

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 2024-86-WO	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/EP2024/071694	International filing date (day/month/year) 31.07.2024	Priority date (day/month/year) 31.07.2023	
International Patent Classification (IPC) or national classification and IPC INV. A61K35/741			
Applicant SIGNIFER S.L.			
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>10</u> sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ a total of <u>9</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which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).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 ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in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 30.05.2025		Date of completion of this report 24.07.2025	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0		Authorized officer Böhmerova, Eva Telephone No. +49 89 2399-7859 	

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

1-42 as originally filed

Claims, Numbers

1-6 filed in electronic form on 26-06-2025

Drawings, Sheets

1/7-7/7 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 66.4bis 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 15.07.2025 (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>1-6</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-6</u>
	No: Claims	
Industrial applicability (IA)	Yes: Claims	<u>1-6</u>
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V.

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

1 Cited documents

1.1 Reference is made to the following documents; the numbering will be adhered to in the rest of the procedure. Unless indicated otherwise, reference is made to the passages cited in the search report.

- D1 CN 113 925 884 A (CHAMBIO CO LTD) 14 January 2022 (2022-01-14)
- D2 GUENICHE AUDREY ET AL: "Advances in Microbiome-Derived Solutions and Methodologies Are Founding a New Era in Skin Health and Care", PATHOGENS, vol. 11, no. 2, 20 January 2022 (2022-01-20), page 121, XP055943801, DOI: 10.3390/pathogens11020121
- D3 WO 2013/153358 A1 (UNIV MANCHESTER [GB]) 17 October 2013 (2013-10-17)
- D4 CN 107 184 958 A (TAICANG YOUHUO BIOTECHNOLOGY CO LTD) 22 September 2017 (2017-09-22)
- D5 US 2022/096573 A1 (LIN MEEI-YN [TW] ET AL) 31 March 2022 (2022-03-31)
- D6 KR 2022 0109664 A (NONGHYUP FEED INC [KR]) 5 August 2022 (2022-08-05)
- D7 BRANDI JESSICA ET AL: "Exploring the wound healing, anti-inflammatory, anti-pathogenic and proteomic effects of lactic acid bacteria on keratinocytes", SCIENTIFIC REPORTS, vol. 10, no. 1, 1 December 2020 (2020-12-01), XP055884268, DOI: 10.1038/s41598-020-68483-4
Retrieved from the Internet:
URL:<https://www.nature.com/articles/s41598-020-68483-4.pdf>

- D8 CN 104 472 887 A (PIZHOU CITY XIAOHE TECHNOLOGY DEV CO LTD ET AL.) 1 April 2015 (2015-04-01)
- D9 WO 2018/227146 A1 (ALLERGYINTELLECT INC [US]) 13 December 2018 (2018-12-13)
- D10 WO 2012/170915 A1 (PROTHERA INC [US]; OLMSTEAD STEPHEN F [US]) 13 December 2012 (2012-12-13)
- D11 WO 2021/137491 A1 (SHIM MIN BO [KR]) 8 July 2021 (2021-07-08)

2 Disclosure of the cited documents

- 2.1 The disclosure of the cited documents is summarised herein below. The bacterial strains present in the postbiotic composition as currently claimed are indicated in bold. The effects of the bacterial strains are underlined.
- 2.2 D1 discloses a postbiotic extract for use in promoting skin regeneration and antiaging effects [abstract] wherein the probiotic is selected from the group consisting of *Bacillus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Bifidobacterium* spp., *Saccharomyces* spp., and combinations thereof [claims 1, 4]. D1 further discloses that postbiotics are defined as inactivated probiotics, non-viable probiotics or probiotic lysates ([0006]), and that the currently most commonly used probiotics include: *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Lactococcus*, *Saccharomyces* and *Streptococcus* ([0004]). In Example 1, extracts of the bacterial strains *Lactobacillus plantarum* CB102, ***Lactobacillus acidophilus*** JCM1132, ***Lactobacillus casei*** JCM1134, *Bifidobacterium bifidum* JCM1255, ***Bifidobacterium lactis*** JCM10602 and *Bifidobacterium longum* CB108 were prepared. Example 3 discloses an in vitro study of postbiotic extracts of ***Lactobacillus plantarum*** and ***Bifidobacterium longum*** both showing an effect in promoting skin regeneration by measuring the levels of TGF- β secretion and cell proliferation in treated Hs68 cells.
- 2.3 D2 discloses the use of postbiotics in cosmetics wherein the postbiotics can be ferments, lysates, extracts, filtrates or any combination of these ingredients that are not living but which have been obtained by means of probiotic bacteria [page 11, paragraphs 5-6]. D2 teaches that ***Lactobacillus paracasei*** NCC2461 (ST11) conferred benefits to the skin to reinforce the skin barrier function and decrease skin sensitivity and dandruff conditions. D2 further teaches that bacterial extracts from ***Bacillus coagulans***, ***L. casei***, and ***L. acidophilus*** have

