Bacteria belonging to *Eubacterium hallii* et rel. include relatively fast-growing anaerobes. They have the metabolic capacity to convert lactate into butyrate in a process that needs acetate (Munoz-Tamayo et al 2011 FEMS Microbiol Ecolo 76: 615-624). Lactate and acetate are abundant metabolites in the upper intestinal tract that is colonized by among others streptococci and lactobacilli that can produce these compounds (Booijink et al 2010, vide supra).

In db/db mice (a model of obesity, diabetes, and dyslipidemia) distinct effect were found of short term oral *E. hallii* L2-7 supplementation to the small intestine on normalization of insulin resistance (as detected by HOMA calculation and postprandial glucose metabolism by AUC of oral glucose tolerance curve) as well as fasting lipid profiles in db/db mice (WO2013/032328).

Culturing obligate anaerobic gut microorganisms is notoriously difficult, and high biomass yields are hardly ever obtained.

Hitherto, *E. hallii* has been cultured in complex media, and relatively low biomass yields have been obtained (WO2013/032328; Duncan et al. Appl. Environm. Microbiol. 70:5810).

There is a need in the art for a method of culturing *E. hallii*, an obligate anaerobe, that provides a composition suitable for administration to humans and that gives high biomass yields. Preferably, such composition should comprise all food grade sources, should be free of animal components, and/or should be kosher. Preferably, the culturing method is based on a simple medium.

Summary

The present inventors have developed a method of preparing an *E. hallii* preparation that is suitable for administration or ingestion to humans and that gives high biomass yields, preferably yields suitable for commercial production. Ideally, the medium comprises food grade components only, is free of any animal sources, and/or is kosher. The method of the invention is based on a simple medium.

In one aspect, the present disclosure refers to a method of preparing an E. hallii preparation suitable for ingestion by a human, said method comprising the steps of:

a) providing a medium comprising:

- 1-100, preferably 5-40, g/kg of a sugar, preferably glucose,
- 1-100, preferably 5-40, g/kg of a source of nitrogen and growth factors, such as yeast extract,
- 0.1-50 g/kg plant peptone, preferably soy peptone,
- acetate,
- a buffer system at pH6-8,

- optionally, cysteine,
- optionally, magnesium ions,
- optionally, calcium ions,
- optionally, potassium ions,
- optionally, vitamins;

b) inoculating said medium with E. hallii;

c) allowing said E. hallii to grow in said medium; and

d) harvesting said E. hallii preparation from said medium.

In another aspect, the present disclosure refers to an *E. hallii* preparation obtainable by the method as disclosed herein, wherein the preparation comprises medium as disclosed herein.

In yet another aspect, the present disclosure refers to an *E. hallii* preparation as disclosed herein for use as a medicament.

In yet another aspect, the present disclosure refers to the use of the *E. hallii* preparation of claim 8 in the manufacture of a medicament for preventing and/or treating insulin resistance and/or insulin resistance-related complications such as metabolic syndrome, dyslipidemia, type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatosis hepatis (NASH), and insulin resistance in endocrine diseases (e.g., obese subjects with type 1 diabetes mellitus, Cushing's disease and lipodystrophy syndromes), for preventing and/or treating lactic-acidosis, short bowel syndrome or inflammatory bowel disease, for preventing, reducing the incidence or reducing the severity of colorectal cancer or colitis.

In yet another aspect, the present disclosure refers the use of the *E. hallii* preparation as disclosed herein in the manufacture of a medicament for promoting butyric acid formation in the intestine of a mammal.

Definitions

As used herein, the term "effective amount" refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, e.g., an amount, which results in the treatment and/or prevention of the diseases and conditions taught herein. In the context of therapeutic or prophylactic applications, the amount of *E. hallii* bacteria administered to the animal subject, preferably human subject, will depend on the type and severity of the disease or condition and on the characteristics of the subject, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine

CLAIMS

1. A method of preparing an *E. hallii* preparation suitable for ingestion by a human, said method comprising the steps of:

a) providing a medium comprising:

- 1-100, preferably 5-40, g/kg of a sugar, preferably glucose,
- 1-100, preferably 5-40, g/kg of a source of nitrogen and growth factors, such as yeast extract,
- 0.1-50 g/kg plant peptone, preferably soy peptone,
- acetate,
- a buffer system at pH6-8,
- optionally, cysteine,
- optionally, magnesium ions,
- optionally, calcium ions,
- optionally, potassium ions,
- optionally, vitamins;

b) inoculating said medium with *E. hallii*;

c) allowing said *E. hallii* to grow in said medium; and

d) harvesting said *E. hallii* preparation from said medium.

2. The method of claim 1, which is carried out under anaerobic conditions.

3. The method of claim 1 or 2, which is carried out at a temperature in the range of 30 – 42 ºC, preferably in the range of 34 – 40 ºC.

4. The method of any of claims 1 – 3, wherein said *E. hallii* preparation is harvested from said medium using filtration.

5. The method of claim 4, wherein the filtrate is further washed with phosphate buffered saline solution.

6. The method of any of claims 1 – 5, which further comprises the step of lyophilizing said *E. hallii* to obtain a lyophilized *E. hallii* preparation.

7. The method of claim 6, wherein said lyophilizing is carried out using a cryoprotectant selected from the group consisting of sucrose, maltose, maltodextrin, trehalose, mannitol, inuline, glycerol, DMSO, ethylene glycol, propylene glycol, 2-methyl-2,4-pentanediol,

polyethylene glycol, polyvinylpyrrolidone, polyvinyl alcohol, polyglycerol, or any combination thereof.

8. *E. hallii* preparation obtainable by the method of any of the preceding claims, wherein the preparation comprises medium as defined in claim 1.

9. *E. hallii* preparation of claim 8 for use as a medicament.

10. Use of the *E. hallii* preparation of claim 8 in the manufacture of a medicament for preventing and/or treating insulin resistance and/or insulin resistance-related complications such as metabolic syndrome, dyslipidemia, type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatosis hepatis (NASH), and insulin resistance in endocrine diseases (e.g., obese subjects with type 1 diabetes mellitus, Cushing's disease and lipodystrophy syndromes), for preventing and/or treating lactic-acidosis, short bowel syndrome or inflammatory bowel disease, for preventing, reducing the incidence or reducing the severity of colorectal cancer or colitis.

11. Use of the *E. hallii* preparation according to claim 8 in the manufacture of a medicament for promoting butyric acid formation in the intestine of a mammal.