 2 and comprises two additional amino acids at the C terminal end.

D2 discloses antimicrobial peptides derived from vicilin, originally identified in *Macadamia*. The document also discloses the sequence of Cocoa vicilin (SEQ ID NO: 7) and defines two peptides which meet the general motif of cysteine residues as defined in D2 (p.4). The peptide disclosed in the present application covers both peptides disclosed in D2.

Similarly, D3 discloses the antimicrobial peptides derived from *Macadamia* (MiAMP1 a to d) and *Theobroma* (TcAMP1a and TcAMP1b). The peptide comprising the four motifs from *Macadamia* (MiAMPall) has less activity than the peptides comprising only one motif (figure 2 and table 2).

- 4 Claim 1 is directed to a peptide which has an amino acid sequence according to SEQ ID N° 1 or exhibits at least 98% sequence identity to SEQ ID N° 2 or differs with maximally 2 amino acids from the sequence according to SEQ ID N° 2.

The peptide disclosed in D1 differs from SEQ ID NO: 1 in the lack of the N-terminal tyrosine residue. It differs from SEQ ID NO: 2 in the the lack of the N-terminal tyrosine residue and the lysine and asparagine at the C-terminus and therefore has less than 98% overall identity to SEQ ID NO: 2.

Novelty (Article 33(2) PCT) of claim 1 therefore can be acknowledged.

- 5 The remaining claims are considered novel by reference to claim 1 (Article 33(2) PCT).
- 6 It is shown in the present application that the claimed peptide, which comprises both cysteine motifs (TcAMP1a and TcAMP1b) has a higher activity than the individual peptides TcAMP1a or TcAMP1b. In view of the results for the *Macadamia* homolog presented in D3, this effect was not predictable. Inventive step for claims 1 to 26 therefore can be acknowledged (Article 33(3) PCT).

Box No. I Basis of the report

1. With regard to the **language**, this report is based on
- ☒ 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 report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

Description, Pages

1-49 as originally filed

Sequence listings, SEQ ID NO

1-25 as originally filed

Claims, Numbers

1-26 filed with the demand for preliminary international examination

Drawings, Sheets

1/4-4/4 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 report has been established as if (some of) the amendments annexed to this report and 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*):

5. ☐ This report has been established:
- ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
 - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).
6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
- ☒ A top-up search was carried out by this Authority on 03.04.2024 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
 - ☐ Additional relevant documents have been discovered during the top-up search.
 - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.
7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* If item 4 applies, some or all of those sheets may be marked "superseded".

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>1-26</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-26</u>
	No: Claims	
Industrial applicability (IA)	Yes: Claims	<u>1-26</u>
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box 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.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination,
 - ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
 - c. ☐ furnished to this Authority as an amendment* under PCT Article 34 on .
 2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established to the extent that a meaningful opinion referred to in Article 33(1) could be formed without a WIPO Standard ST.26 compliant sequence listing.
 3. Additional comments:
- * *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

CLAIMS

1. A peptide, wherein said peptide has an amino acid sequence according to SEQ ID N° 1 or that exhibits at least 98% sequence identity to SEQ ID N° 2 or that differs with maximally 2 amino acids from the sequence according to SEQ ID N° 2.
2. Peptide according to claim 1, wherein said peptide is a peptide according to SEQ ID N° 1 or N° 2.
3. The peptide according to claim 1 or 2, wherein said peptide is fused to a signal peptide at its N terminus
4. The peptide according to claim 3, wherein said signal peptide has a sequence identity of 95% to SEQ ID N° 3 or has a sequence according to SEQ ID N° 3.
5. The peptide according to any of the previous claims 1 to 4 wherein, said peptide has antibacterial and/or antifungal activity.
6. The peptide according to claim 5, wherein said activity is against Gram-positive and Gram-negative bacteria and/or fungi, preferably hyphal fungi and yeasts.
7. The peptide according to any of the previous claims wherein, said peptide is derivable from or isolated from *Theobroma cacao*, preferably *Theobroma cacao* variety CCN-51.
8. The peptide according to any of previous claims, wherein said peptide is a recombinant or a synthetic peptide.
9. A composition comprising a peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 and an excipient.
10. The composition of claim 9 wherein said peptide comprises a signal peptide having a sequence identity of at least 95% to SEQ ID N° 3 or has a sequence according to SEQ ID N° 3.
11. The composition according to any of the claims 9 or 10, wherein said composition is a liquid, a semisolid, a solid, or in gaseous form and/or wherein said composition is in a dosage form of a tablet, capsule, powder, granulate, aerosol, paste, syrup, suspension, emulsion or solution.
12. Peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 or a composition according to any of the claims 9 to 11 for therapeutic use.
13. The peptide or composition for therapeutic use according to claim 12, wherein said peptide is fused to a signal peptide at its N-terminus, said signal peptide

