MYCELIUM OF FILAMENTOUS FUNGI AND METHOD FOR PRODUCING THE SAME

BACKGROUND

Field of Invention

[0001] The present disclosure relates to a method for producing a mycelium of fungi, in particular to a mycelium of filamentous fungi and a method for producing the same.

Description of Related Art

[0002] For the reasons such as health, epidemic prevention, weight management, animal welfare or animal rights, religious beliefs, environmental protection and/or resource usage reduction, more and more people choose vegan, vegetarian or flexitarian diet. Among them, the flexitarian diet refers to a large intake of non-animal foods such as plant, fungal and/or algae foods, and a moderate intake of fish, poultry, eggs, and dairy products, but a small intake of red meat and processed meat, or an intake of the vegan diet at least one day a week. According to a survey conducted by a global market research company in 2021, a population of flexitarian was more than 40% of the total population.

[0003] One of sources of vegan protein is beans. In addition to being edible directly, beans can also be further processed into soy milk, dried tofu, tofu skin, tofu and vegetarian meat products. However, cultivation of beans not only consumes a large amount of land resources, but also requires use of pesticides and/or chemical fertilizers during the cultivation process, thereby polluting the environment. Secondly, in order to provide the vegetarian meat products made

from beans the fibrous mouthfeel, the vegetarian meat products need to be further extruded, resulting in a large amount of carbon emissions.

[0004] A mycelium of filamentous fungi contains high protein, high dietary fiber and low fat, and can improve intestinal flora, and thus the mycelium of filamentous fungi is a common material for health food. Secondly, the filamentous fungi have fibrous mouthfeel, and may be cultivated in large quantities in a fermentation tank through a fermentation step. Thus, application of the mycelium of filamentous fungi does not have the aforementioned disadvantages of cultivation of beans, so that mycelium of filamentous fungi may be used in the food industry as an excellent source of protein for vegan, vegetarian and flexitarian diets. However, the filamentous fungi used in the food industry are terrestrial and thus require use of fresh water for the fermentation step. In regions with insufficient and/or uneven rainfall, where fresh water is precious, cultivation of the filamentous fungi can have negatively environmental impacts.

[0005] Therefore, there is an urgent need for a method for producing a mycelium of filamentous fungi to solve the above problems.

SUMMARY

[0006] Therefore, one aspect of the present disclosure is to provide a method for producing a mycelium of filamentous fungi, in which seawater is added to culture media, so as to promote growth of the mycelium of the filamentous fungi and increase a dry biomass yield and a protein yield of the same. Therefore, the mycelium of the filamentous fungi can become an excellent vegan protein source, thereby solving problems such as consumption of land resources, fresh water resources and/or carbon emissions.

[0007] Another aspect of the present disclosure is to provide a mycelium of filamentous fungi obtained by the aforementioned producing method, in which the mycelium has a relatively high content of protein.

[0008] According to the above aspect of the present disclosure, a method for producing a mycelium of filamentous fungi is provided. First, a solid-state culture step is performed on a first mycelium of *Fusarium venenatum* by using a solid medium at 15°C to 30°C for 3 days to 5 days, so as to obtain a second mycelium. Next, a liquid-state culture step is performed on the second mycelium by using a first liquid medium at 15°C to 30°C, pH 5 to pH 8, and a shaking speed of 10 rpm to 100 rpm for 2 days to 7 days, so as to obtain a third mycelium.

[0009] Subsequently, a fermentation step is performed on the third mycelium by using a second liquid medium at 15°C to 30°C, pH 5 to pH 8, a rotational speed of 10 rpm to 100 rpm for 2 days to 7 days, so as to obtain a fermented product. The solid medium, the first liquid medium and/or the second liquid medium is added with seawater or diluted seawater. Next, a solid-liquid separation step is performed on the fermented product to obtain the mycelium.

[0010] According to one embodiment of the present disclosure, *Fusarium venenatum* was deposited in American Type Culture Collection (ATCC) with an accession number of ATCC 20334.

[0011] According to one embodiment of the present disclosure, the first liquid medium and the second liquid medium further include grains, beans, inorganic salts, carbohydrates, yeast extracts and/or malt extracts.

[0012] According to one embodiment of the present disclosure, the diluted seawater is obtained by diluting the seawater with fresh water, and a

concentration of the seawater in the diluted seawater is greater than 0% by volume (vol%) and less than 100 vol%.

[0013] According to one embodiment of the present disclosure, the fermentation step is performed in a fermentation tank, and performing the fermentation step further includes introducing a gas into the fermentation tank. According to one embodiment of the present disclosure, the gas is selected from the group consisting of air, oxygen, carbon dioxide, helium or any combination thereof.

[0014] According to one embodiment of the present disclosure, the method further includes performing a drying step and/or a grinding step on the fermented product to obtain a dried product of the mycelium.

[0015] According to one embodiment of the present disclosure, the seawater is subjected to a sedimentation step and a seawater filtration step.

[0016] According to one embodiment of the present disclosure, a volume ratio of the first liquid medium to the second liquid medium is 1:100 to 1:200.

[0017] According to one embodiment of the present disclosure, a dry biomass yield of the mycelium is 1.00% by weight (wt%) to 1.50 wt%.

