

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
04 June 2020 (04.06.2020)



(10) International Publication Number
WO 2020/109480 A1

(51) International Patent Classification:

A61K 31/20 (2006.01) *A61K 35/742* (2015.01)
A61K 31/202 (2006.01) *A61P 29/00* (2006.01)
A61K 31/557 (2006.01)

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/EP2019/082935

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

(22) International Filing Date:

28 November 2019 (28.11.2019)

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

18209497.9 30 November 2018 (30.11.2018) EP

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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: PREPARATION FOR USE TO INCREASE THE FORMATION OF ONE OR MORE SPECIALIZED PRO-RESOLVING LIPID MEDIATORS (SPM)

(57) Abstract: The invention relates to preparation comprising at least one omega-3 or omega-6 fatty acid component selected from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), alpha linolenic acid, stearidonic acid, eicosatetraenoic acid, docosapentaenoic acid, linoleic acid, γ -linolenic acid, or arachidonic acid (ARA) and/or derivatives thereof for use to increase the formation of one or more specialized pro-resolving lipid mediators (SPM) by gastrointestinal microorganisms in the gastrointestinal tract of humans or animals wherein the polyunsaturated fatty acid component comprises an omega-3 or omega-6 fatty acid salt.



Preparation for use to increase the formation of one or more specialized pro-resolving lipid mediators (SPM)

The current invention concerns a preparation comprising at least one omega-3 or omega-6 fatty acid component selected from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), alpha
5 linolenic acid, stearidonic acid, eicosatetraenoic acid, docosapentaenoic acid, linoleic acid, γ -linolenic acid, or arachidonic acid (ARA) and/or derivatives thereof for use to increase the formation of one or more specialized pro-resolving lipid mediators (SPM) by microorganisms in the gastrointestinal tract of humans or animals.

Dietary intake of omega-3 fatty acids, namely alpha-linoleic acid (ALA), EPA and DHA, is beneficial
10 for human health, in particular with respect to e.g. the amelioration of rheumatoid arthritis and reduction of cardiovascular disease risk factors [1, 2]. Various seafood products are a source of dietary EPA/DHA, but their consumption is often not sufficient to meet the recommended dietary allowance (typically 500 mg EPA and DHA per day) [3]. This gap is closed by the widespread use of dietary supplements or fortified foods containing omega-3 fatty acids [4]. Dietary supplements
15 are concentrated sources of nutrients or other substances with a nutritional or physiological effect, whose purpose is to supplement the normal diet (www.efsa.europa.eu/en/topics/topic/food-supplements). For example, omega-3 fatty acid supplements often contain either triglycerides or omega-3 ethyl esters of EPA/DHA from fish oil, krill oil, or algae.

Omega-3 fatty acids in general have anti-inflammatory, cardio- and neuroprotective effects [2, 5].
20 Their modes of action involve e.g. direct scavenging of reactive oxygen species, alteration of cell membrane fluidity, which subsequently affects cellular signaling events, modulation of the activity of transcription factors such as PPAR γ and NF κ B that orchestrate the biosynthesis of pro- and anti-inflammatory cytokines, and competitive exclusion of substrates that are converted to proinflammatory cytokines by cyclooxygenases and lipoxygenases.

More recently, several oxygenation products of omega-3 and omega-6 fatty acids have been
25 identified and functionally characterized as crucial mediators of their beneficial health effects, in particular with respect to the amelioration of chronic inflammatory conditions [6]. These products include maresins (MaR), E- and D-series resolvins (RvE and RvD), protectins, lipoxins, and precursors thereof such as 18-hydroxy-eicosapentaenoic acid (18-HEPE), 17-hydroxy-
30 docosahexaenoic acid (17-HDHA), and 17,18-epoxyeicosatetraenoic acid (17,18-EEQ), collectively referred to as specialized pro-resolving lipid mediators (SPM). SPM are endogenously formed by lipoxygenases, cyclooxygenase-2, and cytochrome P450 monooxygenases (CYP450), and act as potent agonists of active inflammation resolution, signaling via G-protein coupled receptors at nanomolar concentrations. The effectiveness of SPM against a multitude of infectious and
35 inflammatory diseases has been demonstrated in studies with rodents [6]. For example, RvE1, RvD2, protectin D1 (PD1), and LXA₄ enhance the clearance of pathogenic *Pseudomonas gingivalis* [7], *E.coli* [8], *Herpes simplex* [9], *Candida* [10], H5N1 Influenza [11].

LXA₄, LXB₄, RvE1, RvE3, RvD1-5, RvD2, PD1, MaR1, MaR2 are protective in models of
40 periodontitis, cystic fibrosis, neuroinflammation, ischemic stroke, Alzheimer's disease [12], atherosclerosis [13], non-alcoholic fatty liver disease [14], corneal injury [15], retinopathy [16],

glaucoma [17], colitis [18], asthma [19, 20], insulin resistance [14], arthritis [21], and pain [22]. Moreover, several precursors of SPM have themselves been shown to exert pro-resolving effects. For example, 18-hydroxy-eicosapentaenoic acid (18-HEPE) counteracts the development of cardiovascular diseases by inhibiting monocyte adhesion to vascular endothelial cells [23] and by
5 inhibiting pressure overload-induced maladaptive cardiac remodeling [24]. Similarly, 17,18-EEQ has cardio-protective, anti-arrhythmic, vasodilatory, and anti-inflammatory properties [5]. Paracrine secretion of ARA-derived 15-HETE by enteric glial cells supports gut barrier function, a process that is impaired in e.g. Crohn's disease [25].

Translation of these promising preclinical findings towards improving human health has however
10 shown to be challenging. Direct delivery of SPM by intravenous or intraperitoneal injection, as has been done in experimental studies, is not feasible for humans, particularly not in the context of preventive approaches. Oral delivery of SPM- or SPM precursor-containing supplements or foods is not reasonable because of their relatively short half-life in biological fluids, which are therefore unlikely to reach their target tissue. In this regard WO 2017/041094 discloses that a concentrated
15 esterified fish oil contains only ~ 0.0005 % 18-HEPE and 17-HDHA, and even enrichment of these SPM precursors by supercritical fluid extraction yields not more than 0.05 % (18-HEPE + 17-HDHA)/total omega-3.

Clinical trials with EPA/DHA have yielded inconclusive or null results, especially for patients with inflammatory bowel diseases, asthma, and traits of the metabolic syndrome [2]. This lack of benefit
20 for humans contrasts with the effective treatment of the respective animal disease models by SPM [6]. We reason that the conversion of omega-3 (and omega-6) to SPM is a crucial step which is decisive for delivering successful outcomes from any interventions aiming to prevent, cure, or treat inflammatory diseases with polyunsaturated fatty acids (PUFA). We also conceive that the SPM-producing machinery is dysfunctional under certain conditions. This idea is supported by findings of
25 reduced (local or circulating) SPM levels in diabetic wounds [26], metabolic syndrome [27], asthma [19, 28], ulcerative colitis [29], Crohn's disease [25], and periodontitis [30], as well as reduced expression or activity of SPM-producing enzymes in severe asthma [28], ulcerative colitis [29], cystic fibrosis [31], periodontitis [30], and Alzheimer's disease [12].