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preferably having a sequence identity of at least 95% to SEQ ID N° 3 or having a sequence according to SEQ ID N° 3.

14. Peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 or composition according to claims 9 to 11 for use in the treatment of bacterial and/or fungal infections in a subject in need thereof.
15. The peptide or composition for use according to claim 14, wherein said peptide is fused to a signal peptide at its N-terminus, preferably having a sequence identity of 95% to SEQ ID N° 3 or having a sequence according to SEQ ID N° 3.
16. The peptide or composition for use according to claims 14 to 15, wherein said subject is a human or animal.
17. Use of a peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 or composition according to claim 9 to 11, in crop protection, as a feed or food additive, a preservative, as a decontaminant or in cosmetical applications.
18. Use according to claim 17, wherein said peptide is fused to a signal peptide at its N-terminus, said signal peptide preferably having a sequence identity of 95% to SEQ ID N° 3 or having a sequence according to SEQ ID N° 3.
19. Use according to any of the claims 17 to 18, wherein said peptide or composition is used for controlling a pathogen in a plant, preferably in a crop.
20. Use according to claim 19, wherein said pathogen is selected from the group of Gram-positive bacteria, Gram-negative bacteria, and/or fungi.
21. Method for producing a peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2, wherein said peptide is produced by peptide recombination techniques, by synthesis, or by extraction or precipitation from *Theobroma cacao*.
22. The method according to claim 21, wherein the peptide is extracted from cocoa beans slurry.
23. A vector comprising a coding sequence for a peptide having a sequence identity of at least 95% with SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2, and optionally fused to a sequence for an N terminal signal peptide, said signal peptide sequence preferably having a sequence identity of 95%

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to SEQ ID N° 3, wherein said vector is designed to allow expression of said peptide in an expression system.

24. A transgenic plant expressing a peptide according to any of the claims 1 to 8.

5 25. A method of producing the transgenic plant of claim 24, wherein said method comprises the step of introducing a nucleic acid encoding for said peptide into a plant cell thereby producing a transformed plant cell expressing said peptide and regenerating the transformed plant cell into a transgenic plants.

10 26. A seed of a plant coated with the peptide or composition according any of the claims 1 to 11.

CLAIMS

1. A peptide, wherein said peptide has an amino acid sequence according to SEQ ID N° 1 or that exhibits at least 98% sequence identity to SEQ ID N° 2 or that differs with maximally 2 amino acids from the sequence according to SEQ ID N° 2.
2. Peptide according to claim 1, wherein said peptide is a peptide according to SEQ ID N° 1 or N° 2.
3. The peptide according to claim 1 or 2, wherein said peptide ~~comprises~~ is fused to a signal peptide at its N terminus
4. The peptide according to ~~any of the previous claims 1 to~~ claim 3, wherein said signal peptide has a sequence identity of 95% to SEQ ID N° 3 or has a sequence according to SEQ ID N° 3.
5. The peptide according to any of the previous claims 1 to 4 wherein, said peptide has antibacterial and/or antifungal activity.
6. The peptide according to claim 5, wherein said activity is against Gram-positive and Gram-negative bacteria and/or fungi, preferably hyphal fungi and yeasts.
7. The peptide according to any of the previous claims wherein, said peptide is derivable from or isolated from *Theobroma cacao*, preferably *Theobroma cacao* variety CCN-51.
8. The peptide according to any of previous claims, wherein said peptide is a recombinant or a synthetic peptide.
- ~~9. The peptide according to any of the previous claims, wherein said peptide is provided with an affinity tag.~~
- ~~10.9.~~ 10.9. A composition comprising a peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 and an excipient.
- ~~11.10.~~ 11.10. The composition of claim ~~9~~ 10 wherein said peptide comprises a signal peptide having a sequence identity of at least 95% to SEQ ID N° 3 or has a sequence according to SEQ ID N° 3.
- ~~12.11.~~ 12.11. The composition according to any of the claims ~~9~~ 10 or ~~10~~ 11, wherein said composition is a liquid, a semisolid, a solid, or in gaseous form and/or wherein said composition is in a dosage form of a tablet, capsule, powder, granulate, aerosol, paste, syrup, suspension, emulsion or solution.
- ~~13.12.~~ 13.12. Peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence

according to SEQ ID N° 1 or SEQ ID N° 2 or a composition according to any of the claims ~~9~~10 to ~~11~~12 for therapeutic use.

~~14.13.~~ The peptide or composition for therapeutic use according to claim ~~12~~13, wherein said peptide ~~comprises~~is fused to a signal peptide at its N-terminus, said signal peptide preferably having a sequence identity of at least 95% to SEQ ID N° 3 or havings a sequence according to SEQ ID N° 3.

~~15.14.~~ Peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 or composition according to claims ~~10-9~~ to ~~11~~12 for use in the treatment of bacterial and/or fungal infections in a subject in need thereof.

~~16.15.~~ The peptide or composition for use according to claim ~~14~~15, wherein said peptide ~~said peptide~~ is fused to ~~comprises~~ a signal peptide at its N-terminus, preferably having a sequence identity of 95% to SEQ ID N° 3 or havings a sequence according to SEQ ID N° 3.

~~17.16.~~ The peptide or composition for use according to claims ~~15~~14 to ~~16~~15, wherein said subject is a human or animal.

~~18.17.~~ Use of a peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2 or composition according to claim ~~9~~10 to 11, in crop protection, as a feed or food additive, a preservative, as a decontaminant or in cosmetical applications.

~~19.18.~~ Use according to claim ~~17~~18, wherein ~~said peptide~~ said peptide ~~comprises~~is fused to a signal peptide at its N-terminus, said signal peptide preferably having a sequence identity of 95% to SEQ ID N° 3 or havings a sequence according to SEQ ID N° 3.

~~20.19.~~ Use according to any of the claims ~~17~~18 to ~~19~~18, wherein said peptide or composition is used for controlling a pathogen in a plant, preferably in a crop.

~~21.20.~~ Use according to claim ~~19~~20, wherein said pathogen is selected from the group of Gram-positive bacteria, Gram-negative bacteria, and/or fungi.

~~22.21.~~ Method for ~~obtaining~~producing a peptide exhibiting at least 95% sequence identity to SEQ ID N° 1 or SEQ ID N° 2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2, wherein said peptide is ~~a~~ produced by recombinant peptide recombination techniques, by synthesis~~a synthetic peptide~~, or ~~is extracted by~~ extraction or ~~precipitated~~precipitation from *Theobroma cacao*.

~~23.22.~~ The method according to claim ~~21~~22, wherein the peptide is extracted from cocoa beans slurry.

~~24.23.~~ A vector comprising a coding sequence for a peptide having a sequence identity of at least 95% with SEQ ID N° 1 or SEQ ID N°2 or that differs with maximally 5 amino acids from the sequence according to SEQ ID N° 1 or SEQ ID N° 2, and optionally ~~comprising~~ fused to a sequence for an N terminal signal peptide, said signal peptide sequence preferably having a sequence identity of 95% to SEQ ID N° 3, wherein said vector is designed to allow expression of said peptide in an expression system.

~~25.24.~~ A transgenic plant expressing a peptide according to any of the claims 1 to ~~8~~9.

~~26.25.~~ A method of producing the transgenic plant of claim ~~24~~25, wherein said method comprises the step of introducing a nucleic acid encoding for said peptide into a plant cell thereby producing a transformed plant cell expressing said peptide and regenerating the transformed plant cell into a transgenic plants.

~~27.26.~~ A seed of a plant coated with the peptide or composition according any of the claims 1 to ~~11~~12.