Title: COMPOSITIONS AND METHODS FOR PREVENTING INFECTIONS

Abstract: The present invention relates to a dietary supplement or pharmaceutical composition, comprising lyophilized Saccharomyces boulardii as an active ingredient and as sole probiotic, optionally in association with a pharmaceutically acceptable vehicle, wherein the composition is in a closed vial having a first airtight compartment comprising lyophilized S. boulardii powder, and a second compartment comprising a solution, wherein the first and second compartment can be brought in airtight communication with one another to yield a suspension of S. boulardii to be administered to an individual upon opening of the vial.
COMPOSITIONS AND METHODS FOR PREVENTING INFECTIONS

Object of the invention

The present invention relates to compositions and methods useful for preventing undesirable effects occasionally reported with *Saccharomyces boulardii* administration to individuals in need thereof.

Technical background

*Saccharomyces boulardii* is a particular strain of the yeast *Saccharomyces cerevisiae*, also referred to as *Saccharomyces cerevisiae var. boulardii*, which is mainly indicated as a supplement to rehydration for the treatment of diarrhea.


Safety and adverse event data collected during clinical trials, when patients are closely monitored for adverse effects possibly related to the investigational treatment, has documented a remarkable safety profile of *S. boulardii*. However, infrequent cases of *S. boulardii* fungemia have been occasionally reported, essentially in individuals who have central venous catheters. As such, it has sometimes been recommended not to give *S. boulardii* to immunocompromised patients or those with central catheters to reduce this risk (McFarland (2010) *World J Gastroenterol* 16:2202-2222; Santino et al. (2014) *Int J Immunopathol Pharmacol* 27:43-6).

However, these individuals could benefit from *S. boulardii* treatment, as it has notably been shown that *S. boulardii* was useful for managing pathologies such as...
HIV-associated diarrhea as well as enteral nutrition-related diarrhea that may arise in immunocompromised patients.

Accordingly, it is an object of the invention to reinforce the safety of S. boulardii usage by preventing undesirable effects.

Summary of the invention

The present invention arises from the recognition by the present inventors that these undesirable effects arise in part from the volatility of Saccharomyces boulardii powder, especially when lyophilized, which favors dissemination and opportunistic infections of this yeast, and from the finding that suspensions of lyophilized S. boulardii powder do not give rise to volatile dissemination of this yeast.

The present invention thus fulfills the above-defined objective by providing a dietary supplement or pharmaceutical composition, comprising lyophilized Saccharomyces boulardii as an active ingredient and preferably as sole probiotic, optionally in association with a pharmaceutically acceptable vehicle, wherein the composition is in a closed vial having a first airtight compartment comprising lyophilized S. boulardii powder and a second compartment comprising a solution, wherein the first and second compartment can be brought in airtight communication with one another to yield a suspension of S. boulardii to be administered to an individual upon opening of the vial.

As should be clear to one of skill in the art, the expression "wherein the composition is in a closed vial" indicates that the composition according to the invention is comprised in a closed vial. Accordingly, the present invention can be synonymously defined as a closed vial comprising a dietary supplement or pharmaceutical composition, wherein the composition comprises lyophilized Saccharomyces boulardii as an active ingredient, optionally in association with a pharmaceutically acceptable vehicle, and the closed vial has a first airtight compartment comprising lyophilized S. boulardii powder and a second compartment comprising a solution, wherein the first and second compartment can be brought in airtight communication with one another to yield a suspension of S. boulardii to be administered to an individual upon opening of the vial.

Alternatively, the invention can be further equivalently defined as a dietary or pharmaceutical product constituted of a dietary supplement or pharmaceutical composition, comprising lyophilized Saccharomyces boulardii as an active ingredient
and preferably as sole probiotic, optionally in association with a pharmaceutically acceptable vehicle, comprised in a closed vial having a first airtight compartment comprising lyophilized S. boulardii powder and a second compartment comprising a solution, wherein the first and second compartment can be brought in airtight communication with one another to yield a suspension of S. boulardii to be administered to an individual upon opening of the vial.