The objective of this invention is therefore to provide a technology that promotes SPM formation
30 inside an organism to provide a benefit to humans and animals suffering from the above-mentioned conditions and that are in need of novel strategies to prevent, ameliorate or cure such and similar conditions, where supplementation of omega-3s alone has yielded little or no success.

This goal is achieved by the invention providing a for use to increase the formation of one or more specialized pro-resolving lipid mediators (SPM) by microorganisms in the gastrointestinal tract of
35 humans or animals.

Biosynthesis of SPM has been described in detail for eukaryotic cells, in particular for granulocytes and monocytes. Macrophages can express all enzymes that are required for SPM biosynthesis; other cell types expressing only selected enzymes can do so together with complementing cells. ALOX5 is found in mast cells, ALOX12 in skin and epithelial cells, ALOX15 in dendritic and enteric
40 glial cells [25], and COX-2 and CYP450 isoforms in epithelial cells.

Given that the gut microbiota determines an individual's response to food ingredients and subsequently modifies health outcomes, we identified it as a target for our technological approach of facilitated endogenous SPM production. Microbiota-targeted strategies in general include the application of prebiotics and probiotics with the intention to modify the composition and activity of the microbiota. Probiotics are live microorganisms, which confer a health benefit on the host when administered in adequate amounts (FAO-WHO; Probiotics in food. Health and nutritional properties and guidelines for evaluation; FAO Food and Nutritional Paper 85, 2006). Prebiotics support the growth of beneficial microorganisms. Prebiotic effects of omega-3 fatty acids have been described [32, 33], but vice versa, possible metabolic impact of gut microbes on omega-3s remained to be determined and are disclosed in this invention. Occurrence of oxygen-consuming enzymes in gut-residing microorganisms is limited; cyclooxygenases and lipoxygenases appear to be absent from gastrointestinal bacteria and archaea, with the exception of a 15-lipoxygenase expressed by pathogenic *Pseudomonas aeruginosa* [34]. CYP450 monooxygenases have been detected in the Genus *Bacillus* [35]. CYP102A1, also named CYP450BM-3, is a bifunctional enzyme found in the species *Bacillus megaterium* that catalyzes the NADPH-dependent hydroxylation of polyunsaturated fatty acids via consecutive oxygenase and reductase activities. This P450 system consists of a polypeptide chain with two different domains, one containing the hemoprotein and the other one containing a FAD-reductase. This bacterial cytochrome P450 class is soluble and obtains the electrons necessary for the reaction mechanism from an NADH-dependent FAD-containing reductase via an iron-sulphur protein of the [2Fe-2S] type [36]. Purified CYP450BM-3 derived from an expression vector construct has been shown to generate 18-HEPE from EPA in a cell-free reaction [37]. However, a possible application of such reaction in a probiotic or synbiotic strategy, wherein a wildtype probiotic strain is the "catalyst" for activation of extracellular EPA/DHA to 18-HEPE or other SPM, and more important, makes these molecules available to the host, has not been described. Furthermore, oxygenation of omega-3 or omega-6 compounds to any other SPM or bioactive lipid mediator by any other probiotic microorganism has thus far not been described.

The present invention is directed to a preparation comprising at least one omega-3 or omega-6 fatty acid component selected from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), alpha linolenic acid, stearidonic acid, eicosatetraenoic acid, docosapentaenoic acid, linoleic acid, gamma-linolenic acid, or arachidonic acid (ARA) and/or derivatives thereof for use to increase the formation of one or more specialized pro-resolving lipid mediators (SPM) by microorganisms in the gastrointestinal tract of humans or animals, wherein the polyunsaturated fatty acid component comprises an omega-3 or omega-6 fatty acid salt. This preparation promotes the formation of various SPM in the intestinal lumen, whereby they become available to the host and exert physiological functions therein.

In an advantageous configuration of the present invention, the formation of SPM results from biochemical oxygenation reactions by gastrointestinal microorganisms.

This new use promotes the formation of various SPM in the intestinal lumen, whereby they become available to the host and exert physiological functions therein. The oxygen required for biosynthesis of SPM from EPA/DHA is available in the intestinal lumen: gas in the human rectum reportably

contains 0.3 – 1.8 % oxygen [38]. Furthermore, a radial partitioning of intraluminal oxygen, increasing from 1 to 40 mmHg pO₂ towards the (vascularized) cecal mucosa, has been described for mice [39], indicating that aerotolerant microbes associated with the mucosa face a relatively oxygen-rich environment that allows for oxygen-dependent biochemical reactions.

- 5 Bacteria of the species *Bacillus megaterium* were found to be especially suitable for this effect. Therefore, the probiotic strain comprises a strain of this species. It is a crucial feature of the invention that the strains lead to extracellular amounts of SPM, which is a prerequisite for eliciting physiological effects on the host. We disclose *Bacillus megaterium*-dependent production of extracellular SPM at nanomolar levels -whereby some SPM are physiologically active at picomolar
- 10 levels [6]-, exceeding those reported for human plasma [27, 40, 41] and partly for human milk [42]. According to the present invention, it is both feasible to use whole cells and lysed bacterial cells, encompassing all components of the bacterial cell. Cell extracts may also be used.

- Bacillus megaterium* has recently been detected in human fecal [43] and saliva [44] samples, showing that these bacteria are residents of the human gut. The invention therefore also covers the
- 15 use of omega-3 or omega-6 components to promote the formation of various SPM in the gastrointestinal tract by gastrointestinal microbiota through e.g. strains of the species *Bacillus megaterium* as naturally occurring gut inhabitants.

- The cells of the strains of the current invention may be present, in particular in the compositions of the current invention, as spores (which are dormant), as vegetative cells (which are growing), as
- 20 transition state cells (which are transitioning from growth phase to sporulation phase) or as a combination of at least two, in particular all of these types of cells. Therefore, in a preferred embodiment, the probiotic strain is present in a dormant form or as vegetative cells.