- antimicrobial properties that support skin healing, that **L. salivarius** lysates accelerated re-epithelisation by inducing keratinocyte migration, that lysates of **L. rhamnosus** improved the skin barrier function in a reconstructed human epidermis, and that **S. thermophilus** extracts are able to increase ceramide production and improve skin hydration, and that **Lactobacillus acidophilus** extract has been shown in vitro to scavenge reactive oxygen species following UVB-induced oxidative stress in keratinocytes [page 12, paragraphs 4- 9].
- 2.4 D3 discloses a probiotic bacterium or a lysate thereof, wherein the bacterium is **Lactobacillus rhamnosus** [claim 1-2] for use in the treatment or repair of the skin barrier [claim 2] more specifically of wound healing, psoriasis, dermatitis or acne [claim 5]. Example 3 teaches the preparation of probiotic lysates from 10⁸cfu/ml **L. rhamnosus**, showing increases in TEER and increased expression of TJ proteins in keratinocytes suggesting a role in enhancing overall skin barrier function [page 42, lines 6-19; page 48, paragraph 4].
- 2.5 D4 discloses a composition comprising metabolites of the following probiotics: Bifidobacterium, Lactobacillus, Bacillus, and **Saccharomyces cerevisiae** for use in treating pig parasites and bacterial skin diseases [claims 1,3]. The examples show a preparation of metabolites of **Lactobacillus acidophilus** and **Bacillus subtilis** for applying to the pig skin to restore the pig's epidermis and treat parasites and bacterial skin diseases [paragraph 0030].
- 2.6 D5 discloses a postbiotic composition to improve skin condition with a probiotic selected from the group of **Lactobacillus acidophilus**, **Lactobacillus casei**, **Bifidobacterium lactis** and combinations thereof [claim 4]. Six postbiotics extracts comprising Lactobacillus plantarum, **Lactobacillus acidophilus**, **Lactobacillus casei**, Bifidobacterium bifidum, **Bifidobacterium lactis**, and Bifidobacterium longum, respectively, were prepared [example 1]. The postbiotic extracts were shown to be effective in inducing TGF-beta secretion in Hs68 cells (human foreskin cells), and improving proliferation of skin cells, and hence promote skin regeneration, enhance skin quality or appearance and prevent skin ageing [column 14, paragraph 1].
- 2.7 D6 discloses the use of a probiotic bioactive, cosmetic or pharmaceutical composition comprising **Bacillus licheniformis** for preventing or improving skin inflammation [claim 1, 4-5 and 10]. D6 shows concentration-dependent anti-oxidant effects of Bacillus licheniformis with a decrease of NO production by INOS in macrophages stimulated with LPS [Table 4; paragraphs 0093-0096]. Furthermore, [paragraphs 0143 and 163] teach the preparation of a **Bacillus licheniformis** dry powder to be used in the preparation of a lotion of inhibiting

