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(54) **Prevention or treatment of hepatic steatosis**

(57) The invention is concerned with *Anaerobutyricum soehngenii* or relative thereof for use in preventing and/or treating hepatic steatosis, particularly Nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH), wherein the use is for increasing bile acid plasma level for reducing liver inflammation and/or for reducing hepatic necroinflammatory activity score. Said *Anaerobutyricum soehngenii* or relative thereof may be combined with at least one *Bifidobacterium* species, preferably *Bifidobacterium animalis subspecies lactis* or relative thereof and/or *Bifidobacterium breve* or relative thereof. In addition or alternatively, said *Anaerobutyricum soehngenii* or relative thereof may be combined with at least one *Akkermansia* species, preferably *Akkermansia muciniphila* or relative thereof. In addition or alternatively, said *Anaerobutyricum soehngenii* or relative thereof may be combined with at least one *Lactobacillus* species, preferably *Lactobacillus acidophilus* or relative thereof, *Lactobacillus casei* or relative thereof and/or *Lactobacillus reuteri* or relative thereof.

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Prevention or treatment of hepatic steatosis

TECHNICAL FIELD

The present invention relates to the field of preventing and/or treating hepatic steatosis.

BACKGROUND OF THE DISCLOSURE

Non-alcoholic fatty liver disease (NAFLD) is recognized as the most prevalent chronic liver disease worldwide, and its spectrum ranges from simple steatosis (non-alcoholic fatty liver) to non-alcoholic steatohepatitis (NASH), NASH-fibrosis, cirrhosis and hepatocellular carcinoma. The current estimated global prevalence of NAFLD is 25%-30% in the general population, and up to 80% in individuals with metabolic syndrome and Type 2 Diabetes mellitus. By definition, excessive alcohol use precludes a diagnosis of NAFLD.

NAFLD refers to a spectrum of disease in which excess fat accumulates in the liver in patients who drink little or no alcohol. The most common form of NAFLD is called non-alcoholic fatty liver (NAFLD). As the occurrence and progression of NAFLD are strongly driven by insulin resistance, multiple therapeutic strategies in clinical development for NAFLD aim at reducing insulin resistance.

NASH refers to liver inflammation triggered by lipotoxicity in the setting of hepatic steatosis. NASH gives a markedly increased risk of developing cirrhosis and hepatocellular carcinoma (HCC) and it is associated with increased atherosclerotic cardiovascular disease. Since the association between NAFLD/NASH and insulin resistance is well-known, strategies to lower insulin resistance may decrease disease progression or symptoms in NAFLD/NASH.

The gut microbiota has been linked to the development and prevalence of NAFLD and NASH. Disease occurrence is significantly lower in individuals taking a plant-based, low-animal-protein diet, which is thought to be mediated by gut microbiota. Hence, Witjes at al (Hepatology Communications, Vol. 4, no. 11, 2020) propose transplantation of fecal microbiota from lean vegan donors as a potential treatment.

However, there is a need in the art for new and improved interventions in the prevention and treatment of NAFLD and NASH.

It is an object of the present disclosure, amongst other objects, to address the above need in the art to provide a new and/or improved strategy for preventing and/or treating NAFLD and NASH.

SUMMARY OF THE DISCLOSURE

The present inventors surprisingly found that administration of *Anaerobutyricum soehngenii,* or relative thereof, to subjects having hepatic steatosis, increases bile acid plasma levels which reduces liver inflammation. Accordingly, administration of *Anaerobutyricum soehngenii,* or relative thereof may be applied in a strategy for prevention and/or treatment of hepatic steatosis.

In addition, it was found that combining *Anaerobutyricum soehngenii,* or relative thereof, with a *Bifidobacterium* species, an *Akkermansia* species and/or a *Lactobacillus* species provides a synergistic therapeutic effect in the prevention or treatment of hepatic steatosis, in particular in Nonalcoholic fatty liver disease (NAFLD), and/or nonalcoholic steatohepatitis (NASH).

The present disclosure provides a new and improved strategy for preventing and/or treating hepatic steatosis, NAFLD, and/or NASH.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to *Anaerobutyricum soehngenii* or relative thereof having a 16S rRNA gene sequence with at least 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.5, 99.9, 100% sequence identity with SEQ ID NO:1 and/or SEQ ID NO:2, particularly for use in preventing and/or treating hepatic steatosis, and/or for increasing production of propionic acid/propionate and/or butyric acid/butyrate or a derivative thereof in the intestine.

In accordance with the foregoing, the present disclosure relates to a method for preventing and/or treating hepatic steatosis, e.g. in a subject in need thereof, involving administration, e.g. to said subject, of said *Anaerobutyricum soehngenii* or relative thereof.

Hepatic steatosis is a condition where excess fat builds up in the liver. There are two stages of fatty liver disease: non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease. NAFLD is made up of simple fatty liver and non-alcoholic steatohepatitis (NASH).

In the present disclosure, the hepatic steatosis may in a particular be chosen from Nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH).

The term 'Nonalcoholic fatty liver disease' (NAFLD) refers to a group of conditions where there is accumulation of excess fat in the liver of people who drink little or no alcohol. The most common stage of NAFLD is called fatty liver. NAFLD is strongly associated with insulin resistance and type 2 diabetes mellitus, therefore treatments of NAFLD may aim at lowering insulin resistance.

The term 'Nonalcoholic steatohepatitis' (NASH) refers to liver inflammation and damage caused by a buildup of fat in the liver. NASH is associated with a markedly increased risk of developing cirrhosis and hepatocellular carcinoma as well as other diseases not directly associated with liver damage, including increased risk of cardiovascular disease. An association between insulin resistance and the development of NASH (/NAFLD) is well-known, and strategies to lower insulin resistance may decrease disease progression or symptoms in NASH (/NAFLD).

The use according to the disclosure can increase plasma levels of bile acids, in particular primary bile acids (cholic acid and chenodeoxycholic acid) and/or secondary bile acids (deoxycholic acid and lithocholic acid). This, in turn, reduces liver inflammation (e.g. as determined by (sum of) lobular inflammation score 0-3, microgranulomas score 0-1, large lipogranulomas score 0-1, and/or portal inflammation score 0-1 as shown below); or as determined by necroinflammatory activity score (NAS). Hence, the use according to the disclosure can reduce liver inflammation (e.g. as determined by (sum of) lobular inflammation score 0-3, microgranulomas score 0-1, large lipogranulomas score 0-1, and/or portal inflammation score 0-1 as shown below); or as determined by necroinflammatory activity score.

An increase in bile acid plasma level as part of the current disclosure is preferably indicated by one or more of the following methods: thin-layer chromatography, gas chromatography, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) supercritical fluid chromatography and capillary electrophoresis, immunoassays and bioluminescence assays.

In a particularly preferred embodiment, the use according to the present disclosure is for reducing hepatic necroinflammatory activity score.

The term hepatic necroinflammatory activity score is interchangeable with the terms NAFLD score and/or NASH score.

To determine the hepatic necroinflammatory activity score, the NASH Clinical Research Network (NASH-CRN) classification may be used as described by Kleiner et al Volume 41, Issue 6 June 2005), e.g. with use of hematoxylin and eosin–stained slides for steatosis, inflammation and ballooning, and with a sirius red–stained slide for evaluation of fibrosis. The score preferably is the unweighted sum of steatosis grade (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2), see below:

Steatosis		
Grade	Low- to medium-power evaluation of parenchymal involvement by steatosis	
	<5%	0
	5%-33%	1
	>33%-66%	2
	>66%	3
Location	Predominant distribution pattern	
	Zone 3	0
	Zone 1	1
	Azonal	2
	Panacinar	3
Microvesicular steatosis*	Contiguous patches	
	Not present	0
	Present	1
Fibrosis		
Stage		
	None	0
	Perisinusoidal or periportal	1
	Mild, zone 3, perisinusoidal	1A
	Moderate, zone 3, perisinusoidal	1B
	Portal/periportal	1C
	Perisinusoidal and portal/periportal	2
	Bridging fibrosis	3
	Cirrhosis	4
Inflammation		
Lobular inflammation	Overall assessment of all inflammatory foci	
	No foci	0

	<2 foci per 200× field	1
	2-4 foci per 200× field	2
	>4 foci per 200× field	3
Microgranulomas	Small aggregates of macrophages	
	Absent	0
	Present	1
Large lipogranulomas	Usually in portal areas or adjacent to central veins	
	Absent	0
	Present	1
Portal inflammation	Assessed from low magnification	
	None to minimal	0
	Greater than minimal	1
Liver cell injury		
Ballooning*		
	None	0
	Few balloon cells	1
	Many cells/prominent ballooning	2
Acidophil bodies		
	None to rare‡	0
	Many	1
Pigmented macrophages		
	None to rare‡	0
	Many	1
Megamitochondria*		
	None to rare‡	0
	Many	1
Other findings		
Mallory's hyaline	Visible on routine stains	
	None to rare‡	0
	Many	1
Glycogenated nuclei	Contiguous patches	
	None to rare‡	0
	Many	1

Diagnostic classification‡	
Not steatohepatitis	0
Possible/borderline	1
Definite steatohepatitis	2

* Ballooning classification: few indicates rare but definite ballooned hepatocytes as well as case that are diagnostically borderline.

† The "None to rare" category is meant to alleviate the need for time-consuming searches for rare examples or deliberation over diagnostically borderline changes. If the feature is identified after a reasonable search, it should be coded as "many."

‡ Diagnostic classification may not be available on adult biopsy observations.

The use according to the disclosure can also decrease

- steatosis grade score, particularly as defined above (score 1, 2, 3); and/or
- fibrosis stage score, particularly as defined above (score 1, 1A, 1B, 1C, 2, 3, or 4).

The *Anaerobutyricum soehngenii* or relative thereof according to the present disclosure is preferably chosen from *Anaerobutyricum* species or *Eubacterium* species, preferably *Anaerobutyricum soehngenii* (e.g. DSM17630/KCTC15707) and/or *Anaerobutyricum hallii* (DSM3353/ATCC27751).

In a study by Shetty et al (Int J Syst Evol Microbiol. 2018 Dec;68(12):3741-3746), the species formerly known as *Eubacterium hallii* has been reclassified into two groups: *Anaerobutyricum hallii* and *Anaerobutyricum soehngenii.* Both *Anaerobutyricum soehngenii* and/or *Anaerobutyricum hallii* are considered as an anaerobic Gram-positive, catalase-negative bacterium belonging to the clostridial cluster XIVa (also known as *Lachnospiracaea)* of the phylum Firmicutes.

Most preferably the at least one *Anaerobutyricum* species according to the present disclosure is *Anaerobutyricum soehngenii* (e.g. DSM17630/KCTC15707), or a relative thereof having a 16S rRNA gene sequence with at least 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.5, 99.9, 100% sequence identity with the 16S rDNA sequence of *Anaerobutyricum soehngenii* (SEQ ID NO:1). Such cut-off value based on 16S rDNA similarity can define species with similar characteristics and/or functionality.

In addition or alternatively, the *Anaerobutyricum* species according to the present disclosure is *Anaerobutyricum hallii* (e.g. DSM3353/ATCC27751), or a relative thereof having a 16S

rRNA gene sequence with at least 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.5, 99.9, 100% sequence identity with the 16S rDNA sequence of *Anaerobutyricum hallii* (SEQ ID NO:2). Such cut-off value based on 16S rDNA similarity can define species with similar characteristics and/or functionality.

5

Anaerobutyricum soehngenii L2-7 16S rRNA gene sequence
Nucleotide sequence (SEQ ID NO:1)* tgatcctggc tcaggatgaa cgctggcggc gtgcctaaca catgcaagtc gaacgaagca ccttttaaga ttcttcggat gattgatcgg tgactgagtg gcggacgggt gagtaacgcg tgggtaacct gccctgtaca gggggataac agttggaaac ggctgctaat accgcataag cgcacgagag gacatcctct tgtgtgaaaa actccggtgg tacaggatgg gcccgcgtct gattagctgg ttggcagggt aacggcctac caaggcgacg atcagtagcc ggtctgagag gatgaacggc cacattggaa ctgagacacg gtccaaactc ctacgggagg cagcagtggg gaatattgca caatggggga aaccctgatg cagcaacgcc gcgtgagtga agaagtattt cggtatgtaa agctctatca gcagggaaga taatgacggt acctgactaa gaagctccgg ctaaatacgt gccagcagcc gcggtaatac gtatggagca agcgttatcc ggatttactg ggtgtaaagg gtgcgtaggt ggcagtgcaa gtcagatgtg aaaggccggg gctcaacccc ggagctgcat ttgaaactgc atagctagag tacaggagag gcaggcggaa ttcctagtgt agcggtgaaa tgcgtagata ttaggaggaa caccagtggc gaaggcggcc tgctggactg ttactgacac tgaggcacga aagcgtgggg agcaaacagg attagatacc ctggtagtcc acgccgtaaa cgatgaatcc taggtgtcgg ggccgtatag gcttcggtgc cgtcgcaaac gcagtaagta ttccacctgg ggagtacgtt cgcaagaatg aaactcaaag gaattgacgg ggacccgcac aagcggtgga gcatgtggtt taattcgaag caacgcgaag aaccttacca ggtcttgaca tccttctgac cactccgtaa tgggagtctt ccttcgggac agaagagaca ggtggtgcat ggttgtccgt cagctcgtgt cgtgagatgt tgggttaagt cccgcaacga gcgcaacccc tatcttcagt agccagcagg taaggctggg cactctggag agactgccag ggataacctg gaggaaggtg gggacgacgt caaatcatca tgccccttat gatctgggcg acacacgtgc tacaatggcg gtcacaaagt gaggcaaacc tgcgaggggg agcaaaccac aaaaaggccg tcccagttcg gactgtagtc tgcaacccga ctacacgaag ctggaatcgc tagtaatcgc gaatcagaat gtcgcggtga atacgttccc gggtcttgta cacaccgccc gtcacaccat gggagtcgga aatgcccgaa gccagtgacc caacctttg gagggarctg tcgaaggtgg agccggtaac tggggtgaag tcgtaacaag gg
Anaerobutyricum hallii 16S rRNA gene sequence
Nucleotide sequence (SEQ ID NO:2)* tttatttgag agtttgatcc tggctcagga tgaacgctgg cggcgtgcct aacacatgca agtcgaacga agcaccttac cwgattcttc ggatgaaagw ytggtgactg agtggcggac

gggtgagtaa cgcgtgggta acctgccctg tacaggggga taacagctgg aaacggctgc
taataccgca taagcgcacg aggagacatc tccttgtgtg aaaaactccg gtggtacagg
atgggcccgc gtctgattag ctggttggca gggtaacggc ctaccaaggc aacgatcagt
agccggtctg agaggatgaa cggccacatt ggaactgaga cacggtccaa actcctacgg
gaggcagcag tggggaatat tgcacaatgg gggaaaccct gatgcagcaa cgccgcgtga
gtgaagaagt atttcggtat gtaaagctct atcagcaggg aagataatga cggtacctga
ctaagaagct ccggctaaat acgtgccagc agccgcggta atacgtatgg agcaagcgtt
atccggattt actgggtgta aagggtgcgt aggtggcagt gcaagtcaga tgtgaaaggc
cggggctcaa ccccggngct gcatttgaaa ctgcwyrgct agagtacagg agaggcaggc
ggaattccta gtgtagcggt gaaatgcgta gatattagga ggaacaccag tggcgaaggc
ggcctgctgg actgttactg acactgaggc acgaaagcgt ggggagcaaa caggattaga
taccctggta gtccacgccg taaacgatga atactaggtg tcggggccgt ataggctycg
gtgccgccgc taacgcagta agtattccac ctggggagta cgttcgcaag aatgaaactc
aaaggaattg acggggaccc gcacaagcgg tggagcatgt ggtttaattc gaagnaacgc
gaagaacctt accaggtctt gacatccttc tgaccgcacc ttaatcggtg ctttccttcg
ggacagaaga gacaggtggt gcatggttgt cgtcagctcg tgtcgtgaga tgttgggtta
agtcccncaa cgagcgcnac ccctatcttc agtagccagc aggtaaggct gggcactctg
gagagactgc cagggataac ctggaggaag gtggggacga cgtnnaatca tcatgcccct
tatgatctgg gcgacacacg tgctacnatg gcggtcacag agtgaggcga accygcgang
gggagcaanc cacaaaaagg ccgtcccagt tcggactgta gtctgcaacc cgactacacg
aagctggaat cgctagtaat cgcgaatcag aatgtcgcgg tgaatacgtt cccnngtctt
gtacacaccg nccgtcacac catgggagtc ggaaatgccc gaagccagtg acccaacctt
tatggaggga gctgtcgaag gtggagccgg taactgggg

*"n" refers to a, t, c, or g.