In the present invention it is preferred that the total amount of the following lipid mediators in the host via their production by gastrointestinal microorganisms is increased:

- 25 17-hydroxy-DHA (17-HDHA), 14-hydroxy-DHA (14-HDHA), 13-hydroxy-DHA (13-HDHA), 7-hydroxy-DHA (7-HDHA), 4-hydroxy-DHA (4-HDHA), 18-hydroxy-eicosapentaenoic acid (18-HEPE), 15-hydroxy-eicosapentaenoic acid (15-HEPE), 12-hydroxy-eicosapentaenoic acid (12-HEPE), 11-hydroxy-eicosapentaenoic acid (11-HEPE), 8-hydroxy-eicosapentaenoic acid (8-HEPE), 5-hydroxy-eicosapentaenoic acid (5-HEPE), 15-hydroxy-eicosatetraenoic acid (15-HETE), 12-hydroxy-
- 30 eicosatetraenoic acid (12-HETE), 11-hydroxy-eicosatetraenoic acid (11-HETE), 8-hydroxy-eicosatetraenoic acid (8-HETE), 5-hydroxy-eicosatetraenoic acid (5-HETE), 9-hydroxyoctadecadienoic acid (9-HODE), 13-hydroxyoctadecadienoic acid (13-HODE), 19Z-docosahexaenoic acid (PDX), protectin D1 (PD1), Aspirin-triggered PD1 (AT-PD1), maresin 1 (MaR1), maresin 2 (MaR2), leukotriene B₄ (LTB₄), t-LTB₄, resolvin D1-5 (RvD1-5), Aspirin-triggered RvD1 (AT-RvD1), resolvin E1 (RvE1), resolvin E3 (RvE3), lipoxin A₄ (LXA₄), lipoxin A₅ (LXA₅), lipoxin B₄ (LXB₄), lipoxin B₅ (LXB₅),

Therefore, in a further preferred embodiment the SPM is selected from 17-HDHA, 14-HDHA, 13-HDHA, 7-HDHA, 4-HDHA, 18-HEPE, 15-HEPE, 12-HEPE, 11-HEPE, 5-HEPE, 15-HETE, 12-

HETE, 11-HETE, 8-HETE, 5-HETE, 9-HODE, 13-HODE, PDX, PD1, AT-PD1, MaR1, MaR2, LTB4, t-LTB4, RvD1-5, AT-RvD1, RvE1, RvE3, LXA4, LXA5, LXB4, LXB5.

In a preferred embodiment, the omega-3 or omega-6 fatty acids are either in the form of free fatty acids, salts, natural triglycerides, fish oil, phospholipid esters or ethyl esters.

- 5 In a further preferred configuration, the fatty acids are selected from the omega-3 fatty acids EPA and DHA or wherein the omega-6 fatty acid component is ARA.

An additional configuration of the present invention is a combination of any of the above-mentioned compositions with 5-Aminolevulinic Acid, a compound that enhances heme biosynthesis [45] and thereby may trigger oxygenase activities of *Bacillus megaterium*.

- 10 In a preferred embodiment the probiotic strain is selected from one or more of the following: *Bacillus megaterium* DSM 32963, DSM 33296 or DSM 33299.

Bacillus megaterium DSM 32963, DSM 33296 and DSM 33299 have been identified by screening of naturally occurring isolates. They have been deposited with the DSMZ on November 27th, 2018 (DSM 32963) and on October 17th, 2019 under the provisions of the Budapest Treaty on the

- 15 International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure under the Accession Number as mentioned before in the name of Evonik Degussa GmbH.

Thus, the *Bacillus megaterium* strain used for the preparation according to the present invention is selected from the following group:

- a) One of the *Bacillus megaterium* strains as deposited under DSM 32963, DSM 33296 and DSM
20 33299 at the DSMZ;
- b) a mutant of the *Bacillus megaterium* strain as deposited under DSM 32963 having all identifying characteristics of the strain DSM 32963, wherein said mutant preferably has a DNA sequence identity to the strain DSM 32963 of at least 95%, preferably at least 96, 97 or 98 %, more preferably at least 99 or 99.5 %;
- 25 c) a preparation of (a) or (b);
- d) a preparation containing an effective mixture of metabolites as contained in (a), (b) or (c).

The *Bacillus megaterium* strain as deposited under DSM 32963 at the DSMZ exhibits the following characterizing sequences:

- a) a 16S rDNA sequence with a sequence identity of at least 99.5 %, above all 100 %, to the
30 polynucleotide sequence according to SEQ ID NO: 1 or SEQ ID NO: 2;
- b) a yqfD sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 3;
- c) a gyrB sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 4;
- 35 d) an rpoB sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 5;

e) a groEL sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 6.

The *Bacillus megaterium* strain as deposited under DSM 33296 at the DSMZ exhibits the following characterizing sequences:

- 5 a) a 16S rDNA sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 13 or SEQ ID NO: 14;
- b) a yqfD sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 15;
- c) a gyrB sequence with a sequence identity of at least 99.5 %, above all 100 %, to the
- 10 polynucleotide sequence according to SEQ ID NO: 16;
- d) an rpoB sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 17;
- e) a groEL sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 18.

15 The *Bacillus megaterium* strain as deposited under DSM 33299 at the DSMZ exhibits the following characterizing sequences:

- a) a 16S rDNA sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 25 or SEQ ID NO: 26;
- b) a yqfD sequence with a sequence identity of at least 99.5 %, above all 100 %, to the
- 20 polynucleotide sequence according to SEQ ID NO: 27;
- c) a gyrB sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 28;
- d) an rpoB sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 29;
- 25 e) a groEL sequence with a sequence identity of at least 99.5 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 30.

Thus, a further subject of the current invention is a *Bacillus megaterium* strain, in particular a *B. megaterium* strain as mentioned before, exhibiting at least one, preferably all, of the following characteristics:

- 30 a) a 16S rDNA sequence with a sequence identity of at least 99 %, preferably at least 99.5 %, more preferably at least 99.8 or 99.9 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 1 or SEQ ID NO: 2, SEQ ID NO: 13 or SEQ ID NO: 14 or SEQ ID NO: 25 or SEQ ID NO: 26;
- b) a yqfD sequence with a sequence identity of at least 99 %, preferably at least 99.5 %, more
- 35 preferably at least 99.8 or 99.9 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 3, SEQ ID NO: 15 or SEQ ID NO: 27;

c) a *gyrB* sequence with a sequence identity of at least 99 %, preferably at least 99.5 %, more preferably at least 99.8 or 99.9 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 4, SEQ ID NO: 16 or SEQ ID NO: 28.

5 Preferably, this *B. megaterium* strain exhibits at least one, more preferably all, of the following further characteristics:

d) a *rpoB* sequence with a sequence identity of at least 99 %, preferably at least 99.5 %, more preferably at least 99.8 or 99.9 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 5, SEQ ID NO: 17 or SEQ ID NO: 29;

10 e) a *groEL* sequence with a sequence identity of at least 99 %, preferably at least 99.5 %, more preferably at least 99.8 or 99.9 %, above all 100 %, to the polynucleotide sequence according to SEQ ID NO: 6, SEQ ID NO: 18 or SEQ ID NO: 30.

