The present invention relates to a method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata* and a pharmaceutical composition for preventing or treating diabetic diseases and diabetic complications prepared by the preparation method. According to the method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata*, the extract contains a relatively high content of expolsaccharides in comparison with the conventional preparation method according to a prior document by these inventors and thus will be capable of being used as an ingredient for a pharmaceutical composition for diabetic diseases or a relevant functional food.
[Fig. 1]

a) 

>CR
CGGGTGTAGCTGCTGCTTTAAACGAGGTATGTCAGCAGCTGCTCATTCCACTCTCTCAACCTCTCTCTGCGACTTTATGTAAGAAGAACGTCGGTGAAGCCAGCTATTAGTTGTTGATTAAAGCTTCTTATTGATTTACTAACAAACGCTTCAGTTATAGAATTTTACTGTGTATAAACACAAATTATATACAACCTTCAGCAACGGATCCTCTGCTCTGCATCGATGAAAGAACAGCAGCGAACATGCGATAAGTGAATGTGAAATTCACTGGAATCATCGGATCTTTGGAGATATGCTGCTGTTTGAAGTCTCATGGAATTTCTCAAACCCCTAAATTGTGAAATGTTTTATGCTTGAGCTGACCTTTCTTACCTGAAAA

b) 

[Figure showing a phylogenetic tree and a list of species]

[Fig. 2a]

A graph showing pH and residual sugar levels with lactose, sucrose, glucose, fructose, and galactose as labels on the x-axis. The graph includes a line for pH ranging from 3 to 7 and a line for residual sugar (mg/mL) ranging from 0 to 50.
[Fig. 2b]

![Images of bottles containing Fructose, Sucrose, Glucose, Galactose, and Lactose]

[Fig. 3]

![Graph showing Mycelial biomass (g/L) and Exopolysaccharide (g/L) for Lactose, Sucrose, Glucose, Fructose, and Galactose]
[Fig. 16]

[Fig. 17]

[Fig. 18]
METHOD FOR PREPARING EXTRACT FROM CULTURE MEDIUM OF CERIPORIA LACERATA AND PHARMACEUTICAL COMPOSITION PREPARED THEREBY FOR PREVENTING OR TREATING DIABETIC DISEASES AND DIABETIC COMPLICATIONS, WHICH CONTAINS EXTRACT FROM CULTURE MEDIUM OF CERIPORIA LACERATA AS ACTIVE INGREDIENT

FIELD OF THE INVENTION

The present invention relates to a method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata* and a pharmaceutical composition for preventing or treating diabetic diseases and diabetic complications prepared by the preparation method.

DESCRIPTION OF THE PRIOR ART

*Ceriporia lacerate* is a kind of white-rotting fungus and performs co-metabolism, *i.e.*, lignin decomposition, in order to use carbon sources such as cellulose, hemi-cellulose, etc. in the ecosystem.

The presence of *Ceriporia lacerate* was reported first in 2002, and for this reason, there have been very few studies on the industrialization of *Ceriporia lacerata*. Only two types of studies on the use of *Ceriporia lacerate* for preventing soil contamination and bleaching have been reported.

With respect to studies on the use of *Ceriporia lacerate* for foods and drugs, Korean Patent No. 10-1031605 entitled “method for preparing *Ceriporia lacerata* culture extract for prevention and treatment of diabetic disease and *Ceriporia lacerata* culture extract prepared thereby,” which was filed in the name of the present inventors, is the only study in the world. However, the above patent document mentions only the effect of the extract against Type 1 diabetes.

Diabetes therapeutic agents developed to date include blood glucose-lowering agents and insulin injections, but these agents merely delay the progression of diabetes and do not
function as agents for preventing and treating diabetes.

For the treatment of diabetes and diabetic complications, a substance for arresting the progression of the diseases is required to be developed. In addition, a substance for either regenerating pancreatic beta-cells that regulate insulin secretion or promoting the regeneration of pancreatic beta-cells is required to be developed.

The present inventors have discovered that the marker substance, exopolysaccharide, contained in a Ceriporia lacerata mycelium culture and a dried substance thereof is a very effective substance for blocking the progression of diabetes and promoting the regeneration of pancreatic beta-cells. Thus, there is an urgent need for studies on the identification of the structure of exopolysaccharide in Ceriporia lacerata and the discovery of an optimal culture method for increasing the content of the exopolysaccharide.