In a preferred embodiment, the *Anaerobutyricum soehngenii* or relative thereof according the disclosure is combined with at least one *Bifidobacterium* species. It was found that this is a synergistic combination, leading to an unexpected reduction in hepatic necroinflammatory activity score.

The *Bifidobacterium* species may be administered separately, sequentially or simultaneously with said *Anaerobutyricum soehngenii* or relative thereof. Accordingly, said *Bifidobacterium* species may be comprised in the same or in a separate composition with respect to said *Anaerobutyricum soehngenii* or relative thereof.

Bifidobacterium is a genus of gram-positive, typically nonmotile, often branched anaerobic bacteria. They are ubiquitous inhabitants of the gastrointestinal tract, vagina and mouth of mammals, including humans. *Bifidobacteria* are one of the major genera of bacteria that make up the gastrointestinal tract microbiota in mammals. The at least one *Bifidobacterium* species according to the present disclosure is/are preferably able to assimilate human milk oligosaccharides (HMOs).

The at least one *Bifidobacterium* species of the present disclosure preferably includes one or more of

- *Bifidobacterium animalis sub. lactis*, or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifidobacterium animalis sub. lactis* (NCBI accession code NR_040867, SEQ ID NO:3);
- *Bifidobacterium infantis* (able to assimilate HMO), or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifidobacterium infantis* (NCBI accession code D86184, SEQ ID NO:4);
- *Bifidobacterium longum* (able to assimilate HMO), or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifidobacterium longum* (NCBI accession code M58739, SEQ ID NO:5);
- *Bifidobacterium breve* (able to assimilate HMO), or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifidobacterium breve* (NCBI accession code AB006658, SEQ ID NO:6);
- *Bifidobacterium thermophilum,* or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifidobacterium thermophilum* (NCBI accession code AB016246, SEQ ID NO:7);
- *Bifdobacterium bifidum,* or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifdobacterium bifidum* (NCBI accession code M38018, SEQ ID NO:8);
- *Bifidobacterium adolescentis,* or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifidobacterium adolescentis* (NCBI accession code M58729, SEQ ID NO:9);

- *Bifodbacterium catenulatum* or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifodbacterium catenulatum* (NCBI accession code M58732, SEQ ID NO:10);
- *Bifdobacterium pseudocatenulatum* or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Bifdobacterium pseudocatenulatum* (NCBI accession code D86187, SEQ ID NO:11).

In a particularly preferred embodiment, the *Bifidobacterium* species is chosen from:

- - *Bifidobacterium animalis subspecies lactis* or relative thereof having a 16S rRNA gene sequence with at least 90, 95, 97, 99, 100% sequence identity with SEQ ID NO:3; and/or
- - *Bifidobacterium breve* or relative thereof having a 16S rRNA gene sequence with at least 90, 95, 97, 99, 100% sequence identity with SEQ ID NO:6.

Bifidobacterium animalis subspecies lactis 16S rRNA gene (NCBI/Genbank accession code NR_040867, SEQ ID NO:3)
1 agtttgatca tggctcagga tgaacgctgg cggcgtgctt aacacatgca agtcgaacgg
61 gatccctggc agcttgctgt cggggtgaga gtggcgaacg ggtgagtaat gcgtgaccaa
121 cctgccctgt gcaccggaat agctcctgga aacgggtggt aataccggat gctccgctcc
181 atcgcatggt ggggtgggaa atgcttttgc ggcatgggat ggggtcgcgt cctatcagct
241 tgttggcggg gtgatggccc accaaggcgt tgacgggtag ccggcctgag agggtgaccg
301 gccacattgg gactgagata cggcccagac tcctacggga ggcagcagtg gggaatattg
361 cacaatgggc gcaagcctga tgcagcgacg ccgcgtgcgg gatggaggcc ttcgggttgt
421 aaaccgcttt tgttcaaggg caaggcacgg tttcggccgt gttgagtgga ttgttcgaat
481 aagcaccggc taactacgtg ccagcagccg cggtaatacg tagggtgcga gcgttatccg
541 gatttattgg gcgtaaaggg ctcgtaggcg gttcgtcgcg tccggtgtga aagtccatcg
601 cctaacggtg gatctgcgcc gggtacgggc gggctggagt gcggtagggg agactggaat
661 tcccggtgta acggtggaat gtgtagatat cgggaagaac accaatggcg aaggcaggtc
721 tctgggccgt cactgacgct gaggagcgaa agcgtgggga gcgaacagga ttagataccc
781 tggtagtcca cgccgtaaac ggtggatgct ggatgtgggg ccctttccac gggtcccgtg
841 tcggagccaa cgcgttaagc atcccgcctg gggagtacgg ccgcaaggct aaaactcaaa
901 gaaattgacg ggggcccgca caagcggcgg agcatgcgga ttaattcgat gcaacgcgaa
961 gaaccttacc tgggcttgac atgtgccgga tcgccgtgga gacacggttt cccttcgggg
1021 ccggttcaca ggtggtgcat ggtcgtcgtc agctcgtgtc gtgagatgtt gggttaagtc
1081 ccgcaacgag cgcaaccctc gccgcatgtt gccagcgggt gatgccggga actcatgtgg

1141 gaccgccggg gtcaactcgg aggaaggtgg ggatgacgtc agatcatcat gcccttacg
1201 tccagggctt cacgcatgct acaatggccg gtacaacgcg gtgcgacacg gtgacgtggg
1261 gcggatcgct gaaaaccggt ctcagttcgg atcgcagtct gcaactcgac tgcgtgaagg
1321 cggagtcgct agtaatcgcg gatcagcaac gccgcggtga atgcgttccc gggccttgta
1381 cacaccgccc gtcaagtcat gaaagtgggt agcacccgaa gccggtggcc cgacccttgt
1441 ggggggagcc gtctaaggtg agactcgtga ttgggactaa gtcgtaacaa ggtagccgta
1501 ccggaaggtg cggctggatc acctcctta

Bifidobacterium infantis 16S rRNA gene (NCBI/Genbank accession code D86184, SEQ ID NO:4)

1 tttgatcatg gctcaggatg aacgctggcg gcgtgcttaa cacatgcaag tcgaacggga
61 tccatcgggc tttgcttggt ggtgagagtg gcgaacgggt gagtaatgcg tgaccgacct
121 gccccataca ccggaatagc tcctggaaac gggtggtaat gccggatgtt ccagttgatc
181 gcatggtctt ctgggaaagc tttcgcggta tgggatgggg tcgcgtccta tcagcttgac
241 ggcggggtaa cggcccaccg tggcttcgac gggtagccgg cctgagaggg cgaccggcca
301 cattgggact gagatacggc ccagactcct acgggaggca gcagtgggga atattgcaca
361 atgggcgcaa gcctgatgca gcgacgccgc gtgagggatg gaggccttcg ggttgtaaac
421 ctcttttatc ggggagcaag cgtgagtgag tttacccgtt gaataagcac cggctaacta
481 cgtgccagca gccgcggtaa tacgtagggt gcaagcgtta tccggaatta ttgggcgtaa
541 agggctcgta ggcggttcgt cgcgtccggt gtgaaagtcc atcgcttaac ggtggatccg
601 cgccgggtac gggcgggctt gagtgcggta ggggagactg gaattcccgg tgtaacggtg
661 gaatgtgtag atatcgggaa gaacaccaat ggcgaaggca ggtctctggg ccgttactga
721 cgctgaggag cgaaagcgtg gggagcgaac aggattagat accctggtag tccacgccgt
781 aaacggtgga tgctggatgt ggggcccgtt ccacgggttc cgtgtcggag ctaacgcgtt
841 aagcatcccg cctggggagt acggccgcaa ggctaaaact caaagaaatt gacgggggcc
901 cgcacaagcg gcggagcatg cggattaatt cgatgcaacg cgaagaacct tacctgggct
961 tgacatgttc ccgacgatcc cagagatggg gtttcccttc ggggcgggtt cacaggtggt
1021 gcatggtcgt cgtcagctcg tgtcgtgaga tgttgggtta agtcccgcaa cgagcgcaac
1081 cctcgccccg tgttgccagc ggattgtgcc gggaactcac gggggaccgc cggggttaac
1141 tcggaggaag gtggggatga cgtcagatca tcatgcccct tacgtccagg gcttcacgca
1201 tgctacaatg gccggtacaa cgggatgcga cgcggcgacg cggagcggat ccctgaaaac
1261 cggtctcagt tcggatcgca gtctgcaact cgactgcgtg aaggcggagt cgctagtaat
1321 cgcgaatcag caacgtcgcg gtgaatgcgt tcccgggcct tgtacacacc gcccgtcaag
1381 tcatgaaagt gggcagcacc cgaagccggt ggcctaaccc cttgtgggat ggagccgtct
1441 aaggtgaggc tcgtgattgg gactaagtcg taacaaggta gccgtaccgg aaggtgcggc
1501 tggatcacct ccttа

Bifidobacterium longum 16S rRNA gene (NCBI/Genbank accession code M58739, SEQ ID NO:5)*
1 ttttgtggag ggttcgattc tggctcagga tgaacgctgg cggcgtgctt aacacatgca 61 agtcgaacgg gatccatcaa gcttgcttgg tggtgagagt ggcgaacggg tgagtaatgc 121 gtgaccgacc tgccccatac accggaatag ctcctggaaa cgggtggtaa tgccggatgt 181 tccagttgat cgcatggtct tctggngaaa gcntttcgcg gtatgggatg gggtcgcgtc 241 ctatcagctt gacggngggg taacggcnna ccgtggcttc gacgggtagc cggcctgaga 301 gggcgaccgg ccacattggg actgagatac ggcccngact cctacgggag gcagcagtgg 361 ggaatattgc acaatgggcg caagcctgat gcagcgacgc cgcgtgaggg atggaggcct 421 tcgggttgta aacctctttt atcggggagc aagcgagagt gagtttaccc gttgaataag 481 caccggctaa ctacgtgcca gcagccgcgg taatacgtag ggtgcnagcg ttatccggaa 541 ttattgggcg taaagggctc gtaggcggtt cgtcgcgtcc ggtgtgaaag tccatcgctt 601 aacggtggat ccgcgccggg tacgggcggg cttgagtgcg gtaggggaga ctggaattcc 661 cggtgtaacg gtggaatgtg tagatatcgg gaagaacacc aatggcgaag gcaggtctct 721 gggccgttac tgacgctgag gagcgaaagc gtggggagcg aacaggatta gataccctgg 781 tagtccacgc cgtaaacggt ggatgctgga tgtggggccn gttccacggg ttccgtgtcg 841 gagctaacgc gttaagcatc ccgcctgggg agtacggccg caaggctaaa actcaaagaa 901 attgacgggg gccngcacaa gcggcggagc atgcggatta attcgatgna acgcgaagaa 961 ccttacctgg gcttgacatg ttcccgacgg tcgtagagat acggcntccc ttcggggcgg 1021 gttcacaggt ggngcatggt cgtcgtcagc tcgtgtcgtg agatgttggg ttaagtcccg 1081 caacgagcgc aaccctcgcc ccgtgttgcc agcggattat gccggnaact cacgggnnac 1141 cgccggggtt aactcggagg aaggtgggga tgacgtcaga tcatcatgcc ccttacgtcc 1201 agggcttcac gcatgctaca atggccggta caacgggatg cgacgcggcg acgcggagcg 1261 gatccctgaa aaccngtctc agttcggatc gcagtctgca actcgactgc gtgaaggcgg 1321 agtcgctagt aatcgcgaat cagcaacgtc gcggtgaatg cgttcccngg ccttgtacac 1381 accgcccgtc aagncatgaa agtgggcagc acccgaagcc ggtggcctaa ccccttgtgg 1441 ganggagccg tctaaggtga ggctcgtgat tgggac
Bifidobacterium breve 16S rRNA gene (NCBI/Genbank accession code AB006658, SEQ ID NO:6)
1 ttcgattctg gctcaggatg aacgctggcg gcgtgcttaa cacatgcaag tcgaacggga 61 tccatcgggc tttgcttggt ggtgagagtg gcgaacgggt gagtaatgcg tgaccgacct 121 gccccatgca ccggaatagc tcctggaaac gggtggtaat gccggatgct ccatcacacc 181 gcatggtgtg ttgggaaagc ctttgcggca tgggatgggg tcgcgtccta tcagcttgat 241 ggcggggtaa cggcccacca tggcttcgac gggtagccgg cctgagaggg cgaccggcca 301 cattgggact gagatacggc ccagactcct acgggaggca gcagtgggga atattgcaca 361 atgggcgcaa gcctgatgca gcgacgccgc gtgagggatg gaggccttcg ggttgtaaac

421 ctcttttgtt agggagcaag gcactttgtg ttgagtgtac ctttcgaata agcaccggct
481 aactacgtgc cagcagccgc ggtaatacgt agggtgcaag cgttatccgg aattattggg
541 cgtaaagggc tcgtaggcgg ttcgtcgcgt ccggtgtgaa agtccatcgc ttaacggtgg
601 atccgcgccg ggtacgggcg ggcttgagtg cggtagggga gactggaatt cccggtgtaa
661 cggtggaatg tgtagatatc gggaagaaca ccaatggcga aggcaggtct ctgggccgtt
721 actgacgctg aggagcgaaa gcgtggggag cgaacaggat tagataccct ggtagtccac
781 gccgtaaacg gtggatgctg gatgtggggc ccgttccacg ggttccgtgt cggagctaac
841 gcgttaagca tcccgcctgg ggagtacggc cgcaaggcta aaactcaaag aaattgacgg
901 gggcccgcac aagcggcgga gcatgcggat taattcgatg caacgcgaag aaccttacct
961 gggcttgaca tgttcccgac gatcccagag atggggtttc ccttcggggc gggttcacag
1021 gtggtgcatg gtcgtcgtca gctcgtgtcg tgagatgttg ggttaagtcc cgcaacgagc
1081 gcaaccctcg ccccgtgttg ccagcggatt gtgccgggaa ctcacggggg accgccgggg
1141 ttaactcgga ggaaggtggg gatgacgtca gatcatcatg ccccttacgt ccagggcttc
1201 acgcatgcta caatggccgg tacaacggga tgcgacagtg cgagctggag cggatccctg
1261 aaaaccggtc tcagttcgga tcgcagtctg caactcgact gcgtgaaggc ggagtcgcta
1321 gtaatcgcga atcagcaacg tcgcggtgaa tgcgttcccg ggccttgtac acaccgcccg
1381 tcaagtcatg aaagtgggca gcacccgaag ccggtggcct aacccccttgc gggagggagc
1441 cgtctaaggt gaggctcgtg attgggacta agtcgtaaca aggtagccgt accggaaggt
1501 gcggctggat cacctcctta

Bifidobacterium thermophilum 16S rRNA gene (NCBI/Genbank accession code AB016246, SEQ ID NO:7)