[0018] According to another aspect of the present disclosure, a mycelium of filamentous fungi is provided. The mycelium of filamentous fungi is obtained by the aforementioned producing method, in which a protein yield of the mycelium of *Fusarium venenatum* is greater than 45 wt% and less than or equal to 55 wt%.

[0019] The method for producing the mycelium of the filamentous fungi according to the present disclosure is to add the seawater to at least one of the solid medium, the first liquid medium and the second liquid medium of the filamentous fungi, thereby promoting growth of the mycelium of the filamentous fungi and

increasing a dry biomass yield and a protein yield of the same. Therefore, the mycelium of the filamentous fungi can become an excellent vegan protein source, and the problems such as consumption of land resources, fresh water resources and/or carbon emissions can be solved.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] In order to allow the aforementioned and other objects, features, advantages and embodiments of the present disclosure to be more clearly understood, the detailed description of the accompanying drawings is as follows:

[0021] Fig. 1 showed the wet biomass yields of the mycelia of *Fusarium venenatum* obtained by adding different concentrations of sea salt water or seawater to the first and second liquid media according to an embodiment of the present disclosure.

[0022] Fig. 2 showed the dry biomass yields of the mycelia of *Fusarium venenatum* obtained by adding different concentrations of sea salt water or seawater to the first and second liquid media according to an embodiment of the present disclosure.

[0023] Fig. 3 showed the protein yields of the mycelia of *Fusarium venenatum* obtained by adding different concentrations of sea salt water or seawater to the first and second liquid media according to an embodiment of the present disclosure.

DETAILED DESCRIPTION

[0024] As previously mentioned, the present disclosure provides a mycelium of filamentous fungi and a method for producing the same, which may include but not limited to adding seawater to culture media for the filamentous fungi, so as to promote growth of the mycelium of the filamentous fungi and increase a dry biomass yield and/or a protein yield of the same, so that the mycelium of the filamentous fungi can be used as an excellent vegan protein source, and that the problems such as consumption of land resources, fresh water resources and/or carbon emissions can be solved.

[0025] The "vegan protein" described herein refers to non-animal protein. Specifically, the vegan protein may be derived from plants, algae, blue-green algae and/or fungi. Specifically, the aforementioned plants may include but not limited to nuts (e.g., almonds, pistachios, pumpkin seeds), grains (e.g., rice, wheat, corn) and/or legumes (e.g., peanuts, peas, soybeans, red beans, mung beans, pinto beans, chickpeas and/or lima beans). The aforementioned algae may include but not limited to *Chlorella*, *Rhodophylla* and/or *Laminaria*. The aforementioned blue-green algae may include but not limited to *Arthrospira maxima* and/or *Arthrospira platensis*. The aforementioned fungi may include but not limited to a fruiting body, a mycelium of macrofungi and/or a mycelium of filamentous fungi. In some embodiments, the aforementioned vegan protein may be, for example, the mycelium of the filamentous fungi.

[0026] The filamentous fungi described here are also called molds, which refer to fungi that can produce a mycelium with a long chain and/or a branched chain, but do not produce a large fruiting body. The filamentous fungi may be any edible species. In some embodiments, the filamentous fungi may include but not

limited to *Fusarium*, *Aspergillus*, and/or *Neurospora*. In some specific examples, the filamentous fungi may include but not limited to *Fusarium venenatum*, *Aspergillus oryzae* and/or *Neurospora intermedia*.

[0027] The aforementioned *Fusarium venenatum* is also known as *Fusarium aureus* or *Fusarium solani*, and is a fungus widely present in soil. *Fusarium venenatum* has a high content of protein and may be produced on a large scale through a fermentation step, so it is often used in manufacture of food compositions. A strain of *Fusarium venenatum* is not particularly limited, and may be any strain that may be mass-produced through the fermentation step, edible and non-pathogenic. In some specific examples, *Fusarium venenatum* may include but not limited to a strain A3/5 deposited in American Type Culture Collection (ATCC) with an accession number of ATCC 20334, which can be purchased from ATCC.

[0028] The seawater described herein refers to salt water from the ocean, which may include but not limited to 30 g/L to 40 g/L of sea salt and active substances. In some embodiments, the active substances refer to substances that can promote the growth of the mycelium of the filamentous fungi. The source of the aforementioned seawater is not particularly limited, and may be taken from any ocean. In some specific examples, the seawater is shallow (or called euphotic zone) seawater in the sea off Keelung. The aforementioned shallow seawater refers to the seawater 50 m to 200 m below sea level. The concentration of active substances in the shallow seawater is moderate, so that the shallow seawater is conducive to the growth of the mycelium of the filamentous fungi. Moreover, solids in the shallow seawater are less, and thus using the shallow seawater can reduce costs of a sedimentation step and a seawater filtration step.

[0029] In some embodiments, the sedimentation step and the seawater filtration step may be optionally performed on the seawater to remove solids (e.g., marine litter, sand, biological debris and/or humus) in the seawater. In the above embodiments, the seawater filtration step may include but not limited to perform a filtration treatment and/or a centrifugation treatment on the seawater. In some embodiments, the seawater excludes evaporation treatment, crystallization treatment and ion exchange membrane electrodialysis to retain the active substances.

[0030] In some embodiments, the diluted seawater is obtained by mixing fresh water with seawater. In some embodiments, a concentration of the seawater in the diluted seawater may be greater than 0% by volume (vol%) and less than 100 vol%, or 50 vol% to 90 vol%, or 60 vol% to 80 vol%.