DETAILED DESCRIPTION OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

Accordingly, the present invention has been made in order to solve the above-described problems occurring in the prior art, and it is an object of the present invention to provide an improved method for preparing an extract from the mycelial culture medium of Ceriporia lacerata, which can increase the content of exopolysaccharide.

Another object of the present invention is to provide a pharmaceutical composition for preventing or treating diabetic diseases and diabetic complications, which contains, as an active ingredient, an extract from the mycelial culture medium of Ceriporia lacerata having an increased content of exopolysaccharide prepared by the preparation method.

MEANS TO SOLVE THE PROBLEMS
To achieve the above objects, the present invention provides a method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata*, which comprises the steps of: culturing the mycelia of *Ceriporia lacerata* in a liquid, drying the culture to form powder and preparing a solvent extract from the powder, wherein a medium for culturing the mycelia of *Ceriporia lacerata* comprises 1-2 wt% of sugar, 0.2-1 wt% of glucose, 0.2-1 wt% of starch, 0.1-0.5 wt% of sorghum powder, 0.1-0.5 wt% of barley powder, 0.2-2 wt% of soy flour, 0.05-0.1 wt% of magnesium sulfate (MgSO₄), 0.05-0.1 wt% of monopotassium phosphate (KH₂PO₄), 0.05-0.1 wt% of dipotassium phosphate (K₂HPO₄) and 92-98 wt% of water and has a pH of 4.5-6.0.

The culturing is preferably carried out under a blue LED light source.

The culturing is preferably carried out at a carbon dioxide concentration of 1,000-2,000 ppm.

The present invention also provides a pharmaceutical composition for preventing or treating diabetic diseases and diabetic complications, which contains, as an active ingredient, an extract from the mycelial culture medium of *Ceriporia lacerata* having an increased content of exopolysaccharide prepared by the preparation method.

The diabetic diseases may be Type 2 diabetes.

The diabetic complications may be selected from the group consisting of hyperglycemia, atherosclerosis, microangiopathy, diabetic retinopathy and kidney disease.

**EFFECTS OF THE INVENTION**

According to the method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata*, the extract prepared according to the inventive method has a relatively high content of exopolysaccharides in comparison with the extract prepared by the method described in the prior art document of the present inventors, and thus it can be used as an active ingredient for a pharmaceutical composition for diabetic diseases or a relevant functional food.
BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1a and 1b show the results of ITS-5.8S rDNA sequencing of a Ceriporia lacerata strain according to the present invention.

FIG. 2a is a graph showing the content of residual sugar depending on pH and the kind of sugar in the culture of a Ceriporia lacerata strain, and FIG. 2b is a photograph showing culture products.

FIG. 3 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on the kind of sugar.

FIG. 4 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on the concentration of glucose.

FIG. 5 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on a nitrogen source.

FIG. 6 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on the concentration of soy flour as a nitrogen source.

FIG. 7 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on trace elements.

FIG. 8 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on the concentration of the trace element MgSO₄.

FIG. 9 is a graph showing the growth of mycelia and the content of exopolysaccharide depending on culture time in a 5L culture medium.

FIG. 10 is a graph showing the results of measuring the molecular weight of exopolysaccharide in a purified culture.

FIG. 11 schematically shows an experimental process for analyzing the activity of an extract from the mycelial culture medium of Ceriporia lacerata of the present invention against diabetes.

FIG. 12 shows the food intake of Type 2 diabetic mice treated with a Ceriporia
lacerata mycelium culture extract.

FIG. 13 shows the water intake of Type 2 diabetic mice treated with a Ceriporia lacerata mycelium culture extract.

FIG. 14 shows the pattern of bodyweight increase in Type 2 diabetic mice treated with a Ceriporia lacerata mycelium culture extract.

FIG. 15 shows liver conditions of a normal mouse Type 2 diabetic mice and a mouse treated with a Ceriporia lacerata mycelium culture extract.

FIG. 16 is a graph showing blood glucose levels depending on time.

FIG. 17 is a graph showing blood glucose levels depending on time after oral administration of glucose.

FIG. 18 is a graph showing blood glucose levels after sacrifice of mice fed with a Ceriporia lacerata mycelium culture extract for 6 weeks.

FIGs. 19a and 19b are a graph and a micrograph, which show that a Ceriporia lacerata mycelium culture extract promotes adipocyte differentiation in a manner similar to insulin.

FIG. 20 is a graph showing the degree of adipocyte differentiation by a Ceriporia lacerata mycelium culture extract depending on the presence or absence of insulin.