1 agagtttgat catggctcag gatgaacgct ggcggcgtgc ttaacacatg caagtcgaac
61 gggatcctgc gggctttgcc tgcgggtgag agtggcgaac gggtgagtaa tgcgtgacca
121 acctgcccca tgctccggaa tagctcctgg aaacgggtgg taatgccgga tgttcccgcg
181 ccccgcatgg ggtgcgggga aaagcttttg cggcgtggga tggggtcgcg tcctatcagc
241 ttgttggcgg ggtgagggcc caccaaggct tcgacgggta gccggcctga gaaggcgacc
301 ggccacattg ggactgagat acggcccaga ctcctacggg aggcagcagt ggggaatatt
361 gcacaatggg cgcaagcctg atgcagcgac gccgcgtgcg ggatggaggc cttcgggttg
421 taaaccgctt ttgtttggga gcaagccctt cggggtgagt gtacctttcg aataagcacc
481 ggctaaatac gtgccagcag ccgcggtaat aagtagggtg cgagcgttat ccggatttat
541 tgggcgtaaa gggcttgtag gcggtttgtc gcgtccggtg tgaaagtcca tcgcctaacg
601 gtggatttgc gccgggtacg ggcgggctgg agtgcggtag gggagactgg aattcccggt
661 gtaacggtgg aatgtgtaga tatcgggaag aacaccaatg gcgaaggcag gtctttgggc
721 cgttactgac gctgaggagc gaaagcgtgg ggagcgaaca ggattagata ccctggtagt
781 ccacgccgta aacggtggat gctggatgtg gggcccttcc acgggtcccg tgtcggggcc
841 aacgcgttaa gcatcccgcc tggggagtac ggccgcaagg ctaaaactca aagaaattga

901 cgggggcccg cacaagcggc ggagcatgcg gattaattcg atgcaacgcg aaaaacctta
961 cctgggcttg acatgttccc gacgacggca gagatgtcgt ttcccttcgg ggcgggttca
1021 caggtggtgc atggtcgtcg tcagctcgtg tcgtgagatg ttgggtcaag tcccgcaacg
1081 agcgcaaccc tcgccccgtg ttgccagcgc gtcttggcgg gaactcaccg gggaccgccg
1141 gggtttaccc ggaggaaggt ggggatgacg tcagatcatc atgcccctta cgtccagggc
1201 ttcacggcat gctacaatgg ccgggtacag gcggggatgc agacatggtg acatggagcg
1261 ggatccctga aaaccggtct cagttcggga tcggagcgtg caacccggct cggtgaaggc
1321 ggagtcggct aagtaatcgc ggatcagcaa cgccgcggtg aatgcgttcc cgggccttgt
1381 acacaccgcc cgtcaagtca tgaaagtggg cagcacccga agccggtggc ctgaccagta
1441 ttgctggggg gagccgtcta aggtgaggct cgcgattggg agtaagtcgt aacaaggtag
1501 ccgtaccgga aggtgcggct ggatcacctc ctt

Bifdobacterium bifidum 16S rRNA gene (NCBI/Genbank accession code M38018, SEQ ID NO:8)*

1 tttttgtgga gggttcgatt ctggctcagg atgaacgctg gcggcgtgct taacacatgc
61 aagtcgaacg ggatccatca agcttgcttg gtggtgagag tggcgaacgg gtgagtaatg
121 cgtgaccgac ctgccccatg ctccggaata gctcctggaa acgggtggta atgccgnatg
181 ttccacatga tcgcatgtga ttgtgggaaa gattctatcg gcgtgggatg gggtcgngtc
241 ctatcagctt gttggtgagg taacggctca ccaaggcttc gacgggtagc cggcctgaga
301 gggcgaccgg ccacattggg actgagatac ggcccagact cctacgggag gcagcagtgg
361 ggaatattgc acaatgggcg caagcctgat gcagcgacgc cgcgtgaggg atggaggcct
421 tcgggttgta aacctctttt gtttgggagc aagccttcgg gtgagtgtac ctttcgaata
481 agcgccggct aactacgtgc cagcagccgc ggtaatacgt agggnnnnag cgttatccgg
541 atttattggg cgtaaagggc tcgtaggcgg ctcgtcgcgt ccggtgtgaa agtccatcgc
601 ttaacggtgg atctgcgccg ggtacgggcg ggctggagtg cggtagggga gactggaatt
661 cccggtgtaa cggtggaatg tgtagatatc gggaagaaca ccgatggcga aggcaggtct
721 ctgggcngtc actgacgctg aggagcnaaa gcgtggggag cgaacaggat tagataccct
781 ggtagtccac gccgtaaacg gtggacgctg gatgtggggc acgttccacg tgttccgtgt
841 cggagctaac gcgttaagcg tcccgcctgg ggagtacggc cgcaaggcta aaactcaaag
901 aaattgacgg gggccngcac aagcggcgga gcatgcggat taattcgaac naacgcgaag
961 aaccttacct gggcttgaca tgttcccgac gacgccagag atggcgtttc ccttcggggc
1021 gggttcacag gtggtgcatg gtcgtcgtca gctcgtgtcg tgagatgttg ggttaagtcc
1081 cgcaacgagc gcaaccctcg ccccgtgttg ccagcacgtt atggtgggaa ctcacgggnn
1141 accgccgggg ttaacncgga ggaaggtggg gatgacgtca gatcatcatg cccttacgt
1201 ccagggcttc acgcatgcta caatggccgg tacagcggga tgcgacatgg cgacatggag
1261 cggatccctg aaaaccggtc tcagttcgga tcggagcctg caacccggct ccgtgaaggc
1321 ggagtcgcta gtaatcgcgg atcagcaacg ccgcggtgaa tgcgttcccg ggccttgtac

1381 acaccgcccg tcaagtcatg aaagtgggca gcacccgaag ccggtggcct aacccccttgt
1441 gggatggagc cgtctaaggt gaggctcgtg nttgggacta agnngtaaca agnnnnnngt
1501 accggaagnn nnnnnnngat cacctccttt ct

Bifidobacterium adolescentis 16S rRNA gene (NCBI/Genbank accession code M58729, SEQ ID NO:9)*

1 nnnnttgtgg agggttcgat tctggctcag gatnaacgct ngcggcgtgc ttaacacatg
61 caagtcgaac gggatcggct ngagcttgct ccggctgtga gagtggcgaa cgggtgagta
121 atgcgtgacc gacctgcccc atacaccgga atagctcctg gaaacgggtg gtaatgccgg
181 atgctccagt tggatgcatg tccttctggg aaagattcta tcggtatggg atggggtcgc
241 gtcctatcag cttgatggcg gggtaacggc ccnccatggc ttcgacgggn agccggcctg
301 agagggcgac cggccacatt gggactgaga tacggcccng actcctacgg gaggcagcag
361 tgggnaatat tgcacaatgg gcgcaagcct aatgcagcga cgccgcgtgc gggatgacgg
421 ccttcgggtt gtaaaccgct tttgactggg agcaagcctt cggggtgagt gtacctttcg
481 aataagcacc ggctaactac gtgccagcag ccncggtaat acgtagggtg cnagcgttat
541 ccggaattat tgggcgtaaa gggctcgtag gcggttcgtc gcgtccggtg tgaaagtcca
601 tcgcttaacg gtggntccgc gccgggtacg ggcggncttg agtgcggtag ggnagactgg
661 aattccnggt gtaacggtgg aatgtgtaga tatcgggaag aacaccaatg gcgaaggcag
721 gtctctgggc ngtnactgac gctgaggagc gaaagcgtgg ggagcgaaca ggattagata
781 ccctggtagt ccacgccgta aacggtggat gctggatgtg gggaccattc cacggtctcc
841 gtgtcggagc caacgcgtta agcatcccgc ctggggagta cggccgcaag gctaaaactc
901 aaagaaattg acgggnnccn ncacaagcgg cngagcatgc ggattaattc gatnnaacgc
961 gaagaacctt acctgggctt gacatgttcc cgacaggccc cagagatggg nnntccttcg
1021 ggncgggntc acaggtggng catggtcgtc gtcagctcgt gtcgtgagat gttgggttaa
1081 gtcccgcaac gagcgcaacc ctcgccctgt gttgccagca cgtcgtggtg gnaactcacg
1141 ggngaccgcc ggggtcaact cggaggaagg tgggnatgac gtcagatcat catgcccctt
1201 acgtccaggg cttcacgcat gctacaatgg ccggtacaac gggatgcgac ctcgtgaggg
1261 ggagcggatc ccttaaaacc ggnctcagtt cggattggag tctgcaaccc gactccatga
1321 aggcggagtc gctagtaatc gcggatcagc aacgccgcgg tnaatgcgtt cccgggcctt
1381 gtacacaccg cccgtcaagc catgaaagtg ggtagcaccc gaagccggtg gcccnacctt
1441 tttgggggga gccgtctaag gtgagnctcg tgatngg

Bifodbacterium catenulatum 16S rRNA gene (NCBI/Genbank accession code M58732, SEQ ID NO:10)*

1 nnnttttgtg agnggttcga ttctggctca ggatgaacgc tggcggcgtg cttaacacat
61 gcaagtcgaa cgggatcagg cagcttgctg cctggngaga gtggcgaacg ggnnagtaat
121 gcgtgaccna cctgccnnat acaccggaat agctcctgga aacgggtggt aatgccggat

181 gctccgactc ctcgcatggg gtgtcggnaa agatttcatc ggtatgggat ggggtcgngt
241 cctatcaggt agtcggcggg gtaacggcnn nccgagcctn cgacgggtag ccggcctgag
301 agggcgaccg gccacattgg gactgagata cggccnngac tcctacggga ggcagcagtg
361 ggncatattg cacaatgggc gcaagcctna tgcagcgacg cnnngtgcgg gntgacggcc
421 tncgggttgt aaaccncntt tgatcgggag caagccttcg ggtgagtgta ccnttcgaat
481 aagcaccggc taactacgtg ccagcagccg cggtaatacg tagggtgcna gcgttatccg
541 gaattattgg gcgtaaaggg ctcgtaggcg gttcgtcgcg tccggtgtga aagtccatcg
601 cttaacggtg gatctgcgcc gggtacgggc gggctggagt gcggtagggg ngactggaat
661 tcccggtgta acggtggaat gtgtagatat cgggaagaac accaatggcg aaggcnggtc
721 tctgggcngn nactgacgct gaggagcgaa agcgtgggga gcgaacagga ttagataccc
781 tggtagtcca cgccgtaaac ggtggatgct ggatgtgggg cnngttccac gggttccgtg
841 tcggagctaa cgcgttaagc atccngcctg gggngtncgg cngcaaggcn nnnncncaaa
901 gaaattgang ggggccngca caagcggngg agcatgcgga ttnattcgan nnaacgcgaa
961 gaaccttacc tgggcttgac atgttcccga cagccgtaga gatacggnct cccttcgggg
1021 cgggnncaca ggtggngcat ggtcgtcgtc ngctcgtgtc gtgagatgtt gggttaagtc
1081 ccncaacgag cgcaaccctc gccctgtgtt gccgacacgt catgtnggna ctcacgggnn
1141 accgccgggg tcaactcgga ggaaggtggg gatgacgtca gatcatcatg ccccttacgt
1201 ccagggcttc acgcatgcta caatggccgg tacaacggga tgcgacatgg cgacatggag
1261 cggatccctg aaaaccggnc tcagttcgga ttggagtctg caacccgact ccatgaaggc
1321 ggagtcgcta gtaatcgcgg atcagcaacg ccgcggtgaa tgcgttcccg ggccttgtac
1381 acaccgcncg tcaagncatg aaagtgggta gcacccgaag ccggtggcct nacccnttgt
1441 gggatggagc cgtctaaggt gagactcgtg attgggac

Bifdobacterium pseudocatenulatum 16S rRNA gene (NCBI/Genbank accession code D86187, SEQ ID NO:11)

1 gtttcgattc tggctcagga tgaacgctgg cggcgtgctt aacacatgca agtcgaacgg
61 gatccatcag gctttgcttg gtggtgagag tggcgaacgg gtgagtaatg cgtgaccgac
121 ctgccccata caccggaata gctcctggaa acgggtggta atgccggatg ctccgactcc
181 tcgcatgggg tgtcgggaaa gatttcatcg gtatgggatg gggtcgcgtc ctatcaggta
241 gtcggcgggg taacggccca ccgagcctac gacgggtagc cggcctgaga gggcgaccgg
301 ccacattggg actgagatac ggcccagact cctacgggag gcagcagtgg ggaatattgc
361 acaatgggcg caagcctgat gcagcgacgc cgcgtgcggg atgacggcct tcgggttgta
421 aaccgctttt gatcgggagc aagccttcgg gtgagtgtac ctttcgaata agcaccggct
481 aactacgtgc cagcagccgc ggtaatacgt agggtgcaag cgttatccgg aattattggg
541 cgtaaagggc tcgtaggcgg ttcgtcgcgt ccggtgtgaa agtccatcgc ttaacggtgg
601 atctgcgccg ggtacgggcg ggctggagtg cggtagggga gactggaatt cccggtgtaa
661 cggtggaatg tgtagatatc gggaagaaca ccaatggcga aggcaggtct ctgggccgtt

721 actgacgctg aggagcgaaa gcgtggggag cgaacaggat tagataccct ggtagtccac
781 gccgtaaacg gtggatgctg gatgtggggc ccgttccacg ggttccgtgt cggagctaac
841 gcgttaagca tcccgcctgg ggagtacggc cgcaaggcta aaactcaaag aaattgacgg
901 gggcccgcac aagcggcgga gcatgcggat taattcgatg caacgcgaag aaccttacct
961 gggcttgaca tgttcccgac agccgtagag atatggcctc ccttcggggc gggttcacag
1021 gtggtgcatg gtcgtcgtca gctcgtgtcg tgagatgttg ggttaagtcc cgcaacgagc
1081 gcaaccctcg ccctgtgttg ccagcacgtc atggtgggaa ctcacggggg accgccgggg
1141 tcaactcgga ggaaggtggg gatgacgtca gatcatcatg ccccttacgt ccagggcttc
1201 acgcatgcta caatggccgg tacaacggga tgcgacacgg cgacgtggag cggatccctg
1261 aaaaccggtc tcagttcgga ttggagtctg caacccgact ccatgaaggc ggagtcgcta
1321 gtaatcgcgg atcagcaacg ccgcggtgaa tgcgttcccg ggccttgtac acaccgcccg
1381 tcaagtcatg aaagtgggta gcacccgaag ccggtggcct aaccctttgt ggatggagcc
1441 gtctaaggtg agactcgtga ttgggactaa gtcgtaacaa ggtagccgta ccggaaggtg
1501 cggctggatc acctcctta

*"n" refers to a, t, c, or g.

In another particularly preferred embodiment, the *Anaerobutyricum soehngenii* or relative thereof and/or the at least one *Bifidobacterium* species according the disclosure is combined with at least one *Akkermansia* species, preferably wherein said at least one *Akkermansia* species is pasteurized / has been subjected to pasteurization (i.e. heating to 55-99, preferably 65-80 degrees Celsius for 5-60 seconds or 1-60 minutes, preferably 60-80 degrees Celsius for 20-40 minutes, more preferably 65-75 degrees Celsius for 25-35 minutes). It was found that this is a further synergistic combination, leading to an unexpected reduction in hepatic necroinflammatory activity score.

The at least one *Akkermansia* species may be administered separately, sequentially or simultaneously with said *Anaerobutyricum soehngenii* or relative thereof and/or said at least one *Bifidobacterium* species. Accordingly, said *Akkermansia* species may be comprised in the same or in a separate composition with respect to said *Anaerobutyricum soehngenii* or relative thereof and/or the at least one *Bifidobacterium* species.

Preferably, the at least one *Akkermansia* species according to the present disclosure is *Akkermansia muciniphila* or relative thereof having a 16S rRNA sequence with at least 90, 95, 97, 99, or 100% sequence identity with SEQ ID NO:12.

Akkermansia is a genus in the phylum Verrucomicrobia. It was found that *Akkermansia* species improve intestinal mucosal barrier function, or intestinal barrier function, which refers to the property of the intestinal mucosa that ensures adequate containment of undesirable luminal contents within the intestine while preserving the ability to absorb nutrients. Its role in protecting the mucosal tissues and circulatory system from exposure to pro-inflammatory molecules, such as microorganisms, toxins, and antigens is vital for the maintenance of health and well-being. Accordingly, *Akkermansia* species may prevent or be used for treating intestinal mucosal barrier dysfunction, which has been implicated in numerous health conditions such as: food allergy, microbial infection, irritable bowel syndrome, inflammatory bowel disease, celiac disease, metabolic syndrome, non-alcoholic fatty liver disease, diabetes, and septic shock. See Collado et al 2007 (Appl Environ Microbiol 2007 Dec;73(23):7767-70). Or see Appl Environ Microbiol. 2020 Mar 18;86(7):e03004-19.

The at least one *Akkermansia* species of the present disclosure preferably includes one or more of

- *Akkermansia muciniphila* (able to assimilate HMO) or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Akkermansia muciniphila* (NCBI accession code AY271254, SEQ ID NO:12).
- *Akkermansia glycanipila* or relative thereof having a 16S rRNA gene with at least 90, 95, 97, 98, 99, 100% sequence identity with the 16S rRNA gene sequence of the type strain of *Akkermansia glycanipila* (NCBI accession code NR152695, SEQ ID NO:13).

Akkermansia muciniphila 16S rRNA gene (NCBI/Genbank accession code AY271254, SEQ ID NO:12)
1 aacgaacgct ggcggcgtgg ataagacatg caagtcgaac gagagaattg ctagcttgct
61 aataattctc tagtggcgca cgggtgagta acacgtgagt aacctgcccc cgagagcggg
121 atagccctgg gaaactggga ttaataccgc atagtatcga aagattaaag cagcaatgcg
181 cttggggatg ggctcgcggc ctattagtta gttggtgagg taacggctca ccaaggcgat
241 gacgggtagc cggtctgaga ggatgtccgg ccacactgga actgagacac ggtccagaca
301 cctacgggtg gcagcagtcg agaatcattc acaatggggg aaaccctgat ggtgcgacgc
361 cgcgtggggg aatgaaggtc ttcggattgt aaacccctgt catgtgggag caaattaaaa
421 agatagtacc acaagaggaa gagacggcta actctgtgcc agcagccgcg gtaatacaga
481 ggtctcaagc gttgttcgga atcactgggc gtaaagcgtg cgtaggctgt ttcgtaagtc
541 gtgtgtgaaa ggcgcgggct caacccgcgg acggcacatg atactgcgag actagagtaa
601 tggaggggga accggaattc tcggtgtagc agtgaaatgc gtagatatcg agaggaacac
661 tcgtggcgaa ggcgggttcc tggacattaa ctgacgctga ggcacgaagg ccaggggagc

721 gaaagggatt agataccccct gtagtcctgg cagtaaacgg tgcacgcttg gtgtgcgggg
781 aatcgacccc ctgcgtgccg gagtaacgcg ttaagcgtgc cgcctgggga gtacggtcgc
841 aagattaaaa ctcaaagaaa ttgacgggga cccgcacaag cggtggagta tgtggcttaa
901 ttcgatgcaa cgcgaagaac cttacctggg cttgacatgt aatgaacaac atgtgaaagc
961 atgcgactct tcggaggcgt tacacaggtg ctgcatggcc gtcgtcagct cgtgtcgtga
1021 gatgtttggt taagtccagc aacgagcgca acccctgttg ccagttacca gcacgtgaag
1081 gtggggactc tggcgagact gcccagatca actgggagga aggtggggac gacgtcaggt
1141 cagtatggcc cttatgccca gggctgcaca cgtactacaa tgcccagtac agaggggggcc
1201 gaagccgcga ggcggaggaa atcctaaaaa ctgggcccag ttcggactgt aggctgcaac
1261 ccgcctacac gaagccggaa tcgctagtaa tggcgcatca gctacggcgc cgtgaatacg
1321 ttcccgggtc ttgtacacac cgcccgtcac atcatggaag ctggtcgcac ccgaagtatc
1381 tgaagccaac cgcaaggagg cagggtccta aggtgagact ggtaactggg atg

Akkermansia glycanipila 16S rRNA gene (NCBI/Genbank accession code NR152695, SEQ ID NO:13)

1 aacgaacgct ggcggcgtgg ataagacatg caagtcgaac ggagaagcaa tagcttgcta
61 atgcttctta gtggcgcacg ggtgagtaac acgtgagcaa cctgccttcg agacgggaat
121 agccctggga aaccgggatt aatgcccgat agactcgcaa gagtaaacgc agcaatgcgc
181 ttgaagaggg gctcgcggcc tattagttag ttggtgaggt aacggctcac caaggcgatg
241 acgggtagcc ggtctgagag gatgtccggc cacactggaa ctgagacacg gtccagacac
301 ctacgggtgg cagcagtcga gaatcattca caatggggga aaccctgatg gtgcgacgcc
361 gcgtggggga agaaggtctt cggattgtaa acccctgtca tgtgggagca aggcgcaagc
421 ttgatagtac cacaagagga agagacggct aactctgtgc cagcagccgc ggtaatacag
481 aggtctcaag cgttgttcgg aatcactggg cgtaaagggt acgtaggctg catcataagt
541 cgggcgtgaa aggcaggggc tcaacccctg gagtgcgctt gatactgtga tgctagagtc
601 atggaggggg aaccggaact ctcggtgtag cagtgaaatg cgtagatatc gagaagaaca
661 ctcgtggcga aggcgggttc ctggacatgt actgacgctg aggtacgaag gctaggggag
721 cgaaagggat tagataccc tgtagtccta gcagtaaacg gtgcacgctt ggtgtgtggg
781 gaatcgaccc cccacgtgcc ggagcaaacg cgttaagcgt gccgcctggg gagtacggtc
841 gcaagattaa aactcaaaga aattgacggg gacccgcaca agcggtggag tatgtggctt
901 aattcgatgc aacgcgaaga accttacctg ggcttgacat gtgatgaaca acatgtgaaa
961 gcatgtgaca cctcggtggc gtcacacagg tgctgcatgg ccgtcgtcag ctcgtgtcgt
1021 gagatgtttg gttaagtcca gcaacgagcg caacccctgt tgccagttac cagcacgtta
1081 tggtggggac tctggcgaga ctgcccagat caactgggag gaaggtgggg acgacgtcag
1141 gtcagtatgg cccttatgcc cagggctgca cacgtactac aatgcccagt acagagggta
1201 ccgaacccgc gagggggagg caatccatga aaactgggcc cagttcggat tgtaggctgc
1261 aactcgccta catgaagatg gaatcgctag taatggcgca tcagctacgg cgccgtgaat

1321 acgttcccgg gtcttgtaca caccgcccgt cacatcatgg aagccggtcg cacccgaagt 1381 atctgaagcc aaccgcaagg aggcagggtc ctaaggtgag actggtaact gggatgaa

In another particularly preferred embodiment, the *Anaerobutyricum soehngenii* or relative thereof and/or the at least one *Bifidobacterium* species and/or the at least one *Akkermansia* species according the disclosure is combined with at least one *Lactobacillus* species. It was found that this is a further synergistic combination, leading to an unexpected reduction in hepatic necroinflammatory activity score.

The at least one *Lactobacillus* species may be administered separately, sequentially or simultaneously with said *Anaerobutyricum soehngenii* or relative thereof and/or said at least one *Bifidobacterium* species and/or said at least one *Akkermansia* species. Accordingly, said *Lactobacillus* species may be comprised in the same or in a separate composition with respect to said *Anaerobutyricum soehngenii* or relative thereof and/or the at least one *Bifidobacterium* species and/or the at least one *Akkermansia* species.

Said *Lactobacillus* species is preferably chosen from

- *Lactobacillus acidophilus* or relative thereof having a 16S rRNA sequence with at least 90, 95, 97, 99, 100% sequence identity with SEQ ID NO:14;
- *Lactobacillus casei* or relative thereof having a 16S rRNA sequence with at least 90, 95, 97, 99, 100% sequence identity with SEQ ID NO:15;
- *Lactobacillus reuteri* or relative thereof having a 16S rRNA sequence with at least 90, 95, 97, 99, 100% sequence identity with SEQ ID NO:16; and/or
- *Lactobacillus rhamnosus* or relative thereof having a 16S rRNA sequence with at least 90, 95, 97, 99, 100% sequence identity with SEQ ID NO:17.

Lactobacillus acidophilus 16S rRNA sequence (NCBI NR_043182.1) (SEQ ID NO:14)
1 tcctggctca ggacgaacgc tggcggcgtg cctaatacat gcaagtcgag cgagctgaac 61 caacagattc acttcggtga tgacgttggg aacgcgagcg gcggatgggt gagtaacacg 121 tggggaacct gccccatagt ctgggatacc acttggaaac aggtgctaat accggataag 181 aaagcagatc gcatgatcag cttataaaag gcggcgtaag ctgtcgctat gggatggccc 241 cgcggtgcat tagctagttg gtagggtaac ggcctaccaa ggcaatgatg catagccgag 301 ttgagagact gatcggccac attgggactg agacacggcc caaactccta cgggaggcag 361 cagtagggaa tcttccacaa tggacgaaag tctgatggag caacgccgcg tgagtgaaga 421 aggttttcgg atcgtaaagc tctgttgttg gtgaagaagg atagaggtag taactggcct

481 ttatttgacg gtaatcaacc agaaagtcac ggctaactac gtgccagcag ccgcggtaat
541 acgtaggtgg caagcgttgt ccggatttat tgggcgtaaa gcgagcgcag gcggaagaat
601 aagtctgatg tgaaagccct cggcttaacc gaggaactgc atcggaaact gtttttcttg
661 agtgcagaag aggagagtgg aactccatgt gtagcggtgg aatgcgtaga tatatggaag
721 aacaccagtg gcgaaggcgg ctctctggtc tgcaactgac gctgaggctc gaaagcatgg
781 gtagcgaaca ggattagata ccctggtagt ccatgccgta aacgatgagt gctaagtgtt
841 gggaggtttc cgcctctcag tgctgcagct aacgcattaa gcactccgcc tggggagtac
901 gaccgcaagg ttgaaactca aaggaattga cgggggcccg cacaagcggt ggagcatgtg
961 gtttaattcg aagcaacgcg aagaacctta ccaggtcttg acatctagtg caatccgtag
1021 agatacggag ttcccttcgg ggacactaag acaggtggtg catggctgtc gtcagctcgt
1081 gtcgtgagat gttgggttaa gtcccgcaac gagcgcaacc cttgtcatta gttgccagca
1141 ttaagttggg cactctaatg agactgccgg tgacaaaccg gaggaaggtg gggatgacgt
1201 caagtcatca tgccccttat gacctgggct acacacgtgc tacaatggac agtacaacga
1261 ggagcaagcc tgcgaaggca agcgaatctc ttaaagctgt tctcagttcg gactgcagtc
1321 tgcaactcga ctgcacgaag ctggaatcgc tagtaatcgc ggatcagcac gccgcggtga
1381 atacgttccc gggccttgta cacaccgccc gtcacaccat gggagtctgc aatgcccaaa
1441 gccggtggcc taaccttcgg gaaggagccg tctaaggc

Lactobacillus casei 16S rRNA sequence (NCBI MT994696) (SEQ ID NO:15)

1 gttggagaag aatggtcggc agagtaactg ttgtcggcgt gacggtatcc aaccagaaag
61 ccacggctaa ctacgtgcca gcagccgcgg taatacgtag gtggcaagcg ttatccggat
121 ttattgggcg taaagcgagc gcaggcggtt ttttaagtct gatgtgaaag ccctcggctt
181 aaccgaggaa gcgcatcgga aactgggaaa cttgagtgca gaagaggaca gtggaactcc
241 atgtgtagcg gtgaaatgcg tagatatatg gaagaacacc agtggcgaag gcggctgtct
301 ggtctgtaac tgacgctgag gctcgaaagc atgggtagcg aacaggatta gataccctgg
361 tagtccatgc cgtaaacgat gaatgctagg tgttggaggg tttccgccct tcagtgccgc
421 agctaacgca ttaagcattc cgcctgggga gtacgaccgc aaggttgaaa ctcaaaggaa
481 ttgacggggg cccgcacaag cggtggagca tgtggtttaa ttcgaagcaa cgcgaagaac
541 cttaccaggt cttgacatct ttttgatcac tgagagatca ggtttcccct tcgggggcaa
601 aatgacaggt ggtgcatgtt gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc
661 aacgagcgct a

Lactobacillus reuteri 16S rRNA sequence (NCBI NR_025911) (SEQ ID NO:16)

1 agagtttgat cctggctcag gatgaacgcc ggcagtgtgc ctaatacatg caagtcgtac
61 gcactggccc aactaattga tggtgcttgc tgaattgacg atggatcacc agtgagtggc
121 ggacgggtga gtaacacgta ggtaacctgc cccggagcgg ggaataacat ttggaaacag
181 atgctaatac cgcataacaa caaaagccgc atggtttttc tggaaagatg gctttggcta

241 tcactctggg atggacctgc ggtgcattta gctagttggt aaggtaacgg cttacccaag
301 gcgatgatgc atagccgagt tgagagactg atcggccaca atgggaactg agacacggtc
361 cataacttct acgggaggca gcagtaggga atcttccaca atgggcgcaa gctgatggag
421 caacaccgcg ttattaagaa agggtttcgg ccgcttaaac tctgttgttg gagaagaacg
481 tgcgttagag taactgttac gcagtgacgg tatccaacca gaaagtcacg gctaactacg
541 tgccagcagc cgcggtaata cgtaggtggc aagcgttatc cggatttatt gggcgtaaag
601 cgagcgcagg cggttgctta ggtctgatgt ggaaactcgg cttaaccgaa gaagtgcatc
661 ggaaaccggg cgacttgagt gcagaagagg acagtggaac tccatgtgta gcggtggaat
721 gcgtagatat atggaagaac accagtggcg aaggcggctg tctggtctgc aactgacgct
781 gaggctcgaa agcatgggta gcgaacagga ttagataccc tggtagtcca tgccgtaaac
841 gatgagtgct aggtgttgga gggtttccgc ccttcagtgc ctgttctaac gcattaatgc
901 actccgcctg gggagtacga ccgcaaggtt gaaactcaaa ggaattgacg ggggcccgca
961 caagcggtga agcatgtggt ttaattcgaa gctacgcgaa gaaccttacc aggtcttgac
1021 atcttgcgct aaccttagag ataaggcgtt cccttcgggg acgttaatga caggtggtgc
1081 atggtcgtcg tcagctcgtg tcgtgagatg ttgggttaag tcccgcaacg agcgcaaccc
1141 ttgttactag ttgccagcat taagttgggg actctagtga gactgccggt gacaaaccgg
1201 aggaaggtgg ggacgacgtc agatcatcat gccccttatg accctgggct acacacgtgc
1261 tacaatggac ggtacaacga gtcgcaaact cgcgagagta agctaatctc ttaaagccgt
1321 tctcagttcg gactgtaggc tgcaactcgc ctacacgaag tcggaatcgc tagtaatcgc
1381 ggatcagcat gccgcggtga atacgttccc gggccttgta cacaccgccc gtcacaccat
1441 gggagtttgt aacgcccaaa gttcggtggc ctaaccttta tggacgggta ccctaaggcg
1501 ggacagatga tctggggtga agtcgtaaca aggta

Lactobacillus rhamnosus 16S rRNA sequence (NCBI NR_043408.1) (SEQ ID NO:17)

1 grtsaacgct sgcggcgtgc ctaatacatg caagtcgaac gagttctgat tattgaaagg
61 tgcttgcatc ttgatttaat tttgaacgag tggcggacgg gtgagtaaca cgtgggtaac
121 ctgcccttaa gtgggggata acatttggaa acagatgcta ataccgcata aatccaagaa
181 ccgcatggtt cttggctgaa agatggcgta agctatcgct tttggatgga cccgcggcgt
241 attagctagt tggtgaggta acggctcacc aaggcaatga tacgtagccg aactgagagg
301 ttgatcggcc acattgggac tgagacacgg cccaaactct acgggaggca gcagtaggga
361 atcttccaca atggacgcaa gtctgatgga gcaacgccgc gtgagtnaag aaggctttcg
421 ggtcgtaaaa ctctgttgtt ggagaagaat ggtcggcaga gtaactgttg tcggcgtgac
481 ggtatccaac cagaaagcca cggctaacta cgtgccagca gccgcggtaa tacgtaggtg
541 gcaagcgtta tccggattta ttgggcgtaa agcgagcgca ggcggttttt taagtctgat
601 gtgaaagccc tcggcttaac cgaggaagtg catcggaaac tgggaaactt gagtncagaa
661 gaggacagtg gaactccatg tgtagcggtg aaatgcgtag atatatggaa gaacaccagt
721 ggcgaaggcg gctgtctggt ctgtaactga cgctgaggct cgaaagcatg ggtagcgaac

781 aggattagat accctggtag tccatgccgt aaacgatgaa tgctaggtgt tggagggttt
841 ccgcccttca gtgccgcagc taacgcatta agcattccgc ctggggagta cgaccgcaag
901 gttgaaactc aaaggaattg acgggggccc gcacaagcgg tggagcatgt ggtttaattc
961 gaagcaacgc gaagaacctt accaggtctt gacatctttt gatcacctga gagatcaggt
1021 ttcccccttcg ggggcaaaat gacaggtggt gcatggttgt cgtcagctcg tgtcgtgaga
1081 tgttgggtta agtcccgcaa cgagcgcaac ccttatgact agttgccagc atttagttgg
1141 gcactctagt aagactgccg gtgacaaacc ggaggaaggt ggggatgacg tcaaatcatc
1201 atgcccctta tgacctgggc tacacacgtg ctacaatgga tggtacaacg agttgcgaga
1261 ccgcgaggtc aagctaatct cttaaagcca ttctcagttc ggactgtagg ctgcaactcg
1321 cctacacgaa gtcggaatcg ctagtaatcg cggatcagca cgccgcggtg aatacgttcc
1381 cgggccttgt acacaccgcc cgtcacacca tgagagtttg taacacccga agccggtggc
1441 gtaacccttt tagggagcga gccgtctaag gtgggncaaa tgattagggt gaagtcgtaa
1501 caaggtagcc gtaggagaac c

In a preferred embodiment, the present disclosure excludes the use (for example by co-administration) of any *Ruminococcus* species (for example *Ruminococcus flavefaciens*, R. torques or R. faecis) any *Faecalibacterium* species (for example *Faecalibacterium prausnitzii)*, and/or any *Prevotella* species such as *Prevotella copri*.
The present disclosure may include or exclude any *Anaerostipes* species (particularly *Anaerostipes rhamnisovorans)* or any *Faecalibacterium* species (for example *Faecalibacterium prausnitzii)* for improved effect in the prevention and/or treatment according to the present disclosure.

It is envisaged that the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species as according to the present disclosure is/are comprised in fecal matter.

The *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species according to the present disclosure may be or be derived from fecal matter, e.g. obtained from one or more donor subjects. The term “donor” as used herein denotes a subject who donates fecal matter. The fecal matter according to the present disclosure is thus derived from the donor and may be administered to a recipient. Optionally after processing, the fecal matter is administered to the recipient. The one or more donor subjects are preferably mammal, preferably human. Also the recipient is preferably a mammal, preferably a human.

Preferably the fecal matter is obtained from at least one healthy (human) donor, more preferably at least one (human) donor following (or who has followed) a vegetarian diet, most preferably a vegan diet. A vegetarian diet does not include any meat, poultry or seafood, or at most 0.1, 0.5, 1 kg meat, poultry or seafood per month. A vegan diet does not include any meat, poultry, seafood or any food from animal origin, or at most 0.1, 0.5, 1 kg meat, poultry or seafood or food from animal origin per month. A healthy donor can for example be regarded as a donor not having a condition as mentioned in Table 1 of Lise Sofie et al (2019, Transfusion and Apheresis Science, Volume 58, Issue 1, P113-116).

Selected donor subjects preferably have a BMI between 18-27, preferably between 20 to 25 kg/m^2. The term "Body Mass Index" or "BMI" as used herein denotes a value derived from dividing the mass of a person by the square of the person's body height, expressed in kg/m^2. Selected donor subjects preferably have an age below 30 years or below 35 years. The at least one donor subject for example has an age between 18 and 30 years, such as 20 to 25 years. In addition or alternatively, selected donor(s) follow (or have followed) a diet rich in prebiotic fiber (that increases butyrate production in stools), such as WholeFiber, see WO2021/204719 (e.g. at least 0.1, 0.5, 1 kg prebiotic fiber per month).

Additionally or alternatively, the at least one donor subject has a relative abundance of *Bifidobacteriales* species in the fecal matter of at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30% (as compared to the number of species of other genera). Additionally or alternatively, the at least one donor subject has a relative abundance of *Akkermansia* species in the fecal matter of at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30% (as compared to the number of species of other genera).

In a preferred embodiment, at least 10^8, or 10^8 cells of said *Anaerobutyricum soehngenii* or relative thereof are comprised in said fecal matter. Similarly, at least 10^8, or 10^8 cells of said *Bifidobacterium species* are comprised in said fecal matter. Similarly, at least 10^8, or 10^8 cells of said *Akkermansia* species are comprised in said fecal matter. Similarly, at least 10^8, or 10^8 cells of said *Lactobacillus* species are comprised in said fecal matter.

In other words, the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species as according to the present disclosure is preferably enriched in the fecal matter, i.e. the number of *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species cells is higher than in prior art fecal matter, for example *Anaerobutyricum soehngenii* or

relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species cells have been added to the fecal matter, or the fecal matter has been exposed to conditions favoring growth of said *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species. If the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species according to the present disclosure is comprised in fecal matter, preferably at least at least 10^4, 10^5, $2x10^5$, $3x10^5$, $4x10^5$, $5x10^5$, $6x10^5$, $7x10^5$, $8x10^5$, $9x10^5$, 10^6, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$, $8x10^6$, $9x10^6$, 10^7, $2x10^7$, $3x10^7$, $4x10^7$, $5x10^7$, $6x10^7$, $7x10^7$, $8x10^7$, $9x10^7$, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13} cells are comprised in said fecal matter, for example per ml or per g fecal matter. Preferably, the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species is/are the first, second, third, fourth, fifth, sixth, seventh, eighth , ninth, and/or tenth most dominant bacterial species in the fecal matter, i.e. has the highest cell count in comparison to other bacterial species contained in the fecal matter, or is at least in the top 10.

Preferably, in case the composition according to the present disclosure is fecal matter, the fecal matter can be feces or part thereof, preferably a purified part thereof. By purifying the fecal matter, the fecal matter can be more conveniently administered. In a particular embodiment, 50-150 mg fecal matter sample may be combined with 5-15 mL isotonic saline containing e.g. 10% glycerol and can be frozen at -80 C until delivery. For example, 1 mL may be mixed with mother's own milk or pasteurized bank milk to a total volume of 10 mL, and 5 mL can be administered to the recipient.

A part of fecal matter as used herein denotes one or more specific groups of components including, but not limited to: enzymes, proteins, lipids, molecules, microorganisms, viruses, bacteria, fungi, yeast, archaea, compounds, complexes, solids, liquids, particles, and fibers.

A purified part of fecal matter as used herein denotes that undesired groups of components are not present in the fecal matter.

Preferably, the fecal matter for use according to the disclosure is comprised in liquid medium and/or does not comprise solids having a diameter of more than 10, 25, 50, 75, 100, 200, 400, 600, 800, or 1000 µm, preferably obtained by mixing allogenic feces with aqueous medium and subsequent filtering and/or centrifugation. This greatly reduces the viscosity and enhances flow of the fecal matter, facilitating administration of the fecal matter to the receiving subject. The liquid medium can comprise water, or another type of liquid which may be supplemented with other components, such as salts, to provide an isotonic solution.

According to one aspect of the disclosure, the fecal matter according to the disclosure is comprised in a composition, such as a pharmaceutical composition, more preferably a liquid dosage form, facilitating administration of the fecal matter to a recipient.

It is further envisaged that the fecal matter according to the present disclosure is present in lyophilized and/or microencapsulated form (to protect from gastric environment). The use according to the disclosure may involve 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 separate administrations of fecal matter obtained from the at least one donor subject to the recipient, preferably with intervals of at least 1, 2, 3, 4, 5, 6, 7, 8 weeks between said separate administrations.

Alternatively, the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species as according to the present disclosure is/are not comprised in fecal matter.

The at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species as according to the present disclosure may be comprised in a composition.

The composition according to the present disclosure may be administered by enteral, preferably by oral, nasal or rectal administration, and/or by nasoduodenal tube administration.

The composition according to the present disclosure may be used as medicament and/or accompanied by a physiologically acceptable carrier which may be any inert carrier. For instance, non-limiting examples of suitable physiologically or pharmaceutically acceptable carriers include any well-known physiological or pharmaceutical carriers, buffers, diluents, and excipients. It will be appreciated that the choice for a suitable physiological carrier will depend upon the intended mode of administration of the composition as taught herein (e.g., oral). The skilled person knows how to select a physiologically acceptable carrier, which is suitable for or compatible with the compositions for use as taught herein.

It is envisaged that the composition according to the present disclosure is comprised in and/or encapsulated by an (enteric) coating, preferable wherein said coating does not dissolute and/or disintegrate in the gastric environment of the recipient. Such coating may help the composition to reach the intended site for delivery, e.g. the duodenum, without suffering breakdown due to the acidic environment of the stomach. Preferred (enteric) coatings work by presenting a surface that is stable at the highly acidic pH found in the stomach, but breaking

down more rapidly at a lower pH. For example, it will not dissolve in the gastric acids of the stomach (pH ~3), but it will dissolve in the alkaline (pH 7–9) environment present in the small intestine, or duodenum.

In an embodiment, the present disclosure is concerned with the composition for use as a probiotic. Accordingly, 'probiotics' as used herein refers to microorganisms such as intestinal bacteria, which - when administered or ingested in effective amounts - confer health benefits to the host (e.g. humans or mammals). Preferably, probiotics should be alive or viable when administered to a subject so as to allow the probiotics to colonize the large intestine of the host. However, under certain conditions, probiotics may also be dead when administered provided that substances produced by the probiotics still exert probiotic, beneficial effects on the host.

In an embodiment, the present combination as taught herein may be for use as a symbiotic. The term 'symbiotic' or 'symbiotic products' as used herein generally refers to compositions and/or nutritional supplements combining probiotics and one or more compounds that promote the growth and/or activity of GI microorganisms, such as prebiotics, into one product. The symbiotic beneficially affects the host by improving the survival and colonization of the probiotic in the GI tract, by selectively stimulating the growth and/or by activating the metabolism of the probiotic, thus improving host welfare. The skilled person is well-acquainted with symbiotics and knows how to select ingredients that may be combined into a symbiotic.

The present inventors furthermore surprisingly found that micro-encapsulation of the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species as according to the present disclosure, may provide a further synergistic therapeutic effect in the prevention or treatment of hepatic steatosis, NAFLD and/or NASH.

The term 'micro-encapsulation' is used to describe the encapsulation of bacteria in a matrix, coating, or membrane, generally a protective matrix or protective membrane. The (average) diameter of the microcapsules may be between 50 nm and 2 mm, preferably between 100 nm and 1 mm. The matrix, coating or membrane is typically comprised of milk, milk protein, and/or a polymer. The purpose of micro-encapsulation, among other possible purposes, may be to protect bacteria and their components against destruction by the surrounding environment, such as the gastrointestinal environment. The micro-encapsulation of bacteria may also support improved incorporation of bacteria into dairy products, food products,

pharmaceutical formulations, and/or pharmaceutical compositions. The micro-encapsulation of bacteria may also support the therapeutic effect.

Various materials may be used for the micro-encapsulation of bacteria, such as pea protein, milk, milk protein, whey protein, casein, xanthan gum, alginate, gelatin, chitosan, carboxymethyl cellulose, starch, and/or carrageenan, and combinations thereof. In a preferred embodiment, the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species as according to the present disclosure is micro-encapsulated in one or more polymers.

The subject receiving the combination or composition as taught herein may be selected from the group consisting of human being, non-human primate, mouse, rat, dog, cow, and pig. In a preferred embodiment, the subject is a human.

The at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species as according to the present disclosure may be comprised in the combination or composition in an amount ranging from 10^4 to 10^{15} colony forming units (CFU). For instance, the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species may be comprised in the combination in an amount of 10^6 CFU to 10^{13} CFU, preferably 10^7 CFU to 10^{12} CFU, preferably 10^8 CFU to 10^{11} CFU, more preferably 10^9 CFU to 10^{11} CFU, e.g. per dose or per ml or per g of formulation or composition comprising said.

In one of the embodiments, the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species in the combination or composition taught herein may be incorporated in lyophilized form and/or, micro-encapsulated form (reviewed by, for example, Solanki et al. BioMed Res. Int. 2013, Article ID 620719), or any other form preserving the activity and/or viability of the bacterial strain.

In an embodiment, the combination or composition as taught herein may comprise one or more ingredients which are suitable for promoting survival and/or viability of the bacterium or strain derived therefrom as taught herein during storage and/or during exposure to bile and/or during passage through the GI tract of a mammal (e.g. a human being). Non-limiting examples of such ingredients include an enteric coating, and controlled release agents

allowing passage through the stomach. The skilled person knows how to select suitable ingredients for maintaining a bacterium as taught herein viable and functional i.e. able to carry out intended function(s).

It may be advantageous to add one or more prebiotic ingredients to the combination as taught herein, for example, to supplement the effects (e.g. production of propionic acid/propionate and/or butyric acid/butyrate or a derivative thereof) of the bacterium as taught herein. The prebiotic ingredients may also enhance the activity and/or stimulate the growth of the bacterium, or a strain derived therefrom, as taught herein. A 'prebiotic' as used herein generally refers to a non-digestible food ingredient that promotes the growth of beneficial microorganisms in the intestines. Prebiotics or prebiotic products consist mainly of fermentable fibres or non-digestible carbohydrates. The fermentation of these fibres by probiotics promotes the production of beneficial end products, such as SCFAs, particularly butyrate. Non-limiting examples of suitable prebiotics include fibres such as inulin, pectin, and resistant starch, as well as cellobiose, maltose, mannose, salicine, trehalose, amygdalin, arabinose, melibiose, sorbitol, rhamnose and/or xylose. The skilled person is well-acquainted with the field of prebiotics and knows how to select ingredients endowed with prebiotic activity.

In addition or alternative to preventing and/or treating hepatic steatosis, NAFLD and/or NASH, the present disclosure may be used for (enhancing) butyric acid and/or butyrate production, preferably *in situ*, i.e. in the small intestine. Similarly, the combination according to the present disclosure is also capable of decreasing the level of lactate, e.g. *in situ*, in the small intestine (lactate is known to be an undesired compound in the intestinal tract).

The term 'butyrate' or 'butyric acid' (also known under the systematic name butanoic acid) as used herein refers to a carboxylic acid with the structural formula $CH_3CH_2CH_2COOH$. The term may include derivatives thereof, i.e. compounds derived from butyric acid and includes salts and esters of butyric acid, which are known as butyrate or butanoate. Non-limiting examples of butyrate salts include sodium butyrate, calcium butyrate, magnesium butyrate, manganese butyrate, cobalt butyrate, barium butyrate, lithium butyrate, zinc butyrate, potassium butyrate, ferrous butyrate and the like. Non-limiting examples of butyrate esters (i.e. esters of butyric acid) include cellulose acetate butyrate, methyl butyrate, ethyl butyrate, butyl butyrate, pentyl butyrate, and the like.

Without wishing to be bound by any theories, it is believed that the bacterial strain(s) according to the present disclosure, when administered to a human being or when ingested

by a human being in an adequate amount, is/are able to survive and at least transiently colonize the gastrointestinal tract of said human being. This colonization may typically enable greater *in situ* production of butyric acid/butyrate, although other mechanisms cannot be excluded. Increased *in situ* production may underlie, at least in part, the beneficial effects in the combination as taught herein, e.g. preventing and/or treatment of hepatic steatosis, Nonalcoholic fatty liver disease (NAFLD), and/or nonalcoholic steatohepatitis (NASH).

In an embodiment, the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species may be comprised in a food formulation, feed formulation, feed supplement formulation, food supplement formulation or pharmaceutical formulation. At the same time or alternatively, the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species may be comprised in a liquid, liquid beverage (including dairy beverage and fermented beverage), yogurt, cheese, gel, gelatine, gelatine capsule, powder, paste, tablet, or a capsule.

The food or food supplement formulation is preferably a dairy product, more preferably a fermented dairy product, most preferably a yogurt or a yogurt drink.

The pharmaceutical formulation may be for example a liquid or solid form, more preferably a solid form solid dosage form, e.g., may be a capsule, a tablet, or a powder. Preferably, a pharmaceutical formulation does not relate to pure water or aqueous medium comprising more than 99 wt.% water.

The formulations as taught herein comprising the combination for use according to the present disclosure may further comprise any acceptable carrier that is suitable for keeping the *Anaerobutyricum soehngenii* or relative thereof, *Bifidobacterium* species, *Akkermansia* species and/or *Lactobacillus* species as according to the present herein viable until consumption by a subject (e.g. human or animal). For instance, non-limiting examples of acceptable carriers that are suitable for this purpose include any of well-known physiological or pharmaceutical carriers, buffers, and excipients. It will be appreciated that the choice for a suitable physiological or pharmaceutical carrier will depend upon the intended mode of administration of the formulations as taught herein (e.g. oral) and the intended form of the formulations (e.g. beverage, yogurt, powder, capsules, and the like). The skilled person knows how to select a physiological or pharmaceutical carrier, which is suitable for the formulations as taught herein.

The at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species as taught in the present disclosure may be comprised in the composition in an amount ranging from 10^4 to 10^{15} colony forming units (CFU). For instance, the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species may be comprised in the combination in an amount of 10^6 CFU to 10^{13} CFU, preferably 10^7 CFU to 10^{12} CFU, preferably 10^8 CFU to 10^{11} CFU, more preferably 10^9 CFU to 10^{11} CFU, e.g. per dose or per ml or per g of formulation or composition comprising said. Alternatively, the amount of the at least one *Anaerobutyricum soehngenii* or relative thereof, the at least one *Bifidobacterium* species, the at least one *Akkermansia* species and/or the at least one *Lactobacillus* species and/or administration frequency is chosen such that it is between, 10^6 to 10^{13}, preferably 10^7 to 10^{12}, preferably 10^8 to 10^{11}, more preferably 10^9 to 10^{11}, all in CFU per day.

The terms 'comprising' or 'to comprise' and their conjugations, as used herein, refer to a situation wherein said terms are used in their non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. It also encompasses the more limiting verb 'to consist essentially of' and 'to consist of'.

Reference to an element by the indefinite article 'a' or 'an' does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article 'a' or 'an' thus usually means 'at least one'.

The terms 'to increase' and 'increased level' and the terms 'to decrease' and 'decreased level' refer to the ability to significantly increase or significantly decrease or to a significantly increased level or significantly decreased level. Generally, a level is increased or decreased when it is at least 5%, such as 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% higher or lower, respectively, than the corresponding level in a control or reference. Alternatively, a level in a sample may be increased or decreased when it is statistically significantly increased or decreased compared to a level in a control or reference.

As used herein, the term "identity" refers to a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk,

A. M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D. W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER; Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J. Applied Math (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in GUIDE TO HUGE COMPUTERS, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J. Applied Math (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. For example NCBI Nucletide Blast with standard settings (blastn, https://blast.ncbi.nlm.nih.gov/). Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., et al., Nucleic Acids Research (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S. F. et al., J. Molec. Biol. (1990) 215:403).

As an illustration, by a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence, it is intended that the nucleotide sequence is identical to the reference sequence except that there may be up to five point mutations per each 100 nucleotides of the reference polypeptide sequence. In other words, to obtain a nucleotide sequence being at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted and/or substituted with another nucleotide, and/or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. In a sequence listing, a “n” may denote a, t, g, or c.

Should there be an inconsistency between the sequences disclosed in the description and the sequences disclosed in the sequence listing, the sequences disclosed in the description are preferred. Alternatively, the sequences of the sequence listing may be used.

CLAUSES

1. *Anaerobutyricum soehngenii* or relative thereof having a 16S rRNA gene sequence with at least 97% sequence identity with SEQ ID NO:1 or SEQ ID NO:2, for use in preventing and/or treating hepatic steatosis, wherein the use is for increasing bile acid plasma level for reducing liver inflammation.

2. *Anaerobutyricum soehngenii* or relative thereof having a 16S rRNA gene sequence with at least 97% sequence identity with SEQ ID NO:1 or SEQ ID NO:2, for use in preventing and/or treating hepatic steatosis, wherein said *Anaerobutyricum soehngenii* or relative thereof is combined with at least one *Bifidobacterium* species.

3. *Anaerobutyricum soehngenii* or relative thereof for use according to clause 2, wherein the at least one *Bifidobacterium* species is chosen from:
- *Bifidobacterium animalis subspecies lactis* or relative thereof having a 16S rRNA gene sequence with at least 97% sequence identity with SEQ ID NO:3; and/or
- *Bifidobacterium breve* or relative thereof having a 16S rRNA gene sequence with at least 97% sequence identity with SEQ ID NO:6.

3. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, wherein the use is further for reducing hepatic necroinflammatory activity score.

4. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, wherein the hepatic steatosis is Nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH).

5. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, wherein said *Anaerobutyricum soehngenii* or relative thereof is combined with at least one *Akkermansia* species.

6. *Anaerobutyricum soehngenii* or relative thereof for use according to claim 5, wherein said at least one *Akkermansia* species has been subjected to pasteurization.

7. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of clauses 5-6, wherein the at least one *Akkermansia* species is *Akkermansia muciniphila* or relative thereof having a 16S rRNA sequence with at least 97% sequence identity with SEQ ID NO:12.

8. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, wherein said *Anaerobutyricum soehngenii* or relative thereof is combined with at least one *Lactobacillus* species.

9. *Anaerobutyricum soehngenii* or relative thereof for use according to clause 8, wherein said at least one *Lactobacillus* species is chosen from
- *Lactobacillus acidophilus* or relative thereof having a 16S rRNA sequence with at least 97% sequence identity with SEQ ID NO:14;
- *Lactobacillus casei* or relative thereof having a 16S rRNA sequence with at least 97% sequence identity with SEQ ID NO:15;
- *Lactobacillus reuteri* or relative thereof having a 16S rRNA sequence with at least 97% sequence identity with SEQ ID NO:16; and/or
- *Lactobacillus rhamnosus* or relative thereof having a 16S rRNA sequence with at least 97% sequence identity with SEQ ID NO:17.

10. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, which is comprised in fecal matter, preferably wherein said fecal matter is obtained from a healthy donor.

11. *Anaerobutyricum soehngenii* or relative thereof for use according to clause 10, wherein said fecal matter is obtained from a donor following a vegan diet.

12. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of clauses 10-11, wherein at least 10^8 cells of said *Anaerobutyricum soehngenii* or relative are comprised in said fecal matter.

13. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, which is in micro-encapsulated or lyophilized form.

14. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of the previous clauses, which is comprised in a composition preferably comprising a physiologically acceptable carrier.

15. *Anaerobutyricum soehngenii* or relative thereof for use according to clause 14, wherein said *Anaerobutyricum soehngenii* or relative thereof is present in the composition in an amount ranging from 10^4 to 10^{15} colony forming units (CFU).

16. *Anaerobutyricum soehngenii* or relative thereof for use according to any one of clauses 14-15, wherein the composition is
- a pharmaceutical composition, preferably in solid dosage form, such as a capsule, a tablet, or a powder; and/or

- a food composition, preferably a dairy product, more preferably a fermented dairy product, most preferably a yogurt or a yogurt drink.

Figure descriptions

Fig.1 SCFA production in the absence or presence of *Bifidobacterium animalis* subsp *lactis* BLC1.

Fig. 2 SCFA produced in absence or presence of *L.rhamnosus* GG on fucose (25 mM) in YCFA medium.

Fig 3. Histological evaluation of the mice. A-D: Inflammation grade, fibrosis grade, NAS score or global NASH score of the mice, E: CRN classification.

EXPERIMENTAL EXAMPLE 1

It has been shown that *A. soehngenii* can exert effect on glucose metabolism and insulin resistance in the small intestine. In an in vitro model of the Ileum in the presence of a synthetic microbiota *A. soehngenii* contributes only limited to SCFA production. An experiment was performed to see if this SCFA production could be enhanced by supplementation with the commercially available probiotic *Bifidobacterium animalis* subsp *lactis* BLC1 (Bottacini et al 2011, J Bacteriol 193: 6387-6388).

Briefly, a synthetic consortium of bacteria was stabilized for 14 days in an Ileum-M- SHIME model (Simulator of Human Intestinal Microbial Ecosystem) comprising the following upper intestinal bacteria with supporting substrates: *Lactobacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Clostridium nexile*, *Faecalibacterium prausnitzii*, *Veillonella* spp., *Prevotella melaninogenica*, and *Blautia obeum*.

A total of 7 ml of this stabilized consortium was seeded with either *A. soehngenii;* or a combination of *A. soehngenii* and *B. infantis* and incubated under anaerobic conditions in the presence of 3 mM bile salts at 37C. The initial pH of the medium was 7.5.

Samples were taken and analyzed for SCFA (acetate, propionate and butyrate) after 24 hours. The result showed a clear increase of all SCFA in the presence of both *A. soehngenii* and *B. infantis* compared to the level of SCFA in the presence of only *A. soehngenii* (Fig. 1).

This demonstrates the metabolic synergy between A. *soehngenii* and *B. infantis* under conditions of the upper intestinal tract.

EXPERIMENTAL EXAMPLE 2

Similarly, the synergy between *A.soehngenii* L2-7 and various *Lactobacillus* spp. was shown in incubations with various carbon sources. The combination of *A. soehngenii* with the commercial probiotic strain *Lactobacillus rhamnosus* GG (Kankainen et al 2009 106:17193-8) showed a clear synergy during growth on fucose, a common sugar present in the intestinal tract: *A.soehngenii* does not utilize fucose but *L.rhamnosus* GG converts fucose into lactate and acetate while the combination of both strains showed conversion of fucose into butyrate, the major metabolic end product of *A.soehngenii.* See Figure 2.

EXPERIMENTAL EXAMPLE 3

For a period of 20 weeks, two groups of 10 C57BL6/J mice each were placed on a Western diet enriched with 15% fructose in the drinking water (WDF). A control group of 10 mice was placed on a chow diet for the same duration. WDF yielded a diet-induced obesity mouse model (body weight 25% higher than control mice) of non-alcoholic steatohepatitis. From week 12, the DIO-NASH mice were treated with weekly oral gavages of 10^ CFUs of *A. soehngenii* or with placebo. At week 20, mice were killed and blood including portal vein sample, as well as liver and gut samples were collected. The DIO-NASH model induced by WDF worked well in inducing NASH: at week 20 average histological steatosis grade was 3, average NAS score 4 and average fibrosis grade was 1 (pericentral or periportal fibrosis).

Upon administration of *A.soehngenii a* clear reduction in inflammation grade, fibrosis grade,NAS score or global NASH score was observed compared to the placebo. Moreover, the number of mice that showed showed NASH were reduced as compared to the placebo (Fig.3).

EXPERIMENTAL EXAMPLE 4

The present inventors consider that co-administration of *Anaerobutyricum soehngenii* or *Anaerobutyricum hallii* with a *Bifidobacterium species, Akkermansia species and/or*

Lactobacillus species has a beneficial and synergistic effect in patients having or at risk of acquiring hepatic steatosis.

METHODS

Participants

Caucasian, treatment-naïve, omnivorous individuals with hepatic steatosis on ultrasound are included. The main inclusion criteria are age 21-69 years, male or postmenopausal female, body mass index (BMI) > 25 kg/m2 with hepatic steatosis on previous ultrasound with suspicion of NAFLD (based on elevated liver enzymes, impaired glucose tolerance, and severity of steatosis on ultrasound). Exclusion criteria are any history of cardiovascular disease, T2DM, renal disease, cholecystectomy, or compromised immunity; use of proton-pump inhibitors, antibiotics, or anticoagulants in the past 3 months; any current use of medication; a history of moderate to heavy alcohol use (>12 g per day); or other causes of liver disease besides NAFLD (e.g. hemochromatosis, auto-immune hepatitis, cirrhosis, hepatitis B or C, hemochromatosis, alpha-1 antitrypsin deficiency, alcoholic liver disease).

Intervention

Subjects are treated for at least 24 weeks according to the single or combinatorial treatment arms shown in Table 1. The hepatic necroinflammatory activity score (NAFLD activity score) is measured at baseline and after treatment. Microbiota treatment is given in capsule form, at 10^{10} living units per capsule, once daily.

Liver biopsy

Percutaneous liver biopsies are performed on the basis of clinical indications according to local standard procedure. All histologic specimens are scored by a liver pathologist who was blinded to any other results. The NASH Clinical Research Network (NASH-CRN) classification (Kleiner et al Volume41, Issue 6 June 2005) is assessed with use of hematoxylin and eosin–stained slides for steatosis, inflammation and ballooning, and with a sirius red–stained slide for evaluation of fibrosis. The necroinflammatory activity score (NAS) is determined as described herein.

Plasma measurement

Bile acid plasma level is determined by liquid chromatography tandem mass spectrometry (LC–MS/MS).

RESULTS

As shown, the present inventors determine the therapeutic effect of *Anaerobutyricum soehngenii* or *Anaerobutyricum hallii* when administered alone, or when administered in combination with a *Bifidobacterium species, Akkermansia species and/or Lactobacillus species*.

Anaerobutyricum soehngenii or *Anaerobutyricum hallii* alone has limited ability to improve necroinflammatory activity score. Nonetheless, the *Anaerobutyricum soehngenii* or *Anaerobutyricum hallii* alone leads to increased plasma levels of primary bile acids (cholic acid and chenodeoxycholic acid) as well as secondary bile acids (deoxycholic acid and lithocholic acid). These increased plasma levels of bile acids activate Farnesoid-X-Receptor (FXR) and G protein–coupled bile acid receptor GPBAR1 (TGR5) which lead to increased secretion of GLP-1 which reduces lipogenesis in the liver and reduces liver inflammation (Chiang (Liver Res. 2017 Jun; 1(1): 3–9).

The effect on bile acid plasma level and efficacy in reduction of the necroinflammatory activity score following treatment is shown in Table 1 accordingly to the following ranking system, wherein the first rank describes the lowest effect and the last rank describes the highest effect: ‘non-measurable’, ‘very low, ‘low’, ‘low/medium’, ‘medium’, ‘high’, ‘very high’. In healthy subjects, a lower necroinflammatory activity score can prevent onset of hepatic steatosis, NAFLD and/or NASH.

Table 1: treatment scheme and effect on bile acid plasma level / lowered necroinflammatory activity score (NAS)

Bacterium	Placebo	*Bifido-bacterium animalis subspecies lactis*	*Bifido-bacterium breve*	*Akkermansia muciniphila*	*Lactobacillus rhamnosus*	*Lactobacillus casei*
Placebo	No change/ Non-measurable	No change/ Non-measurable	No change/ Non-measurable	Slight increase / Very low effect on NAS	No change/ Non-measurable	No change/ Non-measurable
Anaerobuty-ricum soehngenii	Medium increase in bile acids / Medium effect on NAS	Very high increase in bile acids / Very high effect on NAS	Very high increase in bile acids / Very high effect on NAS	Very high increase in bile acids / Very high effect on NAS	High increase in bile acids / High effect on NAS	High increase in bile acids / High effect on NAS
Anaerobuty-ricum hallii	Medium increase in bile acids / Medium effect on NAS	Very high increase in bile acids / Very high effect on NAS	Very high increase in bile acids / Very high effect on NAS	Very high increase in bile acids / Very high effect on NAS	High increase in bile acids / High effect on NAS	High increase in bile acids / High effect on NAS
Anaerobuty-ricum hallii Ruminococcus flavefaciens, Faecali-bacterium prausnitzii, and *Prevotella copri*	Low increase in bile acids / Low effect on NAS	High increase in bile acids / High effect on NAS	High increase in bile acids / High effect on NAS	High increase in bile acids / High effect on NAS	Medium increase in bile acids / Medium effect on NAS	Medium increase in bile acids / Medium effect on NAS

It is expected that results similar to the putative effects as shown in Table 1 can be obtained with larger patient cohorts.

EXPERIMENTAL EXAMPLE 5

MICRO-ENCAPSULATION

As shown in this experiment, the present inventors compare the effect of non-micro-encapsulated bacteria with the effect of micro-encapsulated bacteria.

The same inclusion criteria of subjects and measurements are used as described in experimental example 4. The same ranking system is used as described in experimental example 4 to show the efficacy. The applied dose of bacteria is 100-fold lower as compared to experimental example 1 to exemplify the effect of bacterial micro-encapsulation. The bacteria are given in capsule form, at 10^8 living units per capsule once daily.

RESULTS

Results are shown in Table 2.

Table 2: treatment scheme

Supplement **Bacterium**	**Effect on necro inflammatory score**
Placebo	Non-measurable
Anaerobutyricum soehngenii	Low
Anaerobutyricum soehngenii micro-encapsulated	Low/medium
Anaerobutyricum soehngenii with *Bifidobacterium animalis subspecies lactis*	Low/medium
Anaerobuty-ricum soehngenii with *Bifidobacterium animalis subspecies lactis* micro-encapsulated	High
Anaerobutyricum soehngenii with *Akkermansia muciniphila*	Low/medium
Anaerobutyricum soehngenii with *Akkermansia muciniphila* micro-encapsulated	High

It is expected that similar effects as shown in Table 2 are also obtained with larger patient cohorts.

5

SEQLTXT

SEQUENCE LISTING

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<120> Prevention or treatment of hepatic steatosis

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tgggtaacct gccctgtaca gggggataac agttggaaac ggctgctaat accgcataag 180

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<213> Bifidobacterium animalis subspecies lactis

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<212> DNA
<213> Bifdobacterium bifidum

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P35306NL00
Aanvraagnr. 2030011

Conclusies

1. *Anaerobutyricum soehngenii* of verwant daarvan met een 16S rRNA-gensequentie met ten minste 97% sequentie-identiteit met SEQ ID NO: 1 of SEQ ID NO: 2 voor gebruik bij het voorkomen en/of behandelen van leversteatose, waarbij genoemde *Anaerobutyricum soehngenii* of verwant daarvan wordt gecombineerd met ten minste één *Bifidobacterium*-soort.

2. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens conclusie 1, waarbij de ten minste één *Bifidobacterium*-soort is gekozen uit:
- *Bifidobacterium animalis subspecies lactis* of verwant daarvan met een 16S rRNA-gensequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:3; en/of
- *Bifidobacterium breve* of verwant daarvan met een 16S rRNA-gensequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:6.

3. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, waarbij het gebruik verder is voor het verlagen van de levernecro-inflammatoire activiteitscore.

4. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, waarbij de leversteatose niet-alcoholische leververvetting (NAFLD) en/of niet-alcoholische steatohepatitis (NASH) is.

5. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, waarbij genoemde *Anaerobutyricum soehngenii* of verwant daarvan is gecombineerd met ten minste één *Akkermansia*-soort.

6. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens conclusie 5, waarbij genoemde ten minste één *Akkermansia*-soort is onderworpen aan pasteurisatie.

7. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens willekeurig welke van conclusies 5-6, waarbij de ten minste één *Akkermansia*-soort *Akkermansia muciniphila* of

verwant daarvan is met een 16S-rRNA-sequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:12.

8. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, waarbij genoemde *Anaerobutyricum soehngenii* of verwant daarvan is gecombineerd met ten minste één *Lactobacillus*-soort.

9. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens conclusie 8, waarbij de genoemde ten minste één *Lactobacillus*-soort is gekozen uit
- *Lactobacillus acidophilus* of verwant daarvan met een 16S rRNA-sequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:14;
- *Lactobacillus casei* of verwant daarvan met een 16S rRNA-sequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:15;
- *Lactobacillus reuteri* of verwant daarvan met een 16S rRNA-sequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:16; en/of
- *Lactobacillus rhamnosus* of verwant daarvan met een 16S rRNA-sequentie met ten minste 97% sequentie-identiteit met SEQ ID NO:17.

10. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, dat aanwezig is in fecale materie, bij voorkeur waarbij genoemde fecale materie is verkregen van een gezonde donor.

11. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens conclusie 10, waarbij de fecale materie is verkregen van een donor die een veganistisch dieet volgt.

12. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de conclusies 10-11, waarbij ten minste 10^8 cellen van genoemde *Anaerobutyricum soehngenii* of verwant in genoemde fecale materie aanwezig zijn.

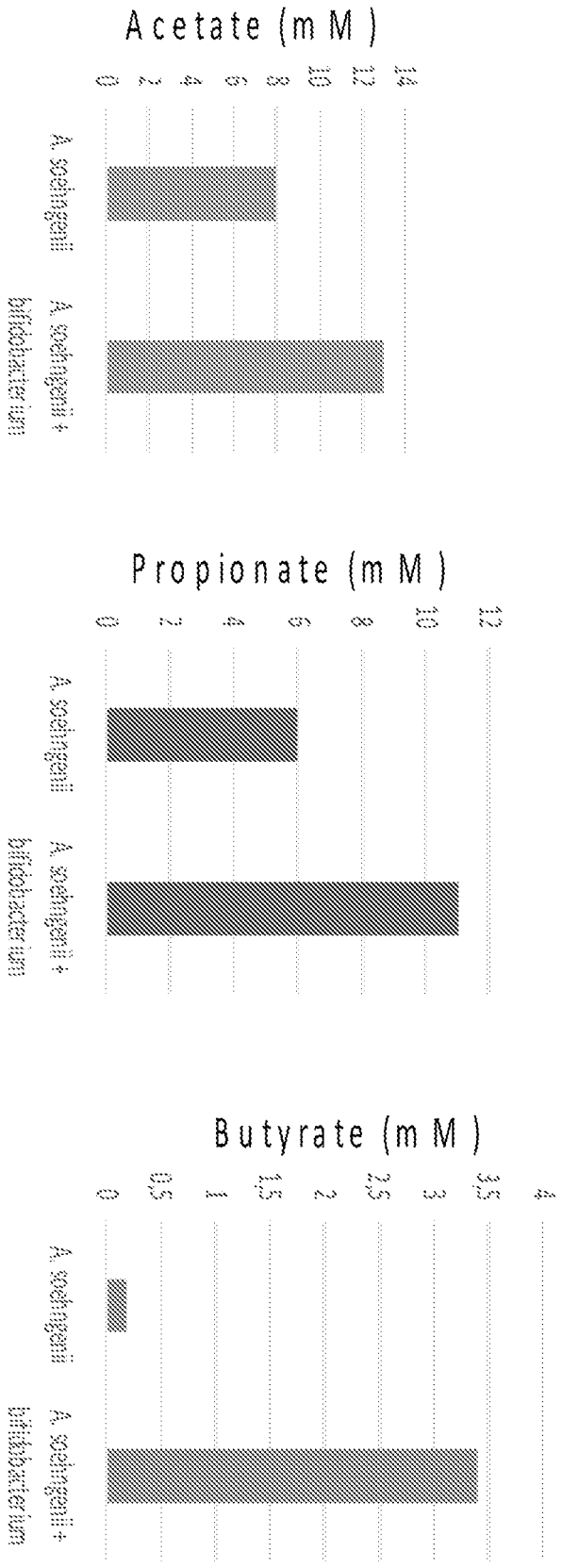
13. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, dat in micro-ingekapselde of gevriesdroogde vorm is.

14. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de voorgaande conclusies, dat is opgenomen in een samenstelling die bij voorkeur een fysiologisch aanvaardbare drager omvat.

15. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens conclusie 14, waarbij genoemd *Anaerobutyricum soehngenii* of verwant daarvan in de samenstelling aanwezig is in een hoeveelheid variërend van 10^4 tot 10^{15} kolonievormende eenheden (CFU).

16. *Anaerobutyricum soehngenii* of verwant daarvan voor gebruik volgens één van de conclusies 14-15, waarbij de samenstelling
- een farmaceutische samenstelling is, bij voorkeur in vaste doseringsvorm, zoals een capsule, een tablet of een poeder; en/of
- een voedingssamenstelling is, bij voorkeur een zuivelproduct, met meer voorkeur een gefermenteerd zuivelproduct, met de meeste voorkeur een yoghurt of een yoghurtdrank.

Fig. 1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4
6
8
10
12
14
A. sebingenii
A. sebingenii + bifidobacterium
Propionate (mM)
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2
4
6
8
10
12
A. sebingenii
A. sebingenii + bifidobacterium
Butyrate (mM)
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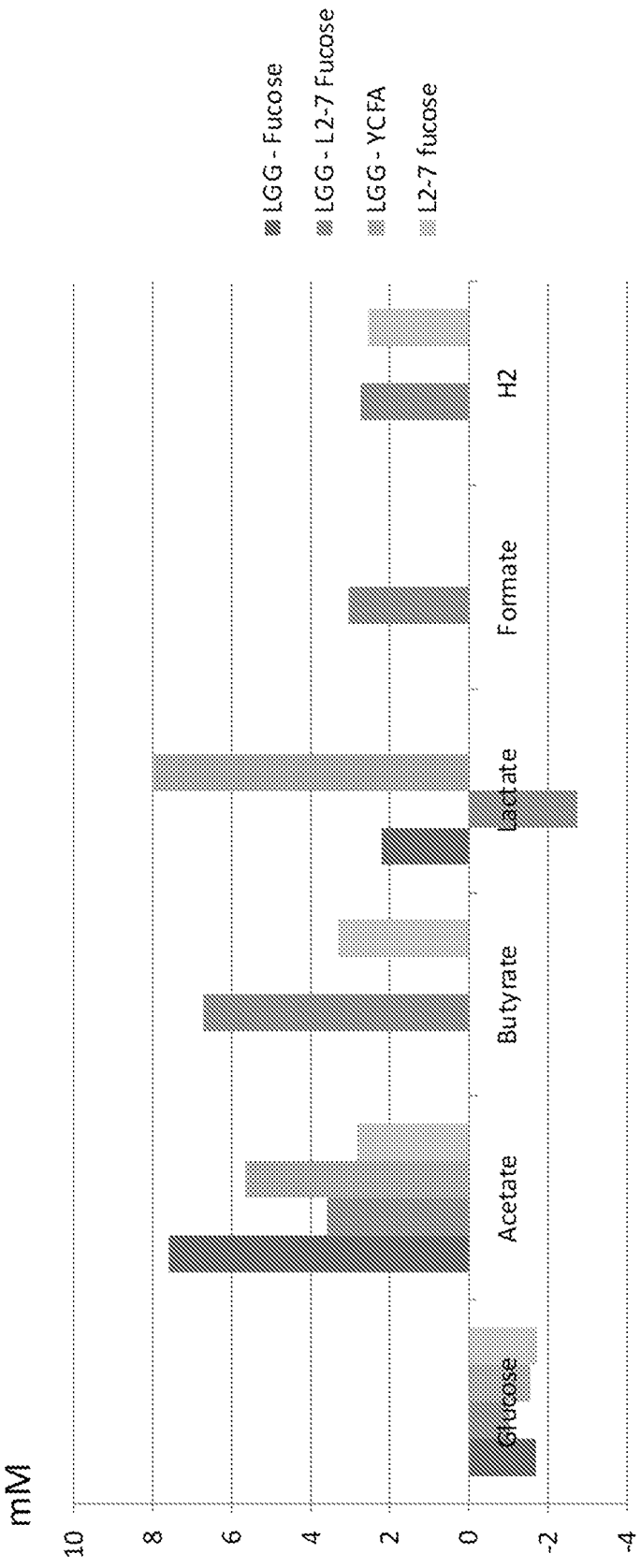
1,5
2
2,5
3
3,5
4
A. sebingenii
A. sebingenii + bifidobacterium

Fig. 2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LGG - L2-7 Fucose
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L2-7 fucose
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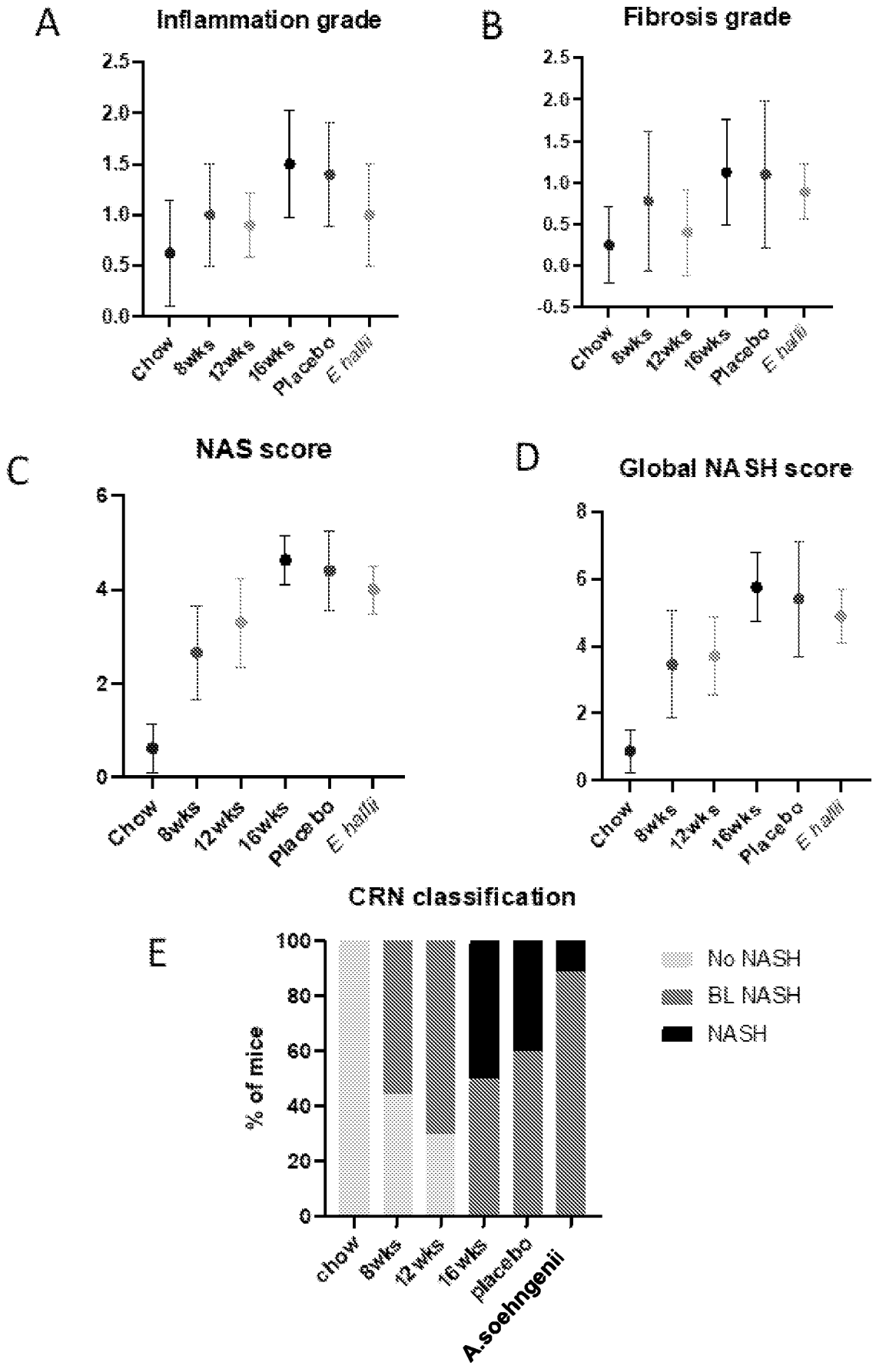
Acetate
Butyrate
Lactate
Formate
H2

Fig. 3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E. hallii
B
Fibrosis grade
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2.0
1.5
1.0
0.5
0.0
-0.5
Chow
8wks
12wks
16wks
Placebo
E. hallii
C
NAS score
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12wks
16wks
Placebo
E. hallii
D
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4
2
0
Chow
8wks
12wks
16wks
Placebo
E. hallii
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placebo
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BL NASH
NASH

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
Nederlands aanvraag nr. **2030011**	Indieningsdatum **03-12-2021**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Caelus Pharmaceuticals B.V., et al**	
Datum van het verzoek voor een onderzoek van internationaal type **04-03-2022**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN80721**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

Zie onderzoeksrapport

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC	**Zie onderzoeksrapport**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐	**GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐	**GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2030011

A. CLASSIFICATIE VAN HET ONDERWERP
INV. A61K35/741 A23L33/135 A61P1/16
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
A61K A23L A61P

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	WO 2020/236979 A1 (PENDULUM THERAPEUTICS INC [US]) 26 november 2020 (2020-11-26)	1,4-7, 10-16
Y	* conclusies; voorbeelden *	2,8
A		3,9

-/--

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
22 juni 2022	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	**Vandenbogaerde, Ann**

1

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2030011

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A,D	SHETTY SUDARSHAN A. ET AL: "Reclassification of Eubacterium hallii as Anaerobutyricum hallii gen. nov., comb. nov., and description of Anaerobutyricum soehngenii sp. nov., a butyrate and propionate-producing bacterium from infant faeces", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, deel 68, nr. 12, 1 december 2018 (2018-12-01), bladzijden 3741-3746, XP055931086, GB ISSN: 1466-5026, DOI: 10.1099/ijsem.0.003041 in de aanvraag genoemd -----	1-16
A	GILIJAMSE PIM W. ET AL: "Treatment with Anaerobutyricum soehngenii: a pilot study of safety and dose-response effects on glucose metabolism in human subjects with metabolic syndrome", NPJ BIOFILMS AND MICROBIOMES, deel 6, nr. 1, 1 december 2020 (2020-12-01), XP055879874, DOI: 10.1038/s41522-020-0127-0 Gevonden op het Internet: URL:https://www.nature.com/articles/s41522-020-0127-0.pdf> -----	1-16
Y	T. ESLAMPARAST ET AL: "Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study", AMERICAN JOURNAL OF CLINICAL NUTRITION, deel 99, nr. 3, 8 januari 2014 (2014-01-08), bladzijden 535-542, XP055432715, ISSN: 0002-9165, DOI: 10.3945/ajcn.113.068890 * samenvatting * -----	2,8

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek

NL 2030011

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
WO 2020236979 A1	26-11-2020	AU 2020278703 A1	27-01-2022
		CA 3140096 A1	26-11-2020
		CN 113993529 A	28-01-2022
		EP 3973047 A1	30-03-2022
		KR 20220011683 A	28-01-2022
		SG 11202112515R A	30-12-2021
		WO 2020236979 A1	26-11-2020

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN80721	03.12.2021		NL2030011

International Patent Classification (IPC)
INV. A61K35/741 A23L33/135 A61P1/16

Applicant
Caelus Pharmaceuticals B.V., et al

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 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 application
- ☐ Box No. VIII Certain observations on the application

	Examiner
	Vandenbogaerde, Ann

Application number

WRITTEN OPINION

NL2030011

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	2, 3, 8, 9
	No:	Claims	1, 4-7, 10-16
Inventive step	Yes:	Claims	3, 9
	No:	Claims	1, 2, 4-8, 10-16
Industrial applicability	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

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

Reference is made to the following documents:

D1 WO 2020/236979 A1 (PENDULUM THERAPEUTICS INC [US]) 26 november 2020 (2020-11-26)

D2 SHETTY SUDARSHAN A. ET AL: "Reclassification of Eubacterium hallii as Anaerobutyricum hallii gen. nov., comb. nov., and description of Anaerobutyricum soehngenii sp. nov., a butyrate and propionate-producing bacterium from infant faeces",
INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY,
deel 68, nr. 12, 1 december 2018 (2018-12-01), bladzijden 3741-3746, XP055931086,
GB
ISSN: 1466-5026, DOI: 10.1099/ijsem.0.003041
in de aanvraag genoemd

D3 Gilijamse Pim W. ET AL: "Treatment with Anaerobutyricum soehngenii: a pilot study of safety and dose-response effects on glucose metabolism in human subjects with metabolic syndrome",
npj Biofilms and Microbiomes,
deel 6, nr. 1, 1 december 2020 (2020-12-01), XP055879874,
DOI: 10.1038/s41522-020-0127-0
Gevonden op het Internet:
URL:https://www.nature.com/articles/s41522-020-0127-0.pdf

D4 T. Eslamparast ET AL: "Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study",
American Journal of Clinical Nutrition,
deel 99, nr. 3, 8 januari 2014 (2014-01-08), bladzijden 535-542, XP055432715,
ISSN: 0002-9165, DOI: 10.3945/ajcn.113.068890

- D1 discloses (cf. claims) microbial composition comprises two or more microbial species selected from the group consisting of: *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis, Bifidobacterium longum, Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis and Eubacterium hallii*, for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD); D1 describes (cf. Example, Fig.2) that that oral administration of wbf11, an encapsulated formulation comprising *Eubacterium hallii* (0.9×10^9 CFU/day) and *Akkermansia muciniphila* (1.2×10^9 CFU/day), during 12 weeks in patients improved the levels of AST and ALT, which are known markers of liver disorders such as NASH and NAFLD.
- D2 discloses that *Eubacterium hallii* (= DSM 3353T = ATCC 27751T) is reclassified as the type strain of a novel genus Anaerobutyricum sp. nov., comb. nov. and that strain L2-7T should be classified as a novel species, Anaerobutyricum soehngenii sp. nov. (= DSM 17630T = KCTC 15707T).
- D3 describes (cf. abstract) that oral intake of *Anaerobutyricum soehngenii* improves peripheral insulin sensitivity after 4 weeks of treatment, which is accompanied by an altered microbiota composition and a change in bile acid metabolism.
- D4 discloses (cf. abstract, p.536 col.1 par.1) a composition comprising *Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium longum and Lactobacillus bulgaricus*, for use in the treatment of NAFLD.

1 Present application

1.1 The relevant Examples, i.e. the Examples relating to the treatment of hepatic steatosis, are Example 3, Example 4 and Example 5.

1.1.1 Example 3 demonstrates that administration of A.soehngenii results in a clear reduction in inflammation grade, fibrosis grade,NAS score or global NASH score in a diet-induced obesity mouse model of nonalchoholic steatohepatitis (NASH); moreover, the number of mice that showed showed NASH were reduced as compared to the placebo (Fig.3).

1.1.2 Example 4 demonstrates (cf. Table 1) that the administration of Anaerobutyricum soehngenii or Anaerobutyricum hallii alone has a beneficial effect in patients with hepatic steatosis.

Example 4 also shows (Table 1) a synergistic effect in patients with hepatic steatosis for the following combinations:

- Anaerobutyricum soehngenii or Anaerobutyricum hallii with Bifidobacterium animalis subspecies lactis;
- Anaerobutyricum soehngenii or Anaerobutyricum hallii with Bifidobacterium breve;
- Anaerobutyricum soehngenii or Anaerobutyricum hallii with Akkermansia muciniphila;
- Anaerobutyricum soehngenii or Anaerobutyricum hallii with Lactobacillus rhamnosus; and
- Anaerobutyricum soehngenii or Anaerobutyricum hallii with Lactobacillus casei

1.1.3 Example 5 shows that the effect of Example 4 is even stronger when the bacteria are micro-encapsulated.

2 **Claims 1 and 3-4,10-16(part) - *A. soehngenii or A. hallii for use in the treatment of hepatic steatosis*: Novelty - Inventive step**

2.1 The Applicant's attention is drawn to the fact that the discovery of a physiological/pharmacological effect or mechanism of action underlying the known medical use of a compound - without ending up in a new purpose reflecting said effect - is not considered as a technical feature which could be used to distinguish the subject-matter of the present claims from the prior art to confer novelty. In other words, the feature "wherein the use is for increasing bile acid plasma level for reducing liver inflammation" of claim 1 is considered as a possible mechanism of action explaining the known therapeutical application for the treatment of liver steatosis.

The same applies to "wherein the use is further for reducing hepatic necroinflammatory activity score" of claim 3.

2.2 The subject-matter of claim 1 is not novel over the teaching of D1.

D1 discloses (cf. claims) microbial composition comprises two or more microbial species selected from the group consisting of: *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis, Bifidobacterium longum, Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis and Eubacterium hallii*, for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD); D1 describes (cf. Example, Fig.2) that that oral administration of wbf11, an encapsulated formulation comprising *Eubacterium hallii* (0.9×10^9

CFU/day) and *Akkermansia muciniphila* (1.2 x 10^9 CFU/day), during 12 weeks in patients improved the levels of AST and ALT, which are known markers of liver disorders such as NASH and NAFLD.

As it is known from D2 that Eubacterium hallii is identical to *Anaerobutyricum soehngenii*, D1 discloses the use of *Anaerobutyricum soehngenii* for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD).

Consequently, D1 is novelty-destroying for claim 1.

2.3 Dependent claims 3-4,10-16(part) do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements with respect to novelty and/or inventive step in view of the teaching of D1.

3 **Claims 2 and 3-4,10-16(part) - *A. soehngenii or A. hallii combined with Bifidobacterium, in particular B. animalis or B. breve, for use in the treatment of hepatic steatosis: Novelty - Inventive step***

3.1 The subject-matter of claims 2 and 3-4,10-16(part) is novel over the teaching of the cited prior art documents.

3.2 However, the subject-matter of claim 2 cannot be considered as involving an inventive step for the following reasons.

Closest prior art document D1 discloses the use of *Anaerobutyricum soehngenii* for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD). D1 also discloses (cf. Example, Fig.2) that oral administration of wbf10, an encapsulated formulation comprising *Clostridium butyricum* (3.3 x 10^9 CFU/day), *Clostridium beijerinckii* (1.2 x 10^{10} CFU/day) and *Bifidobacterium infantis* (2 x 10^9 CFU/day), during 12 weeks in patients improved the levels of AST and ALT, which are known markers of liver disorders such as NASH and NAFLD.

The medical use of *Anaerobutyricum soehngenii* (see D1), *Bifidobacterium infantis* (see D1), *Bifidobacterium breve* (see D4) and *Bifidobacterium longum* (see D4) in the treatment of liver disorders including NASH and/or NAFLD have been disclosed. Consequently, an inventive step can only be seen if the combination of the 2 active ingredients results in a *superior* effect, i.e. a synergistic effect.

It is not clear to a skilled person whether the problem has been solved for the combination of *Anaerobutyricum soehngenii* with ***any*** Bifidobacterium species.

Therefore, the subject-matter cannot be considered as involving an inventive step.

3.3 Importantly, the subject-matter of claim 3 seems to be novel and involving an inventive step.

4 **Claims 5-7 and 10-16(part)- *A. soehngenii or A. hallii combined with Akkermanisis, in particular A. muciniphila, for use in the treatment of hepatic steatosis: Novelty - Inventive step***

4.1 The subject-matter of claim 5 is not novel over the teaching of D1.

D1 discloses (cf. claims) microbial composition comprises two or more microbial species selected from the group consisting of: *Akkermansia muciniphila, Bifidobacterium adolescentis, Bifidobacterium infantis, Bifidobacterium longum, Clostridium beijerinckii, Clostridium butyricum, Clostridium indolis and Eubacterium hallii*, for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD); D1 describes (cf. Example, Fig.2) that that oral administration of wbf11, an encapsulated formulation comprising *Eubacterium hallii* (0.9×10^9 CFU/day) and *Akkermansia muciniphila* (1.2×10^9 CFU/day), during 12 weeks in patients improved the levels of AST and ALT, which are known markers of liver disorders such as NASH and NAFLD.

As it is known from D2 that Eubacterium hallii is identical to *Anaerobutyricum soehngenii*, D1 discloses the use of the combination of *Anaerobutyricum soehngenii* and *Akkermansia muciniphila* for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD).

4.2 Dependent claims 6-7 and 10-16(part) do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements with respect to novelty and/or inventive step in view of the teaching of D1.

5 **Claims 8-9 and 10-16(part)- *A. soehngenii or A. hallii combined with Lactobacillus, in particular L. acidophilus, L. casei, L. reuteri or L. rhamnosus, for use in the treatment of hepatic steatosis: Novelty - Inventive step***

5.1 The subject-matter of claims 8-9 and 10-16(part) is novel over the teaching of the cited prior art documents.

5.2 However, the subject-matter of claim 8 cannot be considered as involving an inventive step for the following reasons.

Closest prior art document D1 discloses the use of *Anaerobutyricum soehngenii* for use in the treatment of liver disorders including nonalchoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD).

The medical use of *Anaerobutyricum soehngenii* (see D1), *Lactobacillus casei* (see D4), *Lactobacillus rhamnosus* (see D4), *Lactobacillus acidophilus* (see D4) and *Lactobacillus bulgaricus* (see D4) in the treatment of liver disorders including NASH and/or NAFLD have been disclosed. Consequently, an inventive step can only be seen if the combination of the 2 active ingredients results in a *superior* effect, i.e. a synergistic effect.

It is not clear to a skilled person whether the problem has been solved for the combination of *Anaerobutyricum soehngenii* with ***any*** Lactobacillus species.

Therefore, the subject-matter cannot be considered as involving an inventive step.

5.3 Importantly, the subject-matter of claim 9 seems to be novel and involving an inventive step.

6 **Patentable subject-matter**

It seems that patentable subject-matter is present in the application, i.e. claim 3 and claim 9.