In an embodiment of the invention, the dietary or pharmaceutical product, the dietary supplement or pharmaceutical composition as defined above, is for use for maintaining the balance of the intestinal flora, for keeping intestines functioning well, for maintaining normal bowel function, and/or for promoting intestinal health of the individual.

In another embodiment of the invention, the dietary or pharmaceutical product, the dietary supplement or pharmaceutical composition as defined above, is for use (i) in the prevention or treatment of microbial imbalance of the digestive tract, in particular for use in the prevention or treatment of diarrhea, such as antibiotic-associated diarrhea, Traveler’s diarrhea, enteral-nutrition diarrhea, acute gastroenteritis in adult or children, HIV-related diarrhea, or giardiasis, more particularly for use as an additional symptomatic treatment of diarrhea in complement to rehydration, and/or (ii) in the prevention or treatment of bacterial, fungal or protozoan infection, such as Clostridium difficile infection, Helicobacter pylori infection, Salmonella infection, Shigella infection, Cryptosporidium infection, or oral candidiasis, and/or (iii) in the prevention or treatment of inflammatory bowel disease or irritable bowel syndrome, in the individual.

In another embodiment of the invention, the dietary or pharmaceutical product, the dietary supplement or pharmaceutical composition as defined above or for use as defined above, is for use in the prevention of S. boulardii fungemia.

In yet another embodiment of the invention, the dietary or pharmaceutical product, the dietary supplement or pharmaceutical composition as defined above or for use as defined above, is not for use in the dentistry field or in the treatment of diseases of the oral cavity, such as diseases of the oral mucosa, of the gums, and of the tooth-support tissues, and in particular is not for use for re-establishing eubiosis in gingivitis or periodontitis or for reducing halitosis.
The present invention further relates to a method for administering *S. boulardii* to an individual in need thereof and optionally to prevent *S. boulardii* fungemia in the individual, comprising:

- providing a dietary supplement or pharmaceutical composition, comprising lyophilized *Saccharomyces boulardii* as an active ingredient and preferably as sole probiotic, optionally in association with a pharmaceutically acceptable vehicle, wherein the composition is in a closed vial having a first airtight compartment comprising lyophilized *S. boulardii* powder and a second compartment comprising a solution;
- bringing the first and second compartment in airtight communication with one another to yield a suspension of *S. boulardii*;
- administering the suspension to the individual.

The present invention also relates to a method for maintaining the balance of the intestinal flora, for keeping intestines functioning well, for maintaining normal bowel function, and/or for promoting intestinal health of an individual, comprising administering the individual an effective quantity of *Saccharomyces boulardii* with the above-defined method for administering *S. boulardii*.

The present invention also relates to a method for (i) the prevention or treatment of microbial imbalance of the digestive tract, in particular for the prevention or treatment of diarrhea, such as antibiotic-associated diarrhea, traveler's diarrhea, enteral-nutrition diarrhea, acute adult or children diarrhea, HIV-related diarrhea, or giardiasis, more particularly for use as an additional symptomatic treatment of diarrhea in complement of rehydration, and/or (ii) for the prevention or treatment of bacterial, fungal or protozoan infection, such as *Clostridium difficile* infection, *Helicobacter pylori* infection, *Salmonella* infection, *Shigella* infection *Cryptosporidium* infection, or oral candidiasis, and/or (iii) in the prevention or treatment of inflammatory bowel disease or irritable bowel syndrome, in an individual, comprising administering the individual a prophylactically or therapeutically effective quantity of *S. boulardii* with the above-defined method for administering *S. boulardii*.

**Brief description of the drawing**

Figure 1 depicts a cross section of a vial according to the invention.