Thus, a particular subject of the current invention is also a *Bacillus megaterium* strain, exhibiting the following characteristics:

15 a) a 16S rDNA sequence according to SEQ ID NO: 1 or SEQ ID NO: 2, SEQ ID NO: 13 or SEQ ID NO: 14 or SEQ ID NO: 25 or SEQ ID NO: 26;

b) a *yqfD* sequence according to SEQ ID NO: 3, SEQ ID NO: 15 or SEQ ID NO: 27;

c) a *gyrB* sequence according to SEQ ID NO: 4, SEQ ID NO: 16 or SEQ ID NO: 28.

Preferably, this *B. megaterium* strain exhibits the following further characteristics:

d) an *rpoB* sequence according to SEQ ID NO: 5, SEQ ID NO: 17 or SEQ ID NO: 29;

20 e) a *groEL* sequence according to SEQ ID NO: 6, SEQ ID NO: 18 or SEQ ID NO: 30.

In an advantageous configuration EPA and DHA are either in the form of free fatty acids, salts, natural triglycerides, fish oil, phospholipid esters or omega-3 ethyl esters.

25 EPA and DHA were effectively transformed by probiotic strains to SPM when they were added as fatty acid salts, whose production and application were disclosed previously. WO2016102323A1 describes compositions comprising polyunsaturated omega-3 fatty acid salts that can be stabilized against oxidation. WO2017202935A1 discloses a method for preparing a composition comprising omega-3 fatty acid salts and amines wherein a paste comprising one or more omega-3 fatty acid(s), one or more basic amine(s) and 20% by weight or less water, based on the total weight of
30 the paste, is kneaded until a homogenous paste is obtained.

Therefore, in a preferred configuration of the present invention the omega-3 component comprises an omega-3 fatty acid amino acid salt, wherein the amino acid is chosen from basic amino acids selected from lysine, arginine, ornithine, histidine, citrulline, choline and mixtures of the same.

35 In a further preferred configuration, the amino acid is chosen from basic amino acids selected from lysine, arginine, ornithine, choline and mixtures of the same.

It is most preferable to use amino acid salts of lysine.

Another preferred configuration of the present invention are formulations of omega-3 dispersions (presumably liposomes) to further improve bioavailability to probiotic strains. Such dispersion formulations preferably consist of phospholipid mixtures (e.g. deoiled sunflower lecithin) or defined phospholipids, e.g. Dioleoylphosphatidylcholine (DOPC). Most preferred forms of such dispersion formulations contain free omega-3 fatty acid salts or free omega-3 fatty acids.

Therefore, in this preferred embodiment the polyunsaturated fatty acid component comprises a preparation comprising a dispersion of at least one phospholipid and at least one omega-3 fatty acid.

In a further preferred embodiment, the polyunsaturated fatty acid component comprises a preparation comprising a dispersion of at least one phospholipid and at least one fatty acid salt of a cation with an anion derived from an omega-3 or omega-6 fatty acid. It is particularly preferred to use omega-3 fatty acids.

In an alternative configuration of the present invention the phospholipid is a deoiled phospholipid comprising a phosphatidylcholine content of greater than 40 weight %, preferably 70 weight %, more preferably greater 90 weight % and a phosphatidylethanolamine content of lower than 5 weight %, preferably lower than 1 weight %.

In an alternative embodiment the phospholipid is a non-hydrogenated phospholipid having an oleic and/or linoleic acid content of greater than 70 weight % of total fatty acids.

In a further preferred configuration of the present invention the mass ratio of phospholipid to fatty acid salt is greater than 0.001, preferably greater than 0.05, more preferably greater than 0.01, more preferably greater than 0.09, most preferably greater than 0.39.

In an alternative embodiment the preparation is in the form of a powder or of a liquid that result in colloidal dispersions with mean particle sizes of smaller than 1 μm , preferably smaller than 500 nm, most preferably smaller than 250 nm when mixed with water at a pH value between pH 6.5 and 7.5.

In another embodiment the components are finely dispersed in each other so that both phospholipid and fatty acid salts are present and detectable in amounts of 100 μg and smaller.

A preferred formulation for enteral delivery of a preparation of this invention is a formulation that provides protection against gastric conditions, or a formulation that provides targeted release of the preparation in the small intestine or a formulation that provides targeted release of the preparation in the large intestine. Therefore, in a preferred embodiment, the preparation comprises a coating for delayed release or enteric or colonic release.

In an alternative configuration the preparation according to the present invention comprising an omega-3 fatty acid amino acid salt, is a solid composition.

Working Examples

Example 1: The strains *Bacillus megaterium* DSM 32963, DSM 33296 and DSM 33299 each possess genetic sequences for a cytochrome P450 monooxygenases (CYP450)

The strains *Bacillus megaterium* DSM 32963, DSM 33296 and DSM 33299 were isolated each
 5 from a soil sample from a pristine garden in east Westphalia. They have been deposited with the
 DSMZ on November 27th, 2018 (DSM 32963) and on October 17th, 2019 under the provisions of
 the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the
 Purpose of Patent Procedure under the accession number as mentioned before in the name of
 Evonik Degussa GmbH.

10 The genome sequence of *B. megaterium* DSM 32963 contains a gene [SEQ ID No: 7] encoding a
 protein with an identity of 97,9 % at the amino acid level to P450 BM3 (CYP102A1) of *B.*
megaterium ATCC 14581 (AAA87602.1). This enzyme incorporates both, a P450 oxygenase and a
 NADPH:P-450 reductase[46]. The natural substrates of P450 BM3 were analyzed to be long chain
 15 fatty acids (C12 to C20), which are exclusively hydroxylated at the subterminal positions (ω -1 to ω -
 3)[47].

Lower sequence similarities on amino acid level ranging from 21,1 % to 30,5 % on partial sequence
 hits were observed to sequences identified within the genome sequence of *B. megaterium* DSM
 32963 compared to P450 BM3. These further potential cytochrome genes might have similar
 functions compared to P450 BM3 [Seq ID No: 8 - 12] (see table 1).

Query sequence	Hit	E-value	Score	HSP length	%Identity	%Gaps	Function based on BLAST
CYP102_Bm_AAA87602	DSM 32963_00731 [SEQ ID No 7]	0	5.461,00	1.049,00	97,9	0	Seq ID 7
CYP102_Bm_AAA87602	DSM 32963_00276 [SEQ ID No 8]	8,35E-62	558	568	30,49	7,87	sulfite reductase (NADPH) flavoprotein alpha-component
CYP102_Bm_AAA87602	DSM 32963_02107 [SEQ ID No 9]	4,03E-12	161	187	26,9	6,6	cytochrome P450
CYP102_Bm_AAA87602	DSM 32963_01384 [SEQ ID No 10]	1,08E-10	149	285	24,56	18,42	cytochrome P450
CYP102_Bm_AAA87602	DSM 32963_02065 [SEQ ID No 11]	2,17E-08	130	210	24,38	14,05	cytochrome P450
CYP102_Bm_AAA87602	DSM 32963_02258 [SEQ ID No 12]	5,04E-04	94	292	21,08	21,08	cytochrome P450 (BM1)

20 Table 1: BLASTp of P450 BM3 against protein sequences of *Bacillus megaterium* DSM 32963 (SEQ ID in the hit column refers to the corresponding nucleotide sequence)

The genome sequence of *B. megaterium* DSM 33296 contains a gene [SEQ ID No: 19] encoding a protein with an identity of 98,9 % at the amino acid level to P450 BM3 (CYP102A1) of *B.*

megaterium ATCC 14581 (AAA87602.1). This enzyme incorporates both, a P450 oxygenase and a NADPH:P-450 reductase[46]. The natural substrates of P450 BM3 were analyzed to be long chain fatty acids (C12 to C20), which are exclusively hydroxylated at the subterminal positions (ω -1 to ω -3)[47].

