PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

FOR FURTHER ACTION
See paragraph 2 below

Applicant's or agent's file reference
see form PCT/ISA/220

International application No. PCT/ FI/2019/050199
International filing date (day/month/year) 11.03.2019
Priority date (day/month/year) 19.03.2018

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/63 C12N9/10 C12N15/82

Applicant
TEKNOLOGIAN TUTKIMUSKESKUS VTT OY

1. This 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 43bis.1(a)(i) with regard to novelty, inventive step and 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

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:
European Patent Office
D-80296 Munich
Tel. +49 89 2399 - 0
Fax: +49 89 2399 - 4465

Date of completion of this opinion see form PCT/ISA/210

Authorized Officer
Weinberg, Suzanna
Telephone No. +49 89 2399-0

Form PCT/ISA237 (Cover Sheet) (January 2015)
Box No. I  Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of:
   (X) 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 (Rules 12.3(a) and 23.1(b)).

2. ( ) 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 43bis.1(a))

3. (X) With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of a sequence listing:
   a. (X) forming part of the international application as filed:
      (X) in the form of an Annex CST.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 CST.25 text file.
   c. ( ) furnished subsequent to the international filing date for the purposes of international search only:
      ( ) in the form of an Annex CST.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).

4. ( ) 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.

5. Additional comments:

Box No. II  Priority

1. (X) The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.

2. ( ) This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
Box No. V  Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)  
Yes: Claims 1-18
No: Claims

Inventive step (IS)  
Yes: Claims
No: Claims 1-18

Industrial applicability (IA)  
Yes: Claims 1-18
No: Claims

2. Citations and explanations

see separate sheet

Box No. VI  Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

and/or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

see form 210
The present application is directed to the heterologous expression of the psilocybin biosynthetic enzymes PsiD, PsiH, PsiK and PsiM in a host cell, and production of psilocybin by the host cell.

Reference is made to the following document:


**Re Item V**

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

**Novelty**

**3.1** The present application meets the criteria of Article 33(2) PCT, because the subject-matter of **Claims 1-18** appears to be new.

**3.2** None of the cited prior art discloses expression of the psilocybin biosynthetic enzymes PsiD, PsiH, PsiK and PsiM in a host cell, and hence the subject-matter of the independent **Claims 1, 16 and 18**, as well as their dependent claims, is new.

**Inventive step**

**3.3** The present application does not meet the criteria of Article 33(3) PCT, because the subject-matter of **Claims 1-18** does not involve an inventive step.

**3.4** **Claim 1** is directed to a recombinant host cell comprising and capable of expressing heterologous polynucleotides encoding PsiD, PsiH, PsiK and PsiM, wherein the cell is capable of producing psilocybin.

**D1** discloses that the four enzymes for psilocybin biosynthesis are PsiD, PsiH, PsiK and PsiM (abstract). **D1** discloses the *in vitro* production of psilocybin using the enzymes and with tryptophan as a starting substrate (Figures 3 and 4). **D1** teaches that this information sets the stage for the heterologous production of psilocybin with engineered microbial hosts (page 12355, left column, last sentence).

The host cell of **Claim 1** differs from **D1** only in that it defines the host cell suggested by **D1**.
Since the host cell of Claim 1 is clearly suggested by D1, the skilled person could, and would, consider providing such a host cell, and Claim 1 lacks an inventive step over D1 alone.

Since the features of dependent Claims 2-5 and 11, and related aspects of Claims 13, 14, 17 and 18 relate only to standard variants in the field, which variants are not associated with any surprising technical effect over the disclosure of D1, the host cell of these claims also lacks an inventive step.

3.5 Claim 5 relates to the inclusion of a further genetic element in the cell to increase biosynthetic production of L-tryptophan in the cell. However the identity of the genetic element is not defined. As such, the feature is merely a formulation of a technical goal, lacking the technical feature which could represent a means for achieving the goal. As such, no inventive step can be acknowledged over D1 alone.

3.6 Claims 6-10, 12, 15 and 16 define the identity of the further genetic element in terms of the specific enzyme encoded. The Examples disclose data relating to cells expressing one or some of these enzymes, namely ARO4, Trp2 and Trp3.

Although the applicant considers that a strain expressing only ARO4 had increased L-tryptophan pathway flux on day 1 of cultivation (Figure 7), the data do not clearly support this conclusion. The data provided are not unambiguously numerical, and there is no indication of the statistical deviation; nonetheless it appears that for the background strain (BS), there is great variation in tryptophan production between day 1 and day 2. The variation in production for the A4M strain (i.e. expressing ARO4), is similar, such that no clear increase in tryptophan production on either of the days can be identified.

Furthermore, for the data presented in Figures 9A, 9B, 10A and 10B, all strains comprising the A4M designation (i.e. comprising the ARO4 gene) produce less psilocybin than the background strain, where the background strain comprises only the genes for psilocybin production.

Consequently, it appears that merely adding any gene involved in tryptophan production is not suitable for increasing tryptophan production in a host cell, such that psilocybin production is increased. Rather, the specific genes are an essential feature of solving the technical problem of how to increase biosynthetic production of L-tryptophan in the cell, such that psilocybin production is increased.
Subject-matter relating to the psilocybin-producing cell in which tryptophan production is increased, wherein the increase in tryptophan production is achieved by expression of the Trp2 and Trp3 genes, appears to represent a solution to the technical problem, which solution is not taught or suggested by the prior art and hence is considered to involve an inventive step.

However, since there is no claim which is restricted to this subject-matter, there is no claim for which inventive step can be acknowledged over D1 alone.