**Detailed description of the invention**
Saccharomyces boulardii

Saccharomyces boulardii, abbreviated S. boulardii, is a yeast well known to a person skilled in the art and is notably described in Hennequin et al. (2001) J. Clin. Microbiol. 39:551-559. As intended herein "Saccharomyces boulardii" and "Saccharomyces cerevisiae var. boulardii" (abbreviated S. cerevisiae var. boulardii) are considered equivalent.

Preferably, Saccharomyces boulardii cells according to the invention are obtained from medicinal products of the brand Ultra-Levure®, Bioflor®, Codex®, Econorm®, Enflor®, Enterol®, Florastor®, Florati®, Florestor®, Inteflora®, Perenterol®, Perenteryl®, Precosa®, Reflor®, or Ultra-Levura®. Saccharomyces boulardii cells according to the invention can also be obtained from deposits in the American Type Culture Collection (ATCC, USA) under reference 74012, in the Collection Nationale de Culture et de Microorganismes (CNMC, Institut Pasteur, France) under reference I-745 or in the Centraalbureau voor Schimmelcultures (CBS, The Netherlands) under reference Hansen CBS 5926 strain.

The S. boulardii cells according to the invention are lyophilized.

Advantageously, the viability and vitality of S. boulardii cells obtained from lyophilizates are greater than can be obtained with other methods of preservation of yeast cells.

As understood here, "lyophilization", also known as freeze-drying, is a method of preservation in which S. boulardii live cells are frozen and are then submitted to sublimation of the frozen water that they contain to give a lyophilizate in the form of dry yeast powder preferably containing less than 2% of water and more preferably less than 1% of water. Preferably, the lyophilized yeast cells are obtained from concentrates of S. boulardii cells. Any type of method of lyophilization of yeast cells known by a person skilled in the art can be used. However, the S. boulardii cells are preferably lyophilized according to the invention by means of the following method of lyophilization:

- cultivate the S. boulardii cells in a liquid nutrient medium until the cells reach a stationary phase;
- concentrate the cultivated S. boulardii cells and freeze the concentrate, optionally in the presence of a cryoprotectant, such as lactose;
- lyophilize the concentrate.

Lyophilized S. boulardii cells are in the form of a powder.
As intended herein, *S. boulardii* is preferably the sole probiotic comprised in the dietary supplement or pharmaceutical composition of the invention. In other words, no other probiotics, such as lactobacilli, are present in the dietary supplement or pharmaceutical composition of the invention in addition to *S. boulardii*. As defined by the Food and Agriculture Organization of the United Nations, a probiotic is a living (or revivable) microorganism, such as a bacteria or a yeast, which when administered in adequate amounts confer a health benefit to the host.

**Individual**

As intended herein, the individual is a mammal, preferably a human.

The individual may beneficiate from *S. boulardii* administration, *i.e.* may be in need thereof. Preferably, the individual is at risk of *S. boulardii* fungemia, such as an individual with a central venous catheter or an immunocompromised individual.

Besides, the volume of the solution as defined above may be minimized while ensuring complete suspension of the powder thereby facilitating administration to individuals such as babies or young children, or individuals with a deglutition disorder.

**Vial**

As intended herein "airtight" means that substantially no lyophilized *S. boulardii* powder can escape from the airtight first compartment and that substantially no water either liquid or gaseous can enter the airtight first compartment.

The second compartment is preferably also airtight.

As intended herein, when the first and second compartment are in airtight communication, the content of the first compartment can be mixed with the solution of the second compartment, while substantially no lyophilized *S. boulardii* powder can escape from the vial.

Upon mixing of the content of the first compartment and the solution of the second compartment to yield the suspension of *S. boulardii*, the closed vial can be opened so that the suspension can be administered.

Numerous configurations of the vial compatible with the invention can be devised by one of skill in the art.

In a preferred embodiment of the vial according to the invention, such as depicted in Figure 1, the first compartment (1) has a single opening (2) which is fitted
in an airtight manner (e.g. forced or screwed) in the single opening (3) of the second compartment (4), the opening (2) of the first compartment is shut by a removable airtight wall (5), and the vial comprise a means (6) for removing the airtight wall without opening the vial. Upon action of the means for removing the airtight wall, the content (7) of the first compartment (1) and the solution (8) can be mixed to yield a suspension and the first and second compartments can be separated (e.g. pulled or unscrewed) thereby opening the vial and yielding access to the suspension. By way of example, the means for removing the airtight wall (6) can be a cutting edge (9) set on an extremity of the first compartment facing the opening thereof (2) which can be motioned to cut the airtight wall (5), for instance by applying a translational force in direction of the second compartment, optionally combined with a rotational force. In the embodiment shown in Figure 1, the first compartment (1) comprises two parts, a fixed part (1a) and a mobile part (1b), the mobile part holds the cutting edge (9) and closes the extremity of the first compartment (1) opposite to the opening (2) thereof while the fixed part is fitted in the single opening (3) of the second compartment (4). The mobile part (1a) is fitted in an airtight manner (e.g. forced or screwed) in the fixed part (1b) and can be motioned in direction of the second compartment (4).

The vial according to the invention can be made of various materials, such as glass and/or plastic. By way of example, the first compartment can be made of a plastic material while the second compartment can be made of glass. One of skill in the can easily select numerous materials which can be used for making the removable wall, such as aluminum foil.

Additional ingredients can be comprised in the first compartment, such as lactose and/or magnesium stearate, preferably in powder form.

When present, lactose is preferably at a dose of about 0.1 mg to 0.15 mg per mg of lyophilized S. boulardii powder, more preferably at a dose of about 0.132 mg per mg of lyophilized S. boulardii powder. According to the invention, lactose is useful as a cryoprotectant for freeze-drying of S. boulardii.

When present, magnesium stearate is preferably at a dose of about 0.005 mg to 0.015 mg per mg of lyophilized S. boulardii powder or per mg of a mixture of lyophilized S. boulardii powder and lactose, preferably with the above-defined dosage of lactose. According to the invention magnesium stearate is useful as an anti-adherent and a lubricant for filling the first compartment with lyophilized S. boulardii powder. Surprisingly, according to the invention, although magnesium stearate is known to be
insoluble in water, its presence in the first compartment does not impair the mixing of the content of the first compartment and that of the second compartment to yield a suspension of *S. boulardii*.

The solution can have any constitution yielding a suspension of *S. boulardii* and compatible with administration by the oral route. Preferably, the solution comprises water, more preferably purified water. The solution may further comprise at least one of a sweetener, such as fructose, an aroma, such as red-fruit aroma, citric acid, and a preservative, such as potassium sorbate and sodium benzoate. Preferably, the suspension of *S. boulardii* according to the invention is not a mouth-wash liquid or a gel ready for topical use.

The dietary supplement or pharmaceutical composition according to the invention may further comprise at least one mineral, such as zinc or selenium, and/or at least one vitamin, such as vitamin A, either in the first compartment, preferably in powder form, or in the second compartment, preferably as a solute.

*Form, dosage and administration*

As intended herein, a dietary supplement composition is a non-medicament composition intended improve the well-being of an individual through ingestion an active ingredient. As intended herein "dietary supplement" is considered equivalent to "nutraceutic".

As intended herein a pharmaceutical composition is a composition intended to restore the health of an individual and/or to prevent or treat a disease. Besides, a "pharmaceutical product" is considered synonymous to "medicament".

As intended herein the "active ingredient" is the causative agent of the beneficial, preventive or therapeutic effects of the composition of the invention.

The pharmaceutical composition may also comprise a pharmaceutically acceptable vehicle. As intended herein, a "pharmaceutically acceptable vehicle" relates to any compound or group of compounds compatible for administration to an individual without significant adverse effect intended to facilitate administration or action of the active ingredient.

Preferably, the first compartment comprises from 5 mg to 5 g, more preferably from 50 mg to 500 mg, most preferably about 250 mg of lyophilized *S. boulardii powder*.

Preferably the volume of the solution in the second compartment is from 0,5 ml to 10 ml, more preferable from 1 ml to 4 ml, most preferable about 2 ml.
Preferably, the suspension is at a concentration of from 10 mg of lyophilized *S. boulardii* powder per mL of solution to 1 g lyophilized *S. boulardii* powder per mL of solution, more preferably at a concentration of from 50 mg of lyophilized *S. boulardii* powder per mL of solution to 500 mg lyophilized *S. boulardii* powder per mL of solution, most preferably at a concentration of about 125 mg of lyophilized *S. boulardii* powder per mL of solution.

Preferably, the suspension of *Saccharomyces boulardii* obtained according to the invention is intended to be administered, or is administered, by the oral route.

Besides, the composition according to the invention may comprise a unit dose of *S. boulardii* or may comprise a dose of *S. boulardii* adapted for several administrations.

**Example**

By way of example, a vial of the invention is as depicted in [Figure 1](#) and can have the following constitution:

The first compartment comprises 250 mg of lyophilized *S. boulardii* powder along with 32.5 mg lactose powder and 2.85 mg magnesium stearate.

The second compartment comprises a 2 mL solution of purified water and fructose, aroma, citric acid and preservatives (potassium sorbate, sodium benzoate).
CLAIMS

1. A dietary supplement or pharmaceutical composition, comprising lyophylized *Saccharomyces boulardii* as an active ingredient, optionally in association with a pharmaceutically acceptable vehicle, wherein the composition is in a closed vial having a first airtight compartment comprising lyophilized *S. boulardii* powder and a second compartment comprising a solution, wherein the first and second compartment can be brought in airtight communication with one another to yield a suspension of *S. boulardii* to be administered to an individual upon opening of the vial.

2. The dietary supplement or pharmaceutical composition of claim 1, wherein the first compartment also comprises lactose.

3. The dietary supplement or pharmaceutical composition of claim 1 or 2, wherein the first compartment further comprises magnesium stearate.

4. The dietary supplement or pharmaceutical composition of any of claims 1 to 3, further comprising at least one mineral and/or at least one vitamin.

5. The dietary supplement or pharmaceutical composition of any of claim 1 to 4, for use for maintaining the balance of the intestinal flora, for keeping intestines functioning well, for maintaining normal bowel function, and/or for promoting intestinal health.

6. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to claim 5, for use in the prevention or treatment of microbial imbalance of the digestive tract.

7. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to claim 6 or 7, for use in the prevention or treatment of diarrhea, and/or of bacterial, fungal or protozoan infection.

8. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 7, for use in the prevention or treatment of antibiotic-associated diarrhea, traveler's diarrhea, enteral-nutrition diarrhea, acute
adult or children gastroenteritis, HIV-related diarrhea, giardiasis, or as an additional symptomatic treatment of diarrhea in complement to rehydration.

9. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 8, for use in the prevention or treatment of Clostridium difficile infection, Helicobacter pylori infection, Salmonella infection, Shigella infection, Cryptosporidium infection, or oral candidiasis.

10. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 9, for use in the prevention or treatment of inflammatory bowel disease or irritable bowel syndrome.

11. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 10, for use in an individual with a deglutition disorder.

12. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 11, for use for preventing Saccharomyces boulardii fungemia.

13. The dietary supplement or pharmaceutical composition of any of claims 1 to 4 or for use according to any of claims 5 to 12, for use in an individual with a central venous catheter.

14. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 13, for use in an immunocompromised individual.

15. The dietary supplement or pharmaceutical composition of any of claims 1 to 4, or for use according to any of claims 5 to 14, for use by the oral route.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L1/30 A23L3/44 A23L1/00 A61J3/07

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23L A61J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

* Special categories of cited documents:

- **A** document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 6 August 2015
Date of mailing of the international search report: 20/08/2015

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NL - 2280 HV Rijswijk
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Authorized officer: Stiegl, Petra

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