[0031] Noted that the water referred in the present invention also includes water solutions which use water molecules (H_2O) as solvent. The water can be grouped into fresh water and salty water according to the salt contents. The salt content of the salty water is bigger or equal to 0.5 g/L, and the salty water includes seawater. The fresh water is water with a salt content of less than 0.5 g/L. In some specific embodiments, the fresh water has a chloride content of less than or equal to 250 mg/L. In some specific embodiments, the fresh water has a sulfate content of less than or equal to 250 mg/L. A source of the fresh water is not particularly limited, and may include but not limited to ground fresh water, surface fresh water, biological water, desalinated seawater and/or purified sewage. In some embodiments, the surface fresh water may include lake water, river water, and/or glacier water. The surface fresh water may be derived from precipitation from rain, snow, snow particles and/or hail. The fresh water may

be further treated by water purification, in which a method of water purification treatment is not particularly limited. In some embodiments, the water purification treatment may include but not limited to gelation, sedimentation, filtration and disinfection, so as to remove impurities and/or germs in the water, thereby obtaining tap water. In some embodiments, the fresh water is distilled water (dH_2O), double-distilled water (ddH_2O) or pure water that is further treated by reverse osmosis, ion exchange resin and/or activated carbon. In some embodiments, the aforementioned fresh water, ground water, tap water, dH_2O, ddH_2O and pure water may be used interchangeably. In some specific examples, the fresh water is equivalent to water without adding seawater or salt water, in other words, a content of the seawater or the salt water is 0 vol%.

[0032] Experiments have confirmed that compared to adding the fresh water, adding the seawater to the culture medium can promote the growth of the mycelium of the filamentous fungi. However, compared to adding the fresh water, adding salt water to the culture medium, such as sea salt water prepared by using sea salt reconstituted from the seawater, actually inhibits the growth of the mycelium of the filamentous fungi, which means that the active substances contained in the seawater play an important role in increasing the dry biomass yield and the protein yield of the mycelium of *Fusarium venenatum*.

[0033] In some embodiments, the mycelium of *Fusarium venenatum* may be obtained by a multi-step process, in which the multi-step process may include but not limited to a solid-state culture step, a liquid-state culture step, and a fermentation step. The aforementioned solid-state culture step is to perform the solid-state culture step on a first mycelium of *Fusarium venenatum* by using a solid medium, so as to obtain a second mycelium.

[0034] Temperature of the solid-state culture step is not particularly limited, and may be, for example, 15°C to 30°C. If the temperature of the solid-state culture step is too high or too low, growth activity of the second mycelium is reduced or even lost. Duration of the solid-state culture step depends on production efficiency and growth activity of the mycelium. In some embodiments, the duration of the solid-state culture step may be, for example, 3 days to 5 days. Otherwise the second mycelium with better growth activity cannot be obtained.

[0035] The liquid-state culture step is to perform the liquid-state culture step on the second mycelium by using a first liquid medium to obtain a third mycelium. The liquid-state culture step may be performed at a conventionally suitable temperature, for example, 15°C to 30°C. If the temperature of the liquid-state culture step is too high or too low, growth activity of the third mycelium is reduced or even lost. Duration of the liquid-state culture step is not particularly limited, and may depend on production efficiency and growth activity of the mycelium. In some embodiments, the duration of the liquid-state culture step may be, for example, 2 days to 7 days, otherwise the third mycelium with better growth activity cannot be obtained, or under premise of increased time cost, a wet biomass yield, a dry biomass yield and/or a protein yield of the third mycelium is not improved. In some embodiments, a shaking speed of the liquid-state culture step may be, for example, 10 rpm to 100 rpm, to increase an aeration rate of the first liquid medium, thereby promoting growth of the third mycelium.

[0036] In some embodiments, the fermentation step may be performed on the third mycelium by using a second liquid medium, so as to obtain a fermented product. Temperature and duration of the fermentation step may be the same as those of the liquid-state culture step, but production scale thereof are different.

In some embodiments, the fermentation step is performed in a fermentation tank, and may optionally be combined with stirring treatment, in which a rotational speed of the stirring treatment is 10 rpm to 100 rpm, or 10 rpm to 40 rpm. In some specific examples, the aforementioned fermentation tank is a ton-level fermentation tank. In some embodiments, a volume ratio of the first liquid medium to the second liquid medium may be, for example, 1:10 to 1:200, or 1:150 to 1:170, or 1:155 to 1:165.

[0037] However, in some embodiments, the fermentation step may be performed in the fermentation tank. Secondly, during the fermentation step, gas may be selectively introduced into the fermentation tank, in which the gas may be, for example, selected from the group consisting of air, oxygen, carbon dioxide, helium, or any combination thereof. In some specific examples, an aeration rate of the fermentation tank may be, for example, from 0.01 volume of air per unit volume of culture medium per minute (VVM) to 1.5 VVM. In some specific examples, a tank pressure of the fermentation tank may be, for example, 0.1 kg/cm^2 to 1.0 kg/cm^2, or 0.1 kg/cm^2 to 0.5 kg/cm^2, to promote growth of the third mycelium.

