to a placebo effect. Further, the ability of probiotic species to influence established gut microbiota has not been proven. Instead, any probiotic bacteria that do survive transit through the digestive tract are present only in the luminal compartment of the gut for a short period of time, rather than colonising the gut mucosal compartment and affecting the established gut microbiota. This hypothesis may explain why many probiotics fail to demonstrate long term effects on gut health.

Parkinson's Disease is a degenerative neurological disorder characterised by motor system syndromes as well as a range of non-motor symptoms. The mean age of the population is increasing in a wide range of countries around the world, making degenerative disorders such as Parkinson's Disease ever more relevant. Current management of the condition is of limited efficacy and pharmacological therapies are associated with significant side effects. There is therefore a need for alternative means for treating Parkinson's Disease.

The present invention addresses these and other problems by providing a method of promoting SCFA production by gut microbiota, thereby promoting gut health, and providing a method of treating or preventing Parkinson's Disease.

SUMMARY ~~OF INVENTION~~

The importance of the gut microbiota in influencing a person's general wellbeing is becoming ever more apparent. In healthy individuals, changes in gut microbiota have been linked with periods of stress, anxiety and depression, and may cause weight gain and variable responses to diet and pharmaceuticals. These changes may be in the composition of the gut microbiota, changes in its metabolic activity, or both.

For example, a person's gut microbiota may exhibit types and/or relative numbers of gut bacteria which are abnormal compared to a healthy gut, a state also known as "dysbiosis". An unhealthy gut may also be indicated by abnormal changes in SCFA production, which itself may be due to changes in composition of the gut microbiota.

SCFAs are thought to contribute to human health, for example by inhibiting pathogenic growth by lowering pH in the intestinal lumen and improving the ability of epithelial cells to defend against pathogenic *E. coli* infection. In addition, butyrate is a major energy source for host colonocytes and induces differentiation in these cells, which is thought to be

related to a reduced risk of colon cancer. Butyrate and propionate have been reported to induce the differentiation of T-regulatory cells via inhibition of histone deacetylation.

In a subject exhibiting dysbiosis, the gut microbiota may be sufficiently imbalanced that the person's wellbeing suffers, even though they may not have any particular disease condition. Dysbiosis can lead to discomfort and an increased risk of infection. Dysbiosis has also been linked with risk of anxiety, stress, or depression in otherwise healthy individuals, as well as being associated with an increased risk of GI cancers such as colon carcinoma.

As well as impacting on the wellbeing of otherwise healthy individuals, impaired gut health is also often associated with certain disease conditions, even if it is not part of the disease symptoms *per se*. For example, abnormal gut microbiota has been reported for subjects having obesity, diabetes, and chronic fatigue syndrome.

Promoting a healthy gut microbiota can thus make an important contribution to a person's overall wellbeing and health, both when that person is otherwise healthy and also when that person is suffering from a disease or pathological condition.

As demonstrated herein, administration of a liquid, water-based probiotic composition comprising a population of probiotic bacteria is capable of influencing an established gut microbiota population so as to increase the levels of SCFA production, and is also shown to change the proportions of the bacterial taxa making up the gut microbiota.

Probiotic bacteria of the preparation administered according to the ~~invention~~ disclosure are shown to colonise and proliferate in the gut mucosal and luminal environment, where their interactions with the established bacterial populations cause the microbiota as a whole to rebalance. This rebalancing is indicated by increased SCFA production by the gut microbiota. It is further evidenced by the changes in the composition of the gut microbiota at the phylum and family taxonomic levels. This modification of the balance of the gut microbiota as a whole is indicative of improvement in the gut health following administration of the probiotic.

Because administration of the probiotic results in a rebalancing of the established microbiota, both in the lumen and especially in the mucosa, the benefits of the methods of

the ~~invention~~disclosure will be persistent and maintained. This is in contrast with the effect seen with many probiotics, where the inability to influence the gut microbiota as a whole means any effect is restricted to a transient effect on the luminal compartment, since the effect will only occur during the transit of the probiotic bacteria through the digestive tract.

Accordingly, in a first ~~aspect~~ example is provided a method of promoting gastrointestinal health in a subject comprising administering a liquid, non-dairy-based probiotic composition comprising a population of lactic acid bacteria, wherein administration of the probiotic composition promotes production of one or more SCFAs by the subject's gut microbiota, thereby promoting intestinal health.

In a second ~~aspect~~example is provided a method of promoting production of one or more short chain fatty acids (SCFAs) by a subject's gut microbiota comprising administering to the subject a liquid, non-dairy-based probiotic composition comprising a population of lactic acid bacteria.

In a further ~~aspect~~example is provided a method of promoting growth of one or more bacterial phyla selected from Actinobacteria (e.g. Bifidobacteriaceae), Firmicutes (e.g. Veillonellaceae, Lachnospiraceae, Streptococcaceae, Eubacteriaceae, Ruminococcaceae, Erysipelotrichaceae), Proteobacteria (e.g. Enterobacteriaceae) in the gut microbiota of a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria.