Lower sequence similarities on amino acid level ranging from 21,5 % to 30,7 % on partial sequence hits were observed to sequences identified within the genome sequence of *B. megaterium* DSM 33296 compared to P450 BM3. These further potential cytochrome genes might have similar functions compared to P450 BM3 [Seq ID No: 20 - 24] (see table 2).

Query	Hit	E-value	Score	HSP length	%Identity	%Gaps	Function
CYP102_Bm_AAA87602	DSM 33296_54_CDS_DSM 33296_02126 [SEQ ID no 19]	0,00	5.506,00	1.049,00	98,86	0,00	cytochrome P450
CYP102_Bm_AAA87602	DSM 33296_54_CDS_DSM 33296_02261 [SEQ ID no 20]	1,48 E-61	556,00	556,00	30,70	7,72	sulfite reductase (NADPH) flavoprotein alpha-component
CYP102_Bm_AAA87602	DSM 33296_46_CDS_DSM 33296_01053 [SEQ ID no 21]	2,74 E-12	162,00	195,00	26,34	6,34	cytochrome
CYP102_Bm_AAA87602	DSM 33296_70_CDS_DSM 33296_03886 [SEQ ID no 22]	1,06 E-10	149,00	272,00	24,22	17,39	cytochrome P450
CYP102_Bm_AAA87602	DSM 33296_46_CDS_DSM 33296_01002 [SEQ ID no 23]	1,26 E-8	132,00	226,00	24,91	15,85	cytochrome P450
CYP102_Bm_AAA87602	DSM 33296_46_CDS_DSM 33296_01221 [SEQ ID no 24]	1,03 E-3	91,00	288,00	21,45	21,16	cytochrome P450

Table 2: BLASTp of P450 BM3 against protein sequences of *Bacillus megaterium* DSM 33296 (SEQ ID in the hit column refers to the corresponding nucleotide sequence)

The genome sequence of *B. megaterium* DSM 33299 contains a gene [SEQ ID No: 31] encoding a protein with an identity of 96,1 % at the amino acid level to P450 BM3 (CYP102A1) of *B.*

megaterium ATCC 14581 (AAA87602.1). This enzyme incorporates both, a P450 oxygenase and a NADPH:P-450 reductase[46]. The natural substrates of P450 BM3 were analyzed to be long chain fatty acids (C12 to C20), which are exclusively hydroxylated at the subterminal positions (ω -1 to ω -3)[47].

Lower sequence similarities on amino acid level ranging from 20,9 % to 30,5 % on partial sequence hits were observed to sequences identified within the genome sequence of *B. megaterium* DSM 33299 compared to P450 BM3. These further potential cytochrome genes might have similar functions compared to P450 BM3 [Seq ID No: 32 - 37] (see table 3).

Query sequence	Hit	E-value	Score	HSP length	%Identity	%Gaps	Function based on BLAST
CYP102_Bm_AAA87 602	DSM 33299_122_CDS_D SM 33299_05006 [SEQ ID no 31]	0,00	5.390,0 0	1.049,0 0	96,09	0,00	cytochrome P450
CYP102_Bm_AAA87 602	DSM 33299_58_CDS_D SM 33299_02626 [SEQ ID no 32]	6,77 E-62	558,00	568,00	30,49	7,87	sulfite reductase (NADPH) flavoprotein in alpha-component
CYP102_Bm_AAA87 602	DSM 33299_123_CDS_D SM 33299_05123 [SEQ ID no 33]	2,56 E-12	162,00	195,00	25,85	6,34	cytochrome P450
CYP102_Bm_AAA87 602	DSM 33299_15_CDS_D SM 33299_00711 [SEQ ID no 34]	1,55 E-11	156,00	272,00	25,16	17,39	cytochrome P450
CYP102_Bm_AAA87 602	DSM 33299_123_CDS_D SM 33299_05165 [SEQ ID no 35]	4,26 E-8	127,00	210,00	23,65	13,28	cytochrome P450
CYP102_Bm_AAA87 602	DSM 33299_6_CDS_DS M 33299_00407 [SEQ ID no 36]	2,54 E-6	113,00	192,00	25,56	14,35	cytochrome P450
CYP102_Bm_AAA87 603	DSM 33299_87_CDS_D SM 33299_03167 [SEQ ID no 37]	7,19 E-5	101,00	280,00	20,94	21,53	cytochrome P450

Table 3: BLASTp of P450 BM3 against protein sequences of *Bacillus megaterium* DSM 33299 (SEQ ID in the hit column refers to the corresponding nucleotide sequence)

Example 2: Preparation of omega-3 fatty acid dispersions with dioleoylphosphatidylcholine

5 (DOPC) in phosphate buffer

To prepare formulations of omega-3 fatty acid dispersions 0.8 g of dioleoylphosphatidylcholine (DOPC, Lipoid GmbH) were dissolved in 1 ml ethanol. 0.2 g of fish oil (Omega-3 1400, Doppelherz®), omega-3 ethyl ester (PronovaPure® 500:200 EE, BASF) or lysine salt of free omega-3 fatty acid in form of omega-3 lysine salt (AvailOm®, Evonik) were added and dissolved. In the case of free omega-3 fatty acid salt, 20 µl of distilled water were added to dissolve the product completely.

The lysine salt of free omega-3 fatty acid in form of omega-3 lysine salt (AvailOm®, Evonik) contains around 67% of fatty acids and high amounts of the omega-3 fatty acids EPA and DHA and small amounts of the omega-3 fatty acid docosapentaenoic acid and the omega-6 fatty acids arachidonic acid, docosatetraenoic acid and docosaenoic acid isomer.

1 ml of the respective solutions was added dropwise to 20 ml of a 0.1 M phosphate buffer, pH = 8, at a temperature of 45°C and under intense stirring. Afterwards the dispersion was put on ice and sonified for 15 minutes to generate nanometer scale dispersions, presumably liposomes (Branson Sonifier, 100% amplitude, 50% impuls). Finally, the dispersions were sterile filtered through 0.2 µm

syringe filters. The resulting dispersions were characterized with regards to particle size in via dynamic light scattering (DLS) measurements (Zetasizer Nano ZS, Malvern). The dispersions contained 40 g/l phospholipids and 10 g/l omega-3 fatty acids or esters.