[0038] Types of the solid medium, the first liquid medium and the second liquid medium are not particularly limited. In some embodiments, the solid medium may be, for example, malt extract agar medium, Sabouraud's agar medium and/or its modified formulation. In some specific examples, the solid medium is preferably 325 malt extract agar medium, which contains 20.0 g/L of malt extracts, 20.0 g/L of glucose, 1.0 g/L of protein peptone, 20.0 g/L of agar and a balance amount of fresh water, seawater and/or diluted seawater. In some embodiments, the first liquid medium may include but not limited to grains, beans,

inorganic salts, carbohydrates, yeast extracts and/or malt extracts, so as to provide sufficient nitrogen source and/or carbon source for the second mycelium, but may be adjusted according to needs. For example, in other embodiments, the first liquid medium may include but not limited to carbohydrates, organic acid salts and inorganic salts. In the above embodiments, a pH value of the first liquid medium may be, for example, pH 5 to pH 8, otherwise growth activity of the third mycelium is poor.

[0039] In some specific examples, types of the grains may include but not limited to barley, wheat, rye, oats, rice, millet, sorghum and/or *Panicum miliaceum*. In the above specific examples, depending on degree of processing, the rice may include but not limited to white rice, germ rice and/or brown rice. In the above specific examples, depending on types of starch, the rice may include but not limited to glutinous rice, indica rice and/or japonica rice.

[0040] In some specific examples, the beans may include but not limited to red beans, pinto beans, lima beans, soybeans, mung beans and/or macadamia. In some embodiments, the inorganic salts may include but not limited to potassium nitrate (KNO_3), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and/or potassium dihydrogen phosphate (KH_2PO_4). In some specific examples, the carbohydrates may include but not limited to monosaccharides, disaccharides, oligosaccharides and/or polysaccharides, in which the monosaccharides may include but not limited to fructose, glucose, ribose, galactose and/or mannose, and the disaccharides may include but not limited to sucrose, lactose, maltose and/or trehalose, and the oligosaccharides may include but not limited to fructooligosaccharides, maltooligosaccharides, isomaltooligosaccharides,

galactooligosaccharides and/or soybean oligosaccharides, and the polysaccharides may include but not limited to inulin, starch, glycogen, oat β-glucan and/or chitin. In some specific embodiments, the organic acid salt may include but not limited to citrate (e.g., sodium citrate), acetate, malate, tartrate and/or quininate.

[0041] The formulation of the aforementioned second liquid medium may be the same as or different from that of the first liquid medium, and a pH value may be, for example, pH 5 to pH 8, such as pH 5 to pH 6. In some embodiments, the seawater and/or the diluted seawater is added to at least one of the solid medium, the first liquid medium, and the second liquid medium. In some specific examples, the seawater and/or the diluted seawater is added to the solid medium, the first liquid medium and the second liquid medium. In some specific examples, the seawater and/or the diluted seawater is added to the first liquid medium and the second liquid medium, and the solid medium is prepared by using the fresh water. In other examples, the seawater and/or the diluted seawater is added to the solid medium and the first liquid medium, and the second liquid medium is prepared by using the fresh water. In further specific examples, the seawater and/or the diluted seawater is added to the solid medium and the second liquid medium, and the first liquid medium is prepared by using the fresh water. In some specific examples, the seawater and/or the diluted seawater is added to the second liquid medium, and the solid medium and the first liquid medium are prepared by using the fresh water. In some specific examples, the seawater and/or the diluted seawater is added to the first liquid medium, and the solid medium and the second liquid medium are prepared by using the fresh water. In some specific examples, the seawater and/or the diluted seawater is

added to the solid medium, and the first liquid medium and the second liquid medium are prepared by using the fresh water.

[0042] Next, a solid-liquid separation step is performed on the fermented product, so as to obtain the mycelium. In some embodiments, the solid-liquid separation step may include but not limited to centrifugation treatment and/or filtration treatment. In some embodiments, when the solid-liquid separation step is the centrifugation treatment, a pellet may be obtained. In other words, in some embodiments, the mycelium and the pellet may be used interchangeably. The culture medium supplemented with the seawater and/or the diluted seawater may be used to promote growth of the mycelium of the filamentous fungi. "To promote growth of the mycelium of the filamentous fungi" described herein may include but not limited to increasing the wet biomass yield, the dry biomass yield and the protein yield of the mycelium. The wet biomass yield, the dry biomass yield and the protein yield described herein are calculated based on the weight of the fermented product as 100% by weight (wt%). In some embodiments, the dry biomass yield of the mycelium is 1.00 wt% to 1.50 wt%. In some embodiments, the protein yield of the mycelium is greater than 45 wt% and less than or equal to 55 wt%.

[0043] In some embodiments, a drying step and/or a grinding step may be further performed on the mycelium to obtain a dried product of the mycelium of *Fusarium venenatum*. The method of the drying step is not particularly limited, and only part or all of water in the mycelium needs to be removed, such as freeze drying, vacuum drying or spray drying. In some embodiments, after the mycelium of *Fusarium venenatum* is freeze-dried, the dried product obtained is lyophilized powder. In other words, in some embodiments, the lyophilized powder and the

dried product may be used interchangeably. The grinding step may be performed by a conventional method, such as mechanical grinding, drum grinding or pneumatic grinding.