FIGs. 21a and 21b show insulin signaling in adipocytes by a Ceriporia lacerata mycelium culture extract.

FIG. 22 is a graph showing the expression level of GLUT4 in adipocytes by a Ceriporia lacerata mycelium culture extract.

BEST MODE FOR CARRYING OUT THE INVENTION

In the present invention, there is provided a method for preparing an extract from the mycelial culture medium of Ceriporia lacerata, which comprises the steps of: culturing the mycelia of Ceriporia lacerata in a liquid, drying the culture to form powder and preparing a solvent extract from the powder, wherein a medium for culturing the mycelia of Ceriporia lacerata comprises 1-2 wt% of sugar, 0.2-1 wt% of glucose, 0.2-1 wt% of starch,
0.1-0.5 wt% of sorghum powder, 0.1-0.5 wt% of barley powder, 0.2-2 wt% of soy flour, 0.05-0.1 wt% of magnesium sulfate (MgSO₄), 0.05-0.1 wt% of monopotassium phosphate (KH₂PO₄), 0.05-0.1 wt% of dipotassium phosphate (K₂HPO₄) and 92-98 wt% of water and has a pH of 4.5-6.0.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention will be described in detail.

The present inventors have found that a marker substance having excellent effects of blocking the progression of diabetes and diabetic complications and promoting the regeneration of beta-cells is the exopolysaccharide of a *Ceriporia lacerata* mycelium culture extract, and the present inventors have developed a method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata*, which can increase the content of the exopolysaccharide, and a pharmaceutical composition for preventing or treating diabetes and diabetic complications, which contains the extract from the mycelial culture medium of *Ceriporia lacerata*, thereby completing the present invention.

Therefore, the present invention provides a method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata*, which comprises the steps of: culturing the mycelia of *Ceriporia lacerata* in a liquid, drying the culture to form powder and preparing a solvent extract from the powder, wherein a medium for culturing the mycelia of *Ceriporia lacerata* comprises 1-2 wt% of sugar, 0.2-1 wt% of glucose, 0.2-1 wt% of starch, 0.1-0.5 wt% of sorghum powder, 0.1-0.5 wt% of barley powder, 0.2-2 wt% of soy flour, 0.05-0.1 wt% of magnesium sulfate (MgSO₄), 0.05-0.1 wt% of monopotassium phosphate (KH₂PO₄), 0.05-0.1 wt% of dipotassium phosphate (K₂HPO₄) and 92-98 wt% of water and has a pH of 4.5-6.0.

The culturing is preferably carried out under a blue LED light source.

The culturing is preferably carried out at a carbon dioxide concentration of 1,000-2,000 ppm.
In a preferred embodiment of the present invention, the extract from the mycelial culture medium of *Ceriporia lacerata* can be prepared by a method comprising the following steps:

(a) culturing the mycelia of *Ceriporia lacerata* to obtain a mycelia culture of

5 *Ceriporia lacerata*;

(b) vacuum-drying or freeze-drying the culture to form powder; and

(c) subjecting the powder to extraction with one or more solvents selected from the group consisting of water, ethanol and methanol.

The liquid culture of *Ceriporia lacerata* mycelia in step (a) is performed by culturing the mycelia of *Ceriporia lacerata* in a liquid to obtain exopolysaccharide. A medium composition for the liquid culture may comprise 1-2 wt% of sugar, 0.2-1 wt% of glucose, 0.2-1 wt% of starch, 0.1-0.5 wt% of sorghum powder, 0.1-0.5 wt% of barley powder, 0.2-2 wt% of soy flour, 0.05-0.1 wt% of magnesium sulfate (MgSO₄), 0.05-0.1 wt% of monopotassium phosphate (KH₂PO₄), 0.05-0.1 wt% of dipotassium phosphate (K₂HPO₄) and 92-98 wt% of water.

Herein, the liquid culture is preferably performed at a temperature of 20 to 25°C, a pH of 4.5-6.0, an illumination intensity of 0.5 LUX, an air injection rate of 0.5-1.5 kgf/cm² and a carbon dioxide concentration of 1,000-2,000 ppm for 8-13 days using a blue LED light source. Most preferably, the liquid culture is performed under the conditions of 22°C, pH 5, an air injection rate of 1.0 kgf/cm² and a carbon dioxide concentration of 1,500 ppm for 10 days to make the content of exopolysaccharide high.