In certain preferred ~~embodiments~~examples, the growth is in the gut mucosal compartment. In certain preferred ~~embodiment~~examples, the growth is in the gut luminal compartment. In certain preferred ~~embodiment~~examples, the growth is in both the luminal and mucosal compartments.

In a preferred ~~embodiment~~example, the method promotes production of one or more SCFAs.

In a further ~~aspect~~example is provided a method of inhibiting growth of one or more bacterial phyla selected from Actinobacteria (e.g. Coriobacteriaceae, Eggerthellaceae), Bacteroidetes (e.g. Bacteroidaceae, Rikenellaceae, Lachnospiraceae, Ruminococcaceae), Firmicutes (e.g., Acidaminococcaceae, Enterococcaceae, Clostridiaceae, Peptostreptococcaceae), Proteobacteria (e.g. Enterobacteriaceae), Synergistetes (e.g. Synergistaceae) and Verrucomicrobio (e.g. Akkermansiaceae) in the gut microbiota of a subject, the method

comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria.

In certain preferred ~~embodiment~~examples, the growth is inhibited in the gut mucosal compartment. In certain preferred ~~embodiment~~examples, the growth is inhibited in the gut luminal compartment. In certain preferred ~~embodiment~~examples, the growth is inhibited in both the luminal and mucosal compartments.

In a preferred ~~embodiment~~example, the method promotes production of one or more SCFAs.

In certain preferred ~~embodiment~~examples of all ~~aspect~~examples of the ~~invention~~disclosure, the methods promote production of one or more SCFAs selected from butyrate, propionate and acetate. In certain preferred ~~embodiment~~examples, the method promotes production of butyrate.

SCFAs produced following administration of the probiotic preparation according to the ~~invention~~disclosure can be considered to be “postbiotic” compounds, or simply “postbiotics”. Postbiotics are health-associated compounds produced by the microbiota following administration of a probiotic. By promoting the production of health-linked SCFAs by the gut microbiota, administration of the probiotic preparation in accordance with the ~~invention~~disclosure can be considered to have a “postbiotic” effect.

Accordingly, in certain ~~embodiment~~examples, methods of the ~~invention~~disclosure promote production of postbiotic compounds, such as SCFAs.

In a further ~~aspect~~example is provided a method of promoting intestinal barrier integrity in a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria, wherein administration of the probiotic preparation promotes said intestinal barrier integrity. In certain such ~~embodiment~~examples, the method prevents or reduces loss of intestinal barrier integrity. In certain ~~embodiment~~examples, the method promotes intestinal barrier repair.

In a further ~~aspect~~example is provided a method for promoting a tolerogenic gut phenotype in a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria, wherein administration of the probiotic preparation promotes said tolerogenic gut phenotype. In certain ~~embodiment~~examples the

method promotes production of anti-inflammatory molecules by intestinal epithelial cells. In certain ~~embodiment~~examples the method reduces production of pro-inflammatory molecules by intestinal epithelial cells.

In certain preferred ~~embodiment~~examples of methods of the ~~invention~~disclosure, the method is a non-therapeutic method.

In certain preferred ~~embodiment~~examples of methods of the ~~invention~~disclosure the subject is a healthy individual.

In certain preferred ~~embodiment~~examples of methods of the ~~invention~~disclosure the subject is in a state of gastrointestinal dysbiosis.

In certain preferred ~~embodiment~~examples, the subject is suffering from a disease or disorder. In certain preferred ~~embodiment~~examples, the subject has a disease or disorder selected from Parkinson's Disease, liver cirrhosis, inflammatory bowel syndrome (IBD), *Clostridium difficile* infection, MRSA infection, *E. coli* infection, obesity, diabetes, and chronic fatigue syndrome. In certain preferred ~~embodiment~~examples, the subject has Parkinson's Disease. In certain ~~embodiment~~examples the subject has liver cirrhosis.

Parkinson's Disease is a degenerative neurological disorder characterised by motor system syndromes as well as a range of non-motor symptoms. As populations of some countries age, degenerative disorders such as Parkinson's Disease become ever more relevant. Current management is of limited efficacy and pharmacological therapies are associated with significant side effects. There is therefore a need for alternative means for treating Parkinson's Disease.

As reported herein, patients with Parkinson's Disease receiving a probiotic preparation according to the ~~invention~~disclosure show an improvement in motor and non-motor symptoms. The data presented in the Examples further demonstrates that the probiotic preparation is able to promote a healthy gut phenotype in Parkinson's Disease, by reducing the loss of intestinal barrier integrity and the inflammatory gut phenotype exhibited by PD patients. Taken together, these data indicate that administration of the probiotic preparation will provide effective treatment of Parkinson's Disease patients.