[0044] The aforementioned fermented product, the mycelium obtained by the solid-liquid separation step of the fermented product, and the dried product obtained after the mycelium is subjected to the drying step and/or the grinding step may be applied to food compositions or pharmaceutical compositions. In some embodiments, the mycelium may be made into a vegetarian meat product with fibrous mouthfeel without performing an extrusion step. In some embodiments, the dried product of the mycelium may be used as a raw material for the manufacture of high-protein powder. In some embodiments, a dosage form of the aforementioned food compositions or pharmaceutical compositions may be, for example, tablet, capsule, granule, pill, patch, powder, emulsifier, liquid suspension, dispersion or solvent. In some embodiments, the aforementioned food compositions or pharmaceutical compositions may be selectively added with pharmaceutical- and/or food-acceptable additives, in which the additives may include but not limited to carriers, excipients, diluents, adjuvants, solvents, dispersants, coatings, antibacterial and/or antifungal agents.

[0045] The aforementioned additives may be adjusted according to the dosage form. In some embodiments, when the dosage form is the tablet, the carrier may include but not limited to lactose, corn starch, and/or lubricants. In some embodiments, the aforementioned lubricant may include but not limited to magnesium stearate and/or sodium stearyl fumarate. In some embodiments, when the dosage form is the capsule, the diluent may include but not limited to lactose and/or dried corn starch. In some embodiments, when the dosage form

is the liquid suspension or the emulsifier, the emulsifier or the suspending agent used to dissolve or suspend the fermented product, the mycelium and/or the dried product may be oily substance. In some embodiments, sweeteners, flavors and/or pigments may be selectively added to the food compositions or pharmaceutical compositions.

[0046] Several embodiments below are utilized to illustrate the application of the present disclosure, but those are not in order to limit the present disclosure, and those skilled in the art of the present disclosure may make various changes and modifications without departing from the spirit and scope of the present disclosure.

Embodiment 1. Preparation of fermented product of *Fusarium venenatum*

1. Preparation Example 1

[0047] A first mycelium of *Fusarium venenatum* (hereinafter referred to as FV) with an accession number of ATCC 20334 was purchased from American Type Culture Collection (ATCC). The first mycelium of FV was inoculated on a solid medium, and a solid-state culture step was performed at 25°C for 3 to 5 days to obtain a second mycelium. The aforementioned solid medium was 325 malt extract agar medium, which contained 20.0 g/L of malt extracts, 20.0 g/L of glucose, 1.0 g/L of protein peptone, 20.0 g/L of agar and a balance amount of pure water.

[0048] Next, the second mycelium was inoculated into a first liquid medium, followed by a liquid-state culture step at 25°C, pH 7, a rotational speed of 10 rpm to 100 rpm, and an aeration rate of 0.01 VVM to 1.5 VVM for 2 days, so as to obtain a third mycelium. The first liquid medium contained 1.0 wt% of glucose, 0.3 wt% of sodium citrate, 0.3 wt% of potassium nitrate (KNO_3), 0.1 wt% of

ammonium dihydrogen phosphate ($NH_4H_2PO_4$), 0.1 wt% of calcium chloride ($CaCl_2$) and 20 vol% seawater (which was obtained by diluting seawater to 20 vol% with ddH_2O). The seawater was taken from 50 m to 60 m below the sea level in the sea off Keelung. After analysis, the seawater had 3.64 ppm of zinc, 0.71 ppm of manganese, 1.09 ppm of copper, 912.8 ppm of calcium, 12.85 ppm of iron, 11,998 ppm of phosphorus, 4,406 ppm of potassium, 13,927 ppm of sodium, and no nitrite was detected.

[0049] Next, the third mycelium was placed in the fermentation tank, and a fermentation step was performed by using a second liquid medium at 25 °C, pH 7, a rotational speed of 10 rpm to 100 rpm, and an aeration rate of 0.01 VVM to 1.5 VVM for 2 days, so as to obtain a fermented product. The second liquid medium contained 1.0 wt% of glucose, 0.3 wt% of sodium citrate, 0.3 wt% of potassium nitrate (KNO_3), 0.1 wt% of ammonium dihydrogen phosphate ($NH_4H_2PO_4$), 0.1 wt% of calcium chloride ($CaCl_2$) and 20 vol% of seawater. A centrifugation step was performed on the fermented product to obtain a pellet (or the mycelium).

2. Preparation Example 2 to Preparation Example 5

[0050] Producing methods of fermented products of Preparation Example 2 to Preparation Example 5 were the same as the producing method of Preparation Example 1, and the differences were that the first and second liquid media of Preparation Example 2 to Preparation Example 5 were added with 40 vol%, 60% vol%, 80% vol% and 100 vol% of seawater, respectively.

3. Preparation Comparative Example 1 to Preparation Comparative Example 7

[0051] Producing methods of fermented products of Preparation Comparative Example 1 to Preparation Comparative Example 7 were the same as the producing method of Preparation Example 1, and the difference were that the first and second liquid media of Preparation Comparative Example 1 and Preparation Comparative Example 2 were added with ddH_2O (equivalent to 0 vol% of seawater or sea salt water), and the first and second liquid media of Preparation Comparative Example 3 to Preparation Comparative Example 7 were added with 20 vol%, 40 vol%, 60 vol%, 80 vol% and 100 vol% of sea salt water, respectively, in which, 100 vol% of sea salt water contained sea salt with a weight volume concentration of 35 g/L.