5 **Example 3: The strain *B. megaterium* DSM 32963 is able to produce intracellularly 18-hydroxy-eicosapentaenoic acid (18-HEPE)**

- For *B. megaterium* DSM 32963 an associated intracellularly activity of SPM-producing enzyme(s) could be demonstrated. From 10 ml Luria Bertami broth (LB, Thermo Fisher Scientific) with 0.1% Glucose (LBG) a culture of *B. megaterium* DSM 32963 was grown for 24 h at 30°C and 200 rpm in a 100 ml flask. The complete culture was transferred to a 200 ml main culture in LBG. The main culture was grown for 6 h at 30°C and 200 rpm in a 2 l flask. The cell culture was then harvested in 10 ml portions, the supernatant removed by centrifugation (15 min, 4000 rpm, room temperature) and the cell pellet resuspended in 10 ml LBG and 2 ml of supplements (table 2), respectively. These cultures were incubated in 100 ml shaking flasks for 16 h at 30°C and 200 rpm.
- 15 Different forms of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) sources were added to the *B. megaterium* DSM 32963 cell cultures to a final concentration of 0.4 g/l in form of omega-3 lysine salt (AvailOm® by Evonik), fish oil (Omega-3 1400 by Doppelherz®), and omega-3 ethyl ester (PronovaPure® 500:200 EE by BASF). These substances were added as sonicated emulsions and as dispersion formulations (preparation described in example 2), respectively.
- 20 DOPC formulation without omega-3 fatty acid content, and PBS buffer were used as controls without EPA.

Supplement	Preparation of stock solution	EPA content calculated (g/l)
DOPC formulation	undiluted	0
Omega-3 lysine salt	0.6 g in 100 ml PBS buffer	2.04
Fish oil	1 g in 100 ml PBS buffer	2.04
Omega-3 ethyl ester	0.408 g in 100 ml PBS buffer	2.04
Preparation of omega-3 lysine salt dispersion with DOPC	6 ml + 4 ml PBS buffer	2.04
Preparation of fish oil dispersion with DOPC	undiluted	2.04
Preparation of omega-3 ethyl ester dispersion with DOPC	4.08 ml + 5.92 ml PBS buffer	2.04
PBS buffer	undiluted	0

Table 2: Supplements, preparation of stock solutions, and its calculated EPA content (g/l)

- The supernatants were separated by centrifugation (15 min, 4000 rpm, room temperature), and the cell cultures were then each harvested. Afterwards, the supernatants were diluted with a solvent consisting of a water/acetonitrile mixture (ratio supernatants : solvent was 1:2, solvent composition: 65% H₂O, pH8 and 35% MeCN). Pellets were freeze dried overnight and resuspended in a solvent consisting of a water/acetonitrile mixture (ratio pellet : solvent was 1:2, solvent composition: 65% H₂O, pH8 and 35% MeCN). The cell disruption was carried out in Lysing Matrix tubes (0.1 mm silica spheres) in a Ribolyser.

The cell homogenate (and the diluted supernatant) was filtered and then used for the detection of 18-hydroxy-eicosapentaenoic acid (18-HEPE) by LC/ESI-MS analysis (Agilent QQQ 6420, Gemini 3 μ C6-Phenyl) in positive SIM-Mode at m/z 318 as well as the precursor compound EPA at m/z 302.

- 5 The addition of EPA, in form of omega-3 lysine salt, omega-3 ethyl ester, omega-3 lysine salt dispersions with DOPC, or fish oil dispersions with DOPC to the *B. megaterium* cells resulted in a cell associated (= intracellular + extracellular adsorbed) accumulation of 18-HEPE (table 3). By far the highest value of intracellular 18-HEPE was achieved by the addition of omega-3 lysine salt; it was tenfold higher than in the other approaches.

Supplement added	Cell associated 18-HEPE in cellular extract (mg/ml)
PBS buffer	0
DOPC formulation	0
Omega-3 lysine salt	>1.5
Fish oil	0
Omega-3 ethyl ester	>0.01
Preparation of omega-3 lysine salt dispersion with DOPC	>0.15
Preparation of fish oil dispersion with DOPC	>0.10
Preparation of omega-3 ethyl ester dispersion with DOPC	0

- 10 Table 3: Concentration of cell associated 18-HEPE (mg/ml) in cellular extract of *Bacillus megaterium* DSM 32963 cells

Example 4: The synbiotic combination of *Bacillus megaterium* DSM 32963 and dispersion formulation of omega-3 fatty acid salt AvailOm® leads to extracellular amounts of 18-HEPE

- 15 To investigate the amount of extracellularly appearing 18-HEPE, the *B. megaterium* DSM 32963 cells were cultivated as described in example 1. The cells were resuspended in 10 ml LBG or LBG containing 9.76 g/l FeSSIF-V2 (biorelevant.com), which is a mixture of taurocholate, phospholipids and other components designed to simulate bile surfactants, and 2 ml of supplements (table 2) were added, respectively. Additionally, the supplements were also added respectively to the
- 20 the different media in shaking flasks without cells and treated under the same conditions (controls). The 18-HEPE concentrations of the culture supernatants and controls were determined after incubation at 16 h, 30°C and 200 rpm (table 4). It could be shown that the *Bacillus megaterium* DSM 32963 cells are able to synthesize 18-HEPE from omega-3 lysine salt (AvailOm®) dispersions, which is extracellularly detectable. Of note, the omega-3 → 18-HEPE conversion rate
- 25 detected by this method is up to 0.075, which exceeds the basal content of 18-HEPE of 0.0005 % in an esterified fish oil, disclosed by WO 2017/041094. More importantly, we discovered that the omega-3 lysine salt is converted by *Bacillus megaterium* strains to a multitude of (final) SPM products at even higher concentrations than 18-HEPE (see example 6), which is of physiological relevance.