- and improving skin inflammation.
- D6 further shows a decrease of skin inflammatory transcription factors and cytokines by TNF- α by **Bacillus licheniformis** in raw 264.7 macrophages, a model of skin inflammation of atopic dermatitis [paragraph 0101]. Figure 2 shows that **Bacillus licheniformis** decrease protein expression of NFAT5 and P65 in cells treated with TNF- α , leading to a decrease of IL-6 and IL-1 β inflammatory cytokines [paragraph 0101].
- 2.8 D7 discloses the wound healing, anti-inflammatory and anti-pathogenic effects of lactic acid bacteria on keratinocytes [Abstract]. D7 analyses lysates of **Lactobacillus paracasei** (SGL 04) and **Lactobacillus casei** (SGL 15) and their wound healing and anti-inflammatory effects [page 2, paragraph 3]. **L. paracasei** SGL 04 and **L. casei** SGL 15 show effects on tissue regeneration and wound healing in vitro in HaCaT keratinocytes cells, namely they show re-epithelization effects (%) [Figure 1] and increased migration and proliferation of these cells [Figure 2]. **L. paracasei** SGL 04 and **L. casei** SGL 15 also showed anti-inflammatory effects in HaCaT keratinocyte cells, namely both **L. casei** and **L. paracasei** reduced IL-8 production while **L. paracasei** also significantly reduced the production of MCP-1 and RANTES from keratinocytes [Figure 3].
- 2.9 D8 discloses a probiotic feed additive comprising **Bacillus coagulans**, **Bacillus subtilis**, **Bacillus licheniformis**, **Streptococcus thermophilus**, **Lactobacillus acidophilus**, **Lactobacillus bulgaricus** and **Saccharomyces** yeast [claim 2]. The feed is taught to act as a therapeutic agent for diseases such as dermatitis [page 4, paragraph 0009]. Examples 4-9 teach that the bacterial composition contains bacterial powder weighing between 1.4-2.8 kg of **Bacillus coagulans**, 2.2-4.4 kg of **Bacillus subtilis**, 1.6-3.2 kg of **Bacillus licheniformis**, 2-4 kg of **Streptococcus thermophilus**, 1.4-2.8 kg of **Lactobacillus acidophilus**, 1.4-2.8 kg of **Lactobacillus bulgaricus** and 5.1-6.8 kg of yeast. No effects of the composition are shown.
- 2.10 D9 discloses a composition comprising a probiotic compound such as **Bifidobacterium animalis** (synonym of **Bifidobacterium lactis**), , **Lactobacillus rhamnosus**, and **Bacillus mesentericus** [claims 4, 5] directed at the treatment of atopic conditions such as dermatitis and chronic idiopathic urticaria [claim 6]. Examples 1-3 show the effects of compositions comprising *inter alia* **L. rhamnosus**, **L. acidophilus**, or **B. subtilis** on the treatment of dermatitis.

- 2.11 D10 discloses a probiotic composition for use in the treatment of irritable bowel syndrome or gastritis [claim 1]. D8 further teaches the treatment of abdominal cramps and constipation by administering a probiotic formulation containing **Lactobacillus paracasei** 0.5 billion CFU, **Bifidobacterium lactis** 1.0 billion CFU, **Streptococcus thermophilus** 1.0 billion CFU, **Lactobacillus bulgaricus** 1.0 billion CFU, **Lactobacillus casei** 2.5 billion CFU, **Lactobacillus acidophilus** 3.0 billion CFU and **Lactobacillus rhamnosus** 6.0 billion CFU [Example 2].
- 2.12 D11 discloses an anti-inflammatory composition comprising probiotics and antibiotics [claim 1] wherein the probiotic is one of **Lactobacillus casei**, **Lactobacillus acidophilus**, **Lactobacillus paracasei**, **Lactobacillus rhamnosus** [claim 3] or **Bacillus subtilis** or **B. licheniformis** [claim 4] for the treatment of dermatitis or atopic dermatitis [claim 12].
Example 2 teaches an anti-inflammatory treatment comprising a probiotic comprising **Lactobacillus casei** var. **Rhamnosus** or **Bacillus subtilis**, and the antibiotic cefaclor. Fig. 8A to 8D show the effect of injecting the composition in the middle of the ear of a patient with otitis. The use of these compositions is shown to improve inflammation of otitis as well as the size of eardrum perforation.