4. Detection methods of wet biomass yield, dry biomass yield and protein yield of mycelium

[0052] A percentage of a weight of the pellet to a weight of the fermented product was calculated to obtain a wet biomass yield (unit: wt%) of the fermented product.

[0053] Next, the mycelium was dehydrated to obtain a dried product. A weight of the dried product was measured, and a percentage of the weight of the dried product to the weight of the fermented product was calculated, so as to obtain a dry biomass yield (unit: wt%) of the mycelium.

[0054] Next, a percentage of a weight of protein of the dried product and a weight of the fermented liquid was detected to obtain a protein yield (unit: wt%) of the mycelium. The detection of the weight of protein was performed by the Kjeldahl method for nitrogen, which is briefly described as follows. First, sulfuric acid was added to the dried product and then heated, so that the protein of the dried product was decomposed into ammonia gas. Since ammonia gas could further react with sulfuric acid to form ammonium sulfate, a strong base might then be

added, so as to decompose ammonium sulfate into ammonia gas, and ammonia gas was then distilled into a standard acid solution by distillation. Since ammonia gas was alkaline after being dissolved, a nitrogen content in the dried product could be further obtained by detecting degree of neutralization of the standard acid solution by ammonia gas using acid-base titration. Subsequently, the weight of protein in the sample could be obtained by multiplying the nitrogen content by a nitrogen factor (where the dry powder was classified as a general food, and its nitrogen factor was 6.25).

Embodiment 2. Analysis results of wet biomass yield, dry biomass yield and protein yield of mycelium of *Fusarium venenatum*

1. Evaluation of whether seawater could increase wet biomass yield of mycelium of *Fusarium venenatum*

[0055] Regarding whether the first and second liquid media added with the seawater and the sea salt water could improve the wet biomass yield of the mycelium of *Fusarium venenatum* (FV), please refer to Fig. 1, which showed wet biomass yields of mycelia of FV obtained by adding different concentrations of sea salt water or seawater to the first and second liquid media according to an embodiment of the present disclosure. The horizontal axis indicated that the first and second liquid media was added with the seawater or the sea salt water, the vertical axis indicated the wet biomass yield (unit: wt%), straight bar 110, straight bar 120, straight bar 130, straight bar 140, straight bar 150, straight bar 160 represented that the concentrations of the sea salt water or the seawater were 0 vol%, 20 vol%, 40 vol%, 60 vol%, 80 vol% and 100 vol%, respectively, i.e., the straight bars from left to right, are Preparation Comparative Example 2 to Preparation Comparative Example 7, Preparation Comparative Example 1, and

Preparation Example 1 to Preparation Example 5, respectively, and "*" means that there was a statistically significant difference (p<0.05) from Preparation Comparative Example 2.

[0056] As shown in Fig. 1, the wet biomass yield of the mycelium of Preparation Comparative Example 2 was 5.3 wt%, in which the mycelium of Preparation Comparative Example 2 were obtained by using the first and second liquid media added with 0 vol% of the sea salt water. The wet biomass yields of the mycelia of Preparation Comparative Example 3 to Preparation Comparative Example 7 decreased as the concentration of the sea salt water increased, in which the wet biomass yield of the mycelium of Preparation Comparative Example 7 was only 4.4 wt%. Noted that the mycelia of Preparation Comparative Example 3 to Preparation Comparative Example 7 were obtained by using the first and second liquid media added with 20 vol% to 100 vol% of the sea salt water, and the mycelium of Preparation Comparative Example 7 was obtained by using the first and second liquid media added with 100 vol% of the sea salt water. However, there was no statistically significant difference between the wet biomass yield of the mycelium of Preparation Comparative Example 1 from those of Preparation Example 1 to Preparation Example 5, in which the mycelium of Preparation Comparative Example 1 was obtained by using the first and second liquid media added with 0 vol% of the seawater, and the mycelia of Preparation Example 1 to Preparation Example 5 were obtained by using the first and second liquid media added with 20 vol% to 100 vol% of the seawater. The results showed that sea salt water could inhibit growth of the mycelium of FV, but the seawater would not inhibit growth of the mycelium of FV.

2. Evaluation of whether seawater could increase dry biomass yield of mycelium of *Fusarium venenatum*

[0057] Regarding whether the first and second liquid media added with the seawater and the sea salt water could improve the dry biomass yield of the mycelium of *Fusarium venenatum* (FV), please refer to Fig. 2, which showed dry biomass yields of mycelia of FV obtained by adding different concentrations of sea salt water or seawater to the first and second liquid media according to an embodiment of the present disclosure. The horizontal axis indicated that the first and second liquid media was added with the seawater or the sea salt water, the vertical axis indicated the dry biomass yield (unit: wt%), straight bar 210, straight bar 220, straight bar 230, straight bar 240, straight bar 250, straight bar 260 represented that the concentrations of the sea salt water or the seawater were 0 vol%, 20 vol%, 40 vol%, 60 vol%, 80 vol% and 100 vol%, respectively, i.e., the straight bars from left to right were Preparation Comparative Example 2 to Preparation Comparative Example 7, Preparation Comparative Example 1, and Preparation Example 1 to Preparation Example 5, respectively, "*" indicated that there was a statistically significant difference between Preparation Comparative Example 3 to Preparation Comparative Example 7 and Preparation Comparative Example 2, and "**" indicated that there was a statistically significant difference between Preparation Example 1 to Preparation Example 5 and Preparation Comparative Example 1 ($p<0.05$ and $p<0.01$, respectively).

[0058] As shown in Fig. 2, the dry biomass yields of mycelia of Preparation Comparative Example 1 and Preparation Comparative Example 2 were 1.05 wt%, in which the mycelia of Preparation Comparative Example 1 and Preparation Comparative Example 2 were obtained by using the first and second liquid media

added with 0 vol% of the sea salt water and 0 vol% of the sea salt water, respectively. Secondly, the dry biomass yields of the mycelia of Preparation Comparative Example 3 to Preparation Comparative Example 7 decreased as the concentration of the sea salt water increased, in which the mycelia of Preparation Comparative Example 3 to Preparation Comparative Example 7 were obtained by using the first and second liquid media added with 20 vol% 100 vol% of the sea salt water. Noted that the mycelia of Preparation Comparative Example 6 and Preparation Comparative Example 7, which were 0.85 wt% and 0.73 wt%, respectively, were significantly lower than that of Preparation Comparative Example 2, in which , the mycelia of Preparation Comparative Example 6 and Preparation Comparative Example 7 were obtained by using the first and second liquid media added with 80 vol% of the sea salt water and 100 vol% of the sea salt water, respectively, and mycelium of the Preparation Comparative Example 2 was obtained by using the first and second liquid media added with 0 vol% of the sea salt water.

[0059] However, compared to the mycelium of Preparation Comparative Example 1, the dry biomass yields of the mycelia of Preparation Example 1 to Preparation Example 5 increased as the concentration of the sea water increased, in which the mycelium of Preparation Comparative Example 1 were obtained by using the first and second liquid media added with 0 vol% of the seawater, and the mycelia of Preparation Example 1 to Preparation Example 5 were obtained by using the first and second liquid media added with 20 vol% to 100 vol% of the seawater. Among them, the dry biomass yields of the mycelia of Preparation Example 3 and Preparation Example 4 that were 1.25 wt% and 1.23 wt%, respectively, were significantly higher than that of Preparation Comparative Example 1, in which

mycelia of Preparation Example 3 and Preparation Example 4 were obtained by using the first and second liquid media added with 60 vol% of the seawater and 80 vol% of the seawater, respectively, and the mycelia of Preparation Comparative Example 1 was obtained by using the first and second liquid media added with 0 vol% of the seawater. The above results showed that the sea salt water could inhibit growth of the mycelium of FV, but the seawater could promote growth of the mycelium of FV, indicating that active substances contained in the seawater could promote growth of the mycelium of FV, and the seawater and the sea salt water could not equivalently replaced with each other.

3. Evaluation of whether seawater could increase protein yield of mycelium of *Fusarium venenatum*

[0060] Regarding whether the first and second liquid media added with the seawater and the sea salt could improve the protein yield of the mycelium of *Fusarium venenatum* (FV), please refer to Fig. 3, which showed protein yields of mycelia of FV obtained by adding different concentrations of sea salt water or seawater to the first and second liquid media according to an embodiment of the present disclosure. The horizontal axis indicated the compositions of the first and second liquid media, the vertical axis indicated the protein yield (unit: wt%), straight bar 310, straight bar 320, straight bar 330, straight bar 340, straight bar 350, and straight bar 360 represented that the concentrations of the sea salt water or the seawater were 0 vol%, 20 vol%, 40 vol%, 60 vol%, 80 vol% and 100 vol%, respectively, i.e., the straight bars from left to right were Preparation Comparative Example 2 to Preparation Comparative Example 7, Preparation Comparative Example 1, and Preparation Example 1 to Preparation Example 5, respectively, and "*" indicated that there was a statistically significant difference

between Preparation Example 1 to Preparation Example 5 and Preparation Comparative Example 1 (p<0.05, respectively).

[0061] As shown in Fig. 3, the protein yields of the mycelia of Preparation Comparative Example 1 and Preparation Comparative Example 2 were 45 wt%, in which the mycelia of Preparation Comparative Example 1 and Preparation Comparative Example 2 were obtained by using the first and second liquid media added with 0 vol% of the sea salt water and 0 vol% of the sea salt water. The protein yields of the mycelia of Preparation Comparative Example 3 to Preparation Comparative Example 7 were not significantly changed relative to Preparation Comparative Example 2 and even decreased as the concentration of the sea salt water increased, in which the protein yields of the mycelia of Preparation Comparative Example 3 to Preparation Comparative Example 7 were obtained by using the first and second liquid media added with 20 vol% to 100 vol% of the sea salt water , and the mycelia of Preparation Comparative Example 2 was obtained by using the first and second liquid media added with 0 vol% of the sea salt water.

[0062] However, compared to the mycelium of Preparation Comparative Example 1, the protein yields of the mycelia of Preparation Example 1 to Preparation Example 5 increased as the concentration of the seawater increased, in which the mycelium of Preparation Comparative Example 1 was obtained by using the first and second liquid media added with 0 vol% of the seawater, and the mycelia of Preparation Example 1 to Preparation Example 5 were obtained by using the first and second liquid media added with 20 vol% to 100 vol% of the seawater. Noted that the protein yield of the mycelium of Preparation Example 5 that was 52 wt% was significantly higher than that of Preparation Comparative Example 1,

in which the mycelium of Preparation Example 5 was obtained by using the first and second liquid media, and the mycelium of Preparation Comparative Example 1 was obtained by using the first and second liquid media added with 0 vol% of the seawater. The above results indicated that the first and second liquid media added with the sea salt water reduced the protein content of the mycelium of FV, but the first and second liquid media added with the same concentration of the seawater increased the protein content of the mycelium of FV, indicating that the active substances contained in seawater could promote growth of the mycelium of FV, and the seawater and the sea salt water could not equivalently replaced with each other.

[0063] In summary, the aforementioned specific filamentous fungi, specific bacterial strain, specific seawater, specific culture media, specific processes or specific evaluation methods are only used to illustrate the mycelium of the filamentous fungi and the method for producing the same of the present disclosure. However, those skilled in the art of the present disclosure should understand that without departing from the spirit and scope of the present disclosure, other filamentous fungi, other bacterial strain, other seawater, other culture media, other processes or other evaluation methods can also be used to describe the mycelium of the filamentous fungi and the method for producing the same of the present disclosure, and not limited to the above. For example, at least one of the solid-state culture step, the liquid-state culture step and the fermentation step using a culture medium supplemented with seawater and/or diluted seawater can also increase a dry biomass yield and/or a protein yield of the mycelium of the filamentous fungi. Secondly, adding seawater and/or diluted seawater to another conventional culture medium to carry out at least one

of the solid-state culture step, the liquid-state culture step and the fermentation step can also improve a dry biomass yield and/or a protein yield of the mycelium of the filamentous fungi.

[0064] As may be seen from the foregoing embodiments, the mycelium of the filamentous fungi and the method for producing the same of the present disclosure have the advantages of using seawater, which can not only promote growth of the mycelium of the filamentous fungi and improve the dry biomass yield and/or the protein yield of the mycelium of the filamentous fungi, but also reduce consumption of land resources and fresh water resources and/or carbon emissions. Therefore, the mycelium of the filamentous fungi may be used as the excellent source of vegan protein, and can solve problems of food shortage when fresh water resources are scarce.

[0065] Although the present disclosure has been disclosed above with several specific embodiments, various modifications, changes and replacements may be made to the foregoing disclosure, and it should be understood that, without departing from the spirit and scope of the present disclosure, some features of the embodiments of the present disclosure will be used in some cases but not others. Therefore, the spirit of the present disclosure and the scope of claims should not be limited to those described in the above exemplary embodiments.

WHAT IS CLAIMED IS:

1. A method for producing a mycelium of filamentous fungi, comprising:

performing a solid-state culture step on a first mycelium of *Fusarium venenatum* by using a solid medium at 15°C to 30°C for 3 days to 5 days, so as to obtain a second mycelium;

performing a liquid-state culture step on the second mycelium by using a first liquid medium at 15°C to 30°C, pH 5 to pH 8, and a shaking speed of 10 rpm to 100 rpm for 2 days to 7 days, so as to obtain a third mycelium;

performing a fermentation step on the third mycelium by using a second liquid medium at 15°C to 30°C, pH 5 to pH 8, a rotational speed of 10 rpm to 100 rpm for 2 days to 7 days, so as to obtain a fermented product, wherein at least one of the solid medium, the first liquid medium and the second liquid medium is added with seawater or diluted seawater; and

performing a solid-liquid separation step on the fermented product to obtain the mycelium.

2. The method for producing the mycelium of the filamentous fungi of claim 1, wherein *Fusarium venenatum* was deposited in American Type Culture Collection (ATCC) with an accession number of ATCC 20334.

3. The method for producing the mycelium of the filamentous fungi of claim 1, wherein the first liquid medium and the second liquid medium further comprise grains, beans, inorganic salts, carbohydrates, yeast extracts and/or malt extracts.

4. The method for producing the mycelium of the filamentous fungi of claim 1, wherein the diluted seawater is obtained by diluting the seawater with fresh water, and a concentration of the seawater in the diluted seawater is greater than 0% by volume and less than 100% by volume.

5. The method for producing the mycelium of the filamentous fungi of claim 1, wherein the fermentation step is performed in a fermentation tank, and performing the fermentation step further comprises introducing a gas into the fermentation tank.

6. The method for producing the mycelium of the filamentous fungi of claim 5, wherein the gas is selected from the group consisting of air, oxygen, carbon dioxide, helium and any combination thereof.

7. The method for producing the mycelium of the filamentous fungi of claim 1, further comprising performing a drying step and/or a grinding step on the fermented product to obtain a dried product of the mycelium.

8. The method for producing the mycelium of the filamentous fungi of claim 1, wherein the seawater is subjected to a sedimentation step and a seawater filtration step.

9. The method for producing the mycelium of the filamentous fungi of claim 1, wherein a volume ratio of the first liquid medium to the second liquid medium is 1:100 to 1:200.

10. The method for producing the mycelium of the filamentous fungi of claim 1, wherein a dry biomass yield of the mycelium is 1.00% by weight to 1.50% by weight.

11. A mycelium of filamentous fungi, which is obtained by the method for producing the mycelium of the filamentous fungi of any one of claims 1 to 9, wherein a protein yield of the mycelium of *Fusarium venenatum* is greater than 45% by weight and less than or equal to 55% by weight.