Supplement added	bile acids	18-HEPE content (mg/l)		
		supernatant of culture	control without cells	-netto-cellular produced
PBS buffer	-	0.0	0.0	0.0
DOPC formulation	-	0.0	0.0	0.0
Omega-3 lysine salt	-	>0.2	>0.3	0.0
Fish oil	-	0.0	0.0	0.0
Omega-3 ethyl ester	-	0.0	0.0	0.0
Preparation of omega-3 lysine salt dispersion with DOPC	-	>0.5	>0.1; <0.2	>0.3
Preparation of fish oil dispersion with DOPC	-	>0.05	0.0	>0.05
Preparation of omega-3 ethyl ester dispersion with DOPC	-	0.0	>0.1	0.0
PBS buffer	+	0.0	0.0	0.0
DOPC formulation	+	0.0	0.0	0.0
Omega-3 lysine salt	+	>0.4	>0.4	0.0
Fish oil	+	0.0	0.0	0.0
Omega-3 ethyl ester	+	0.0	0.0	0.0
Preparation of omega-3 lysine salt dispersion with DOPC	+	>1.2	>0.5; <1	>0.2
Preparation of fish oil dispersion with DOPC	+	0.0	0.0	0.0
Preparation of omega-3 ethyl ester dispersion with DOPC	+	0.0	0.0	0.0

Table 4: measured 18-HEPE concentrations (mg/l) of culture supernatants and controls

Example 5: Biotransformation of EPA by different Bacillus species

To investigate the ability of different Bacillus species to produce intracellularly 18-HEPE, cells of different species were cultivated as described in example 1. The cells were resuspended in 10 ml LBG containing 9.76 g/l FeSSIF-V2 (biorelevant.com), which is a mixture of taurocholate, phospholipids and other components designed to simulate bile surfactants, and 1.2 ml of the omega-3 lysine salt dispersion with DOPC were added, respectively. The internal 18-HEPE concentrations of the cells after incubation for 16 h at 30°C and 200 rpm were determined as described in example 3.

Only the *Bacillus megaterium* cells were able to synthesize 18-HEPE internally from omega-3 lysine salt (AvailOm®) dispersions, whereby *B.subtilis*, *B. amyloliquefaciens*, *B. pumilus* and *B. licheniformis* were not.

Strain	Species	Internal 18-HEPE content (mg/ml in 10 mg pellet)
DSM23778 (wildtype 168)	<i>B. subtilis</i>	0
	<i>B. subtilis</i>	0
	<i>B. amyloliquefaciens</i>	0

	<i>B. pumilus</i>	0
	<i>B. licheniformis</i>	0
DSM 32963	<i>B. megaterium</i>	>0.15

Table 5: Intracellularly measured 18-HEPE content (mg/ml) of *B. megaterium* DSM 32963 cells

Example 6: The production of SPM by *Bacillus megaterium* DSM 32963 from omega-3 fatty acid salt AvailOm® under different culture conditions

5 Lipidometabolomics:

Bacterial supernatant samples were subjected to lipid extraction using RP-phase solid phase extraction and subsequently analyzed by ultra performance liquid chromatography ESI tandem mass spectrometry (UPLC-MS/MS) according to a published procedure [48]. Under these conditions approximately 40 different LM, including 5-hydroxy-eicosapentaenoic acid 5-HEPE, 8-HEPE, 11-HEPE, 12-HEPE, 15-HEPE, 18-HEPE, 5-hydroxy-eicosatetraenoic acid (5-HETE), 8-10 HETE, 11-HETE, 12-HETE, 15-HETE, 4-hydroxy-DHA (4-HDHA), 7-HDHA, 13-HDHA, 14-HDHA, 17-HDHA, lipoxin A₄ (LXA₄), LXB₄, resolvin E1 (RvE1), RvE3, resolvin D1-5 (RvD1-5), AT-RvD1, RvD2, protectin D1 (PD1), AT-PD1, maresin 1 (MaR1), MaR2, plus 4 fatty acid substrates can be detected with a lower limit of detection of 1 pg.

15 To investigate if *Bacillus megaterium* is capable of producing other PUFA oxygenation products in addition to 18-HEPE, a lipidometabolomics screening of supernatants from *Bacillus megaterium* strain 32963 cultured as in example 4 with AvailOm® was performed, either dissolved or in a dispersion formulation, with or without bile acids. Cell-free preparations of AvailOm® formulations were treated and analyzed in parallel and served as controls for non-enzymatic, spontaneous 20 formation of oxygenation products. Values given in table 6 display net concentrations of products formed, i.e. after subtraction of control values. As can be seen, numerous oxygenation products including SPM have been formed by the bacteria. Concentrations of several SPM exceed by far the concentrations of SPM found in human plasma samples, which are typically in a range of ~ 20-100 pg/ml [27, 40, 41]. For comparison, human breast milk contains ~ 6.000 pg/ml RvE1 and ~ 10.000 25 pg/ml RvD1 [42]. Given the fact that SPM exert receptor-mediated effects *in vitro* and *in vivo* in rodents in the nM or even pM range [6], findings displayed in table 6 strongly imply the physiological and therapeutic importance of our invention.

The type of PUFA formulation had a great impact on product levels, which were generally higher in the presence of PUFA dispersion formulations and/or the addition of bile acids as solubilizers. In 30 parallel, abundance of mono-hydroxylated SPM precursors 5-HEPE, 11-HEPE, 12-HEPE, 15-HEPE, 18-HEPE, 5-HETE, 8-HETE, and 9-HODE was lower in these samples compared to samples treated with AvailOm in absence of dispersion formulation and bile acids. This can be explained by an increased conversion of these precursors to di- and trihydroxylated fatty acids.

	- bile acids	- bile acids	+ bile acids	+ bile acids
	AvailOm	AvailOm dispersion	AvailOm	AvailOm dispersion
Substance	pg/ml	pg/ml	pg/ml	pg/ml
7-HDHA				397
18-HEPE	24463			
15-HEPE	7536			
12-HEPE	4413			
11-HEPE	4659			
5-HEPE	2253			
8-HETE			148	
5-HETE	13		1834	
9-HODE	43			
PDX 1	109	19542	16623	27541
PD1 1	113	3841	3666	6564
AT-PD1 1	96	247		
RvD1	598	1863		
AT-RvD1	519	4932	22	732
RvD2	87	99	40	27
RvD3	178	8409	1115	906
RvD4	3681			
RvD5	68	9016	4750	7071
RvE1	88	57826	22930	34786
RvE3		33092	769	17772
MaR1	114	145	48	189
MaR2	120	197		
LTB4	171		43	71
t-LTB4	360	667		
LXA4				
LXA5	7767	41263	26620	69960
LXB4		76301	26356	7986
LXB5	2376	669042	299105	492508

Table 6: Extracellular concentrations of PUFA oxygenation products of *Bacillus megaterium* DSM 32963 cells

Example 7: The production of SPM from a dispersion formulation of omega-3 fatty acid salt AvailOm® by other *Bacillus megaterium* strains

47 additional *Bacillus megaterium* strains sourced from various habitats were screened for their SPM production capacity to determine if this is a general phenomenon of the species *Bacillus megaterium* and to detect strain-specific differences in types and quantities of SPM being produced. Cells were cultured as detailed in example 4, with dispersion formulation of AvailOm® serving as omega-3 fatty acid source. Cell-free preparations of AvailOm® were treated and

analyzed in parallel and served as controls for non-enzymatic, spontaneous formation of oxygenation products. It was observed that all tested strains produced measurable (> 1pg/ml) amounts of various SPM and precursors thereof, that these amounts were hugely different between the strains (up to 2.000 fold), and that concentrations of RvE₃ were particularly high (up to 1.3 5 µg/ml) in most of the strains.

Values given in table 7 display net concentrations of PUFA oxygenation products formed by two of the top performing strains, *Bacillus megaterium* DSM 33296 and *Bacillus megaterium* DSM 33299.

	<i>Bacillus megaterium</i> DSM 33296	<i>Bacillus megaterium</i> DSM 33299
Substance	pg/ml	pg/ml
7-HDHA	202129	243080
18-HEPE	391397	217725
15-HEPE	256797	151168
12-HEPE	124701	79699
11-HEPE	124860	63306
5-HEPE	395188	989691
8-HETE	24207	15941
5-HETE	15434	9356
PDX 1	78345	138918
PD1 1	99029	75342
AT-PD1 1	4558	10317
RvD1	3626	4389
AT-RvD1	9612	48243
RvD2	265	428
RvD3	381	941
RvD4	1986	2523
RvD5	15079	34168
RvE1	5840	28379
RvE3	1322555	880739
MaR1	1161	1576
MaR2	1522	1233
LTB4	1769	2206
t-LTB4	1780	4181
LXA4	1157	1430
LXA5	4693	128825

Table 7: Extracellular concentrations of PUFA oxygenation products of *Bacillus megaterium* DSM 33296 and *Bacillus megaterium* DSM 33299 cells.

Example 8: Capsules comprising EPA-DHA amino acid salts and *Bacillus megaterium* strain(s) as food supplement or as drug

The following components were filled in HPMC capsules (size 00).

Compound	Capsule I	Capsule II	Capsule III
Omega-3 amino acid* salt	250 mg	50 mg	800 mg
<i>Bacillus megaterium</i> strain#	1x10 ⁷ CFU - 1x10 ¹¹ CFU	1x10 ⁷ CFU - 1x10 ¹¹ CFU	1x10 ⁷ CFU - 1x10 ¹¹ CFU

Table 7: Preparations for filling into HPMC capsules. *Amino acids are selected from L-ornithine, L-

5 lysine and L-arginine. #Strain selected from *Bacillus megaterium* DSM 32963, DSM 33296, DSM 33299.

The capsules may further contain amino acids selected from L-ornithine, L-aspartate, L-lysine and L-arginine.

10 The capsules may further contain further carbohydrate ingredients, selected from arabinoxylans, barley grain fibre, oat grain fibre, rye fibre, wheat bran fibre, inulins, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch, beta-glucans, glucomannans, galactoglucomannans, guar gum and xylooligosaccharides.

15 The capsules may further contain one or more plant extracts, selected from ginger, cinnamon, grapefruit, parsley, turmeric, curcuma, olive fruit, panax ginseng, horseradish, garlic, broccoli, spirulina, pomegranate, cauliflower, kale, cilantro, green tea, onions, and milk thistle.

The capsules may further contain astaxanthin, charcoal, chitosan, glutathione, monacolin K, plant sterols, plant stanols, sulforaphane, collagen, hyalurone, phosphatidylcholine.

20 The capsules may comprise further vitamins selected from biotin, vitamin A, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B9 (folic acid or folate), vitamin C (ascorbic acid), vitamin D (calciferols), vitamin E (tocopherols and tocotrienols) and vitamin K (quinones) or minerals selected from sulfur, iron, chlorine, calcium, chromium, cobalt, copper, magnesium, manganese, molybdenum, iodine, selenium, and zinc.

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Claims

1. Preparation comprising at least one omega-3 or omega-6 fatty acid component selected from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), alpha linolenic acid, stearidonic acid, eicosatetraenoic acid, docosapentaenoic acid, linoleic acid, γ -linolenic acid, or arachidonic acid (ARA) and/or derivatives thereof for use to increase the formation of one or more specialized pro-resolving lipid mediators (SPM) by microorganisms in the gastrointestinal tract of humans or animals, wherein the polyunsaturated fatty acid component comprises an omega-3 or omega-6 fatty acid salt.
2. Preparation according to claim 1, wherein formation of SPM results from biochemical oxygenation reactions by gastrointestinal microorganisms.
3. Preparation according to any one of the preceding claims, wherein the gastrointestinal microorganism is belonging to the genus *Bacillus megaterium*.
4. Preparation according to any one of the preceding claims, wherein the SPM is selected from: 17-HDHA, 14-HDHA, 13-HDHA, 7-HDHA, 4-HDHA, 18-HEPE, 15-HEPE, 12-HEPE, 11-HEPE, 5-HEPE, 15-HETE, 12-HETE, 11-HETE, 8-HETE, 5-HETE, 9-HODE, 13-HODE, PDX, PD1, AT-PD1, MaR1, MaR2, LTB4, t-LTB4, RvD1-5, AT-RvD1, RvE1, RvE3, LXA4, LXA5, LXB4, LXB5.
5. Preparation according to any one of the preceding claims, wherein the omega-3 fatty acid component is selected from EPA or DHA or wherein the omega-6 fatty acid component is ARA.
6. Preparation according to any of the preceding claims, wherein the omega-3 or omega-6 fatty acid component is either in the form of free fatty acids, salts, natural triglycerides, fish oil, phospholipid esters or ethyl esters.
7. Preparation according to any of the preceding claims, wherein the omega-3 component comprises an omega-3 fatty acid amino acid salt, wherein the amino acid is chosen from basic amino acids selected from lysine, arginine, ornithine, choline and mixtures of the same, preferably lysine.
8. Preparation according to any of the preceding claims, wherein the omega-3 or omega-6 component comprises a preparation comprising a dispersion of at least one phospholipid and at least one omega-3 or omega-6 fatty acid.
9. Preparation according to claim 8 and 9, wherein the composition is a solid composition.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/082935

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/20 A61K31/202 A61K31/557 A61K35/742 A61P29/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
A61K A61P
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANNIKA I. OSTERMANN ET AL: "Effects of omega-3 fatty acid supplementation on the pattern of oxylipins: a short review about the modulation of hydroxy-, dihydroxy-, and epoxy-fatty acids", FOOD & FUNCTION, vol. 8, no. 7, 1 January 2017 (2017-01-01) , pages 2355-2367, XP055586102, GB	1,2,4-6, 8,9
Y	ISSN: 2042-6496, DOI: 10.1039/C7F000403F page 2360, left-hand column, paragraph 2 - right-hand column, paragraph 2 page 2362, right-hand column, paragraph 2 ----- -/--	3,7

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 6 April 2020	Date of mailing of the international search report 20/04/2020
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Escolar Blasco, P
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/082935

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	abstract page 1110 - page 1112	3,7
X	----- WO 2013/170006 A2 (SOLUTEX NA LLC [US]; BANNENBERG GERHARDUS LUCAS [ES] ET AL.) 14 November 2013 (2013-11-14) page 3, paragraph 8 - page 5, paragraph 13 page 9, paragraph 32-33; claims	1,2,4-6, 8,9
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