Accordingly, in a further ~~aspect~~example is provided a method of treating or preventing Parkinson's Disease in a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria. In one aspect is provided a use of a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria in the manufacture of a medicament for treating or preventing Parkinson's Disease in a subject, wherein the population of lactic acid bacteria comprises one or more of *Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria, and wherein the medicament is to be administered to the subject. In certain ~~embodiment~~examples, administration of the probiotic preparation improves in the subject one or more of: motor symptoms, non-motor symptoms, systemic inflammatory markers. In certain ~~embodiment~~examples, administration of the probiotic preparation improves non-motor symptoms in the subject, for example improves gastrointestinal non-motor symptoms in the subject. In certain ~~embodiment~~examples, administration of the probiotic preparation slows or prevents the onset of Parkinson's Disease symptoms such as motor symptoms.

In certain preferred ~~embodiment~~examples the subject is in a state of gastrointestinal dysbiosis. In certain preferred ~~embodiment~~examples the subject is exhibiting an elevated level of *Firmicutes* in their gut microbiota compared to healthy controls. In certain preferred ~~embodiment~~examples the subject is exhibiting a reduced level of *Bacteroidetes* in their gut microbiota compared to healthy controls.

In certain preferred ~~embodiment~~examples of all ~~aspect~~examples, the population of lactic acid bacteria comprises at least one of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria. In certain ~~embodiment~~examples, the population of lactic acid bacteria comprises at least two or at least three of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria.

In certain ~~embodiment~~examples, the population of lactic acid bacteria comprises *Lactobacillus rhamnosus*, *Lactobacillus acidophilus,* and *Lactobacillus plantarum* bacteria.

In certain preferred ~~embodiment~~examples, the population of lactic acid bacteria comprises each of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria.

BRIEF DESCRIPTION OF FIGURES

Figure 1: Total bacterial counts (left) and total viable cell counts (right), determined with flow cytometry, for Symprove bacteria upon addition to stomach juice (St0), after 45 min in stomach juice (St45) and after 180 min in small intestinal fluid (SIt180).

CLAIMS:

~~1. A method of stimulating production of one or more short chain fatty acids (SCFAs) by a subject's gut microbiota comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria.~~

~~2. A method of promoting gastrointestinal health in a subject comprising administering a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria, wherein administration of the probiotic preparation promotes production of one or more short chain fatty acids (SCFAs) by the subject's gut microbiota, thereby promoting intestinal health.~~

~~3. A method according to claim 1 or claim 2 wherein the one or more SCFAs is selected from butyrate, propionate and acetate, preferably butyrate.~~

~~4. A method of promoting growth of one or more bacterial phyla selected from Actinobacteria (e.g. Bifidobacteriaceae), Firmicutes (e.g. Veillonellaceae, Lachnospiraceae, Streptococcaceae, Eubacteriaceae, Ruminococcaceae, Erysipelotrichaceae, Clostridiaceae), Proteobacteria (e.g. Enterobacteriaceae) in the gut microbiota of a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria.~~

~~5. A method of inhibiting growth of one or more bacterial phyla selected from Actinobacteria (e.g. Coriobacteriaceae, Eggerthellaceae), Bacteroidetes (e.g. Bacteroidaceae, Rikenellaceae, Lachnospiraceae, Ruminococcaceae), Firmicutes (e.g., Acidaminococcaceae, Enterococcaceae, Clostridiaceae, Peptostreptococcaceae), Proteobacteria (e.g. Enterobacteriaceae), Synergistetes (e.g. Synergistaceae) and Verrucomicrobio (e.g. Akkermansiaceae) in the gut microbiota of a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria~~

~~6. A method according to claim 4 or claim 5, wherein the effect on the growth of the one or more bacterial phyla is in the gut mucosal compartment.~~

~~7. A method according to any one of claims 4-6, wherein the effect on the growth of the one or more bacterial phyla is in the gut luminal compartment.~~

~~8. A method of promoting intestinal barrier integrity in a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria, wherein administration of the probiotic preparation promotes said intestinal barrier integrity.~~

~~9. A method according to claim 8, wherein the method prevents or reduces loss of intestinal barrier integrity in a subject.~~

~~10. A method according to claim 8 or claim 9, wherein the subject is at risk of loss of intestinal barrier integrity.~~

~~11. A method according to claim 8, wherein the method promotes intestinal barrier repair.~~

~~12. A method for promoting a tolerogenic gut phenotype in a subject, the method comprising administering to the subject a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria, wherein administration of the probiotic preparation promotes said tolerogenic gut phenotype.~~

~~13. A method according to any one of the preceding claims wherein the method is a non-therapeutic method.~~

~~14. A method according to any one of the preceding claims wherein the subject is a healthy individual.~~

~~15. A method according to any one of the preceding claims wherein the subject is in a state of intestinal dysbiosis.~~

~~16. A method according to any one of claims 1-12 and 15, wherein the method is a therapeutic method.~~

~~17. A method according to any one of claims 1-12 and 15-16 wherein the subject has a condition selected from Parkinson's Disease, liver cirrhosis, inflammatory bowel disease (IBD), *Clostridium difficile* infection, MRSA infection, *E. coli* infection, salmonella infection, norovirus infection, giardiasis, coeliac disease, chronic kidney disease, HIV/AIDS, cystic fibrosis, type I diabetes, obesity, and chronic fatigue syndrome.~~

~~18. A method according to any one of claims 1-12 and 15-17, wherein the subject has Parkinson's Disease.~~

~~19. A method according to any one of claims 1-12 and 15-17, wherein the subject has liver cirrhosis.~~

~~20. A method according to any one of claims 1-12 and 15-17, wherein the subject has IBD.~~

~~2~~1. Use of a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria in the manufacture of a medicament for ~~A method of~~ treating or preventing Parkinson's Disease in a subject, wherein the population of lactic acid bacteria comprises one or more of *Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria, and wherein~~,~~ the ~~method comprising administering~~medicament is to be administered to the subject ~~a liquid, non-dairy probiotic preparation comprising a population of lactic acid bacteria~~.

2~~2~~. ~~A method~~The use according to claim ~~2~~1, wherein administration of the probiotic preparation improves in the subject one or more of: motor symptoms, non-motor symptoms, systemic inflammatory markers.

~~2~~3. The use ~~A method~~ according to claim ~~2~~1 or claim ~~2~~2, wherein administration of the probiotic preparation improves non-motor symptoms in the subject, optionally improves gastrointestinal non-motor symptoms in the subject.

~~2~~4. The use ~~A method~~ according to any one of claims ~~2~~1-~~2~~3, wherein administration of the probiotic preparation improves intestinal barrier integrity.

~~2~~5. The use ~~A method~~ according to any one of claims ~~2~~1-~~2~~4, wherein administration of the probiotic preparation improves intestinal barrier repair.

~~2~~6. The use ~~A method~~ according to any one of claims ~~2~~1-~~2~~5, wherein administration of the probiotic preparation promotes a tolerogenic gut phenotype in the subject.

~~2~~7. The use ~~A method~~ according to any one of claims ~~2~~1-~~2~~5 wherein administration of the probiotic preparation treats or prevents Parkinson's Disease in the subject by reducing the severity or slowing the progression of motor symptoms or by slowing or preventing the onset of motor symptoms.

~~2~~8. The use ~~A method~~ according to any one of claims ~~2~~1-~~2~~6 wherein the subject is in a state of gastrointestinal dysbiosis, optionally wherein the subject is exhibiting an elevated level of *Firmicutes* and/or a reduced level of *Bacteroidetes* in their gut microbiota compared to healthy controls.

~~2~~9. The use ~~A method~~ according to any one of the preceding claims, wherein the ~~method~~ use promotes production of one or more anti-inflammatory molecules by intestinal epithelial cells, optionally wherein the one or more anti-inflammatory molecules are selected from IL-6 and IL-10.

~~30~~10. The use ~~A method~~ according to any one of the preceding claims, wherein the ~~method~~ use reduces production of one or more pro-inflammatory molecules by intestinal epithelial cells, optionally wherein the one or more pro-inflammatory molecules are selected from CXCL-10, TNFa, IL-8 and MCP-1.

~~31~~11. The use ~~A method~~ according to any one of claims 4-~~30~~10, wherein the ~~method~~use promotes production of one or more SCFAs, preferably acetate, propionate or butyrate, preferably butyrate.

~~32. A method according to any one of the preceding claims wherein the population of lactic acid bacteria comprises one or more of *Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria.~~

~~33~~12. The use ~~A method~~ according to any one of the preceding claims wherein the population of lactic acid bacteria comprises each of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus, Lactobacillus plantarum* and *Enterococcus faecium* bacteria.

~~34~~13. The use ~~A method~~ according to any one of the preceding claims wherein the ~~method~~use reduces production of one or more branched chain fatty acids (BCFA) and/or ammonium by the subject's gut microbiota

~~35~~14. The use ~~A method~~ according to claim 34 wherein the one or more BCFAs is selected from isobutyrate, isovalerate and 2-methylbutyrate.

~~36~~15. The use ~~A method~~ according to any one of the preceding claims wherein the medicament ~~probiotic composition~~ is to be administered to the subject at least once a week, preferably once a day.

~~37~~16. The use ~~A method~~ according to any one of the preceding claims wherein the ~~probiotic composition~~medicament is to be administered to the subject for a period of at least 1 month, at least 2 months or at least 3 months.