3 Novelty

- 3.1 The present application fulfils the criteria of Article 33(1) PCT, because the subject-matter of claims 1-6 is new in the sense of Article 33(2) PCT. None of the cited documents discloses a composition comprising combined lysates of all the 12 bacterial species as currently claimed, let alone the specific percentages thereof.

4 Inventive step

- 4.1 The present application fulfils the criteria of Article 33(1) PCT, because the subject-matter of claims 1-6 is considered to involve an inventive step in the sense of Article 33(3) PCT.
- 4.2 The problem to be solved by the current application is the provision of an agent for the treatment of skin disorders.
- 4.3 Any of D1-D7, D9 or D11 can be considered as the closest prior art as they relate to postbiotic compositions for use for the same therapeutic purpose as the present application, i.e. treatment of skin conditions. These documents

solve the same technical problem as the current application by compositions comprising one or more of the bacterial strains present in the currently claimed composition (for details, see the disclosure of the cited documents above).

4.4 The technical difference between claim 1 over any of D1-D7, D9 or D11 is that the postbiotic composition as claimed consist essentially of lysates of all twelve bacterial strains: *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus mesentericus*, *Bacillus subtilis*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Saccharomyces cerevisiae* and *Streptococcus thermophilus* in the precise amounts by weight as claimed.

4.5 The examples provided by the present application disclose certain effects of claimed postbiotic composition (see Table 2 in page 29 of the application as filed).

Example 2 shows **tissue regeneration** of the caudal fin of a zebra fish model (after amputation) by feeding the fish the postbiotic composition of Table 2. Three days after the amputation of the zebrafish tail, it was shown that zebrafish larvae which were fed the postbiotic composition showed a better tail tissue regeneration mean value (0.185 mm) compared to the control group fed a standard diet (0.173 mm) [Fig. 5 and Table 7].

Example 2 also shows that the composition has dose-dependent **anti-inflammatory effect** *in vitro* by reducing the levels of **IL-8** and **TNF- α** secretion between concentrations of 250ug/mL to 62.5 ug/ml [Fig. 2].

Example 2 also tests the composition for **antioxidant activity** *in vitro* showing increased production of ROS at concentrations ranging from 500 ug/mL to 3.90625 ug/mL and no significant effect at concentrations lower than 5 ug/mL [Fig. 4 and Table 6].

Example 3 relates to *in vitro* skin irritation (RHE model) and phototoxicity assays (Balb/c 3T3 cells) used to determine the efficacy of the postbiotic composition. The postbiotic composition of Table 2 was considered as non-irritant and nonphototoxic.

4.6 Thus, there is *in vitro* **anti-inflammatory** activity, and *in vivo* **tissue regenerative effect** of the claimed composition shown in the present application.

4.7 The objective technical problem is to provide an alternative postbiotic composition for the treatment of skin disorders.

- 4.8 The question to be answered is whether it was obvious to a person skilled in the art looking for alternative compositions for the treatment of skin disorders to arrive at the currently claimed postbiotic composition.
- 4.9 There is no combination of only two cited documents that discloses all the 12 strains of claim 1. A combination of a minimum of three documents, for example documents D7 + D8 + D9 would be necessary in order to obtain a composition comprising all the 12 bacterial strains. Moreover, the main claim has been restricted to a composition essential consisting of the 12 bacterial strains, wherein the compositions of the prior art comprise further bacterial strains not present in the currently claimed composition. By combining three or more cited documents, a skilled person would have arrived at compositions comprising bacterial strains not present in the currently claimed composition. Furthermore, none of the cited documents discloses the specific concentrations in weight of the strains as currently claimed. Therefore, this specific composition is not suggested, hinted at, or derivable from the cited prior art. The prior art, even when considered in combination, fails to disclose or suggest the claimed combination, either in terms of strain selection, or their specific relative proportions. None of the cited documents individually, or in a combination, teach or motivate the skilled person to select the particular 12 strains and to combine them in the precise weight percentages defined in claim 1. It is considered that, without a hindsight, a skilled person could not have arrived at the currently claimed composition. The subject-matter of claims 1-6 is therefore considered to be inventive.

5 Industrial applicability

- 5.1 Claims 1-6 are industrially applicable.