The parent strain that is used in step (a) is a strain obtained by culturing an excellent strain, stored in PDA medium at 4°C, in a shaking incubator at 25°C for 7-9 days using PDB medium in an Erlenmeyer flask. Herein, the amount of the mycelium to be inoculated is most preferably 0.5% of the solution to be incubated. Because an increase in the mycelium amount (%/100 mL) does not lead to an increase in the content of exopolysaccharide, the medium composition should have selective culture conditions which are not based on the best
nutrient ratio and environmental conditions for the growth of mycelia, but the conditions for
maximizing the content of exopolysaccharide.

The culture solution is separated into mycelia and an aqueous solution. The
separation is performed by removing mycelia from the culture solution using a centrifuge and
repeatedly purifying the remaining solution using a multi-sheet filter press and a vibrating
membrane separator (PALLSEP), followed by irradiation with UV rays for 1 minute. Also,
the culture solution should be sealed and stored after the removal of oxygen. This is
because the presence of mycelia in the culture solution results in the change in the content of
the active ingredient due to the growth of the mycelia.

In step (b), the mycelium culture solution prepared in step (a) is vacuum-dried or
freeze-dried to form powder. When the drying is carried out at high temperature, a
significant portion of the active ingredient can be lost. For this reason, the drying is carried
out at a temperature of 40°C or lower, preferably 30°C or lower, for 48-96 hours. In
addition, for the drying in step (b), a vacuum freeze dryer is preferably used compared to a
vacuum dryer in which a relatively high evaporation temperature is set, in order to minimize
the change in the content of the active substance.

In step (c), the dried mycelial culture obtained in step (b) is subjected to extraction
with a solvent, thereby preparing an extract from the mycelial culture medium of Ceriporia
lacerata containing exopolysaccharide according to the present invention.

In step (c), 5 g of the dried powder is sufficiently suspended in 100 mL of distilled
water, and the suspension is centrifuged at 8,000 rpm for 20 minutes. A 2-3-fold amount of
cold alcohol is added to the supernatant, and the solution was placed in a refrigerator at 4°C
and allowed to stand for 12 hours.

The supernatant in the solution which had been allowed to stand is centrifuged again
at 8,000 rpm for 20 minutes, and the precipitate is recovered, thereby preparing crude
exopolysaccharide. The extract is preferably vacuum-freeze-dried at 30°C or lower.

The extract from the mycelial culture medium of Ceriporia lacerata prepared
according to the present invention as described above has significantly high contents of active
ingredients effective for the treatment of steroid-induced diabetes, and thus has an excellent
effect of arresting and treating diabetes-related diseases and complications. More
specifically, the extract from the mycelial culture medium of Ceriporia lacerata according to
the present invention contains exopolysaccharide having anti-diabetic effects in an amount of
0.3 ± 0.03%/1L. In addition, the dried extract contains the exopolysaccharide in an amount
of 5.00 ± 0.02%/100 g.

The present invention also provides a pharmaceutical composition for preventing or
treating diabetes and diabetic complications, which contains, as an active ingredient, an
extract from the mycelial culture medium of Ceriporia lacerata prepared by the above
method.

The diabetic diseases may be Type 2 diabetes.

The diabetic complications may be selected from the group consisting of
hyperglycemia, atherosclerosis, microangiopathy, diabetic retinopathy and kidney disease.

The pharmaceutical composition which contains, as an active ingredient, the extract
from the mycelial culture medium of Ceriporia lacerata prepared by the above method, may
further contain a suitable carrier, excipient or diluent known in the art.

For example, a powder formulation may be prepared by mixing 200 mg of the
extract, 100 mg of rice powder and 10 mg of talc and packing the mixture into an airtight bag.

For example, a tablet formulation is prepared by mixing 100 mg of the extract, 50
mg of rice powder, 10 mg of lactose and 2 mg of magnesium stearate and compressing the
mixture into a tablet.

For example, a liquid formulation is prepared by mixing 100 mL of the extract, 5 g
of isomerized sugar, a suitable amount of pine fragrance and a suitable amount of a
preservative and packing the mixture in a brown bottle. In this case, the resultant material
of step (a) may be used directly instead of the extract.

Hereinafter, the present invention will be described in further detail with reference to
examples. It is to be understood, however, that these examples are for illustrative purposes
and are not intended to limit the scope of the present invention.
Examples

1. Preparation of *Ceriporia lacerata* culture extract

1-1. Preparation of *Ceriporia lacerata* culture

*Ceriporia lacerata* was isolated from *Quercus serrata* and subcultured to obtain a parent strain which was then freeze-stored at -80°C. The stored strain was subcultured 2-3 times in PDA medium (87 plastic bulbs), and then a sufficient amount of a complete strain was selected and stored in a refrigerator at 4°C until use. In addition, 600 mL of PDB medium was placed in an Erlenmeyer flask, and then a PDA culture strain was added thereto and shake-cultured for 8 days. Also, a liquid culture medium comprising 1.5 wt% of sugar, 0.5 wt% of glucose, 0.5 wt% of potato starch, 0.25 wt% of soy flour, 0.25 wt% of sorghum powder, 0.05 wt% of magnesium sulfate (MgSO₄), 0.05 wt% of monopotassium phosphate (KH₂PO₄), 0.05 wt% of dipotassium phosphate (K₂HPO₄) and 96.85 wt% of water was sterilized for 20 minutes in a 800-L fermenter at 121°C and at an air injection rate of 1.5 kgf/cm², and then cooled to 23°C, after which it was inoculated with 600 mL of the PDB culture strain to be used as a starter. Then, *Ceriporia lacerata* mycelia were liquid-cultured in the medium at a temperature of 23°C, an aeration rate of 0.5-1.5 kgf/cm² and a carbon dioxide concentration of 1,000-2,000 ppm for 10 days, thereby preparing a *Ceriporia lacerata* mycelium culture.

1-2. Preparation of *Ceriporia lacerata* culture extract

The prepared *Ceriporia lacerata* mycelium culture was freeze-dried using a vacuum freeze dryer at a temperature of 25°C for 72 hours to form powder. 5 g of the dried powder was suspended sufficiently in 100 mL of distilled water and then centrifuged at 8,000 rpm for 20 minutes, and a 2-3-fold amount of cold alcohol was added to the supernatant. The resulting solution was placed in a refrigerator at 4°C and allowed to stand for 12 hours. The supernatant in the solution which had been allowed to stand was centrifuged again at 8,000
rpm for 20 minutes, and the precipitate was recovered, thereby extracting a crude exopolysaccharide. The crude exopolysaccharide was dried in a freeze dryer for 72 hours, thereby obtaining a complete exopolysaccharide.

2. Optimization of conditions for liquid culture of Ceriporia lacerata

2-1. Experimental method

2-1-1. Culture conditions

For optimization of Ceriporia lacerata liquid culture conditions according to shaking flask culture conditions, physiochemical characteristics depending on kinds and concentrations of carbohydrates and micronutrients and the contents of the mycelium and exopolysaccharide were measured. For evaluation of characteristics, carbon sources including glucose, sucrose, lactose, fructose and galactose were used at a concentration of 3-5%, and nitrogen sources including tryptone, yeast extract, soy flour, L-glutamic acid, ammonium persulfate, malt extract and peptone were used at a concentration of 0.25%. For micronutrients, KH₂PO₄, MgSO₄, ZnSO₄, CuSO₄, FeSO₄ and CaCl₂ were used at a concentration of 0.1-0.5%. Culture was carried out in a 1,000 mL Erlenmeyer flask in a total volume of 800 mL at 25°C and 120 rpm for 8 days, followed by analysis. In a 5 L jar fermenter, culture was carried out in a total volume of 3 L for various periods of time (days), thereby analyzing physicochemical characteristics depending on culture time.

2-1-2. Measurement of pH, acidity and Brix

pH was measured with a pH meter, and Brix was measured using an electronic Brix meter. Acidity was measured by measuring the pH of 10 mL of a culture with a pH meter, adding 0.1 N NaOH until the pH of the culture reached 8.3, and then comparing the used amount of 0.1 N NaOH and the content of tartaric acid.
A culture was centrifuged at 12,000 xg for 20 minutes, and the precipitate was washed three times with distilled water and then filtered. The filtrate was freeze-dried and weighed, thereby measuring the production of mycelia. A culture was centrifuged at 12,000 xg for 20 minutes, and a 2-fold volume of cold isopropyl alcohol was added to the supernatant. Then, the solution was incubated at 4°C overnight and centrifuged again at 12,000 xg for 20 minutes. The precipitate was dissolved in distilled water and freeze-dried and weighed, thereby measuring the production of mycelia.

2-1-4. Measurement of tyrosine content and protease, α-amylase and fibrinolytic enzyme activities

In order to measure the production of peptide in a Ceriporia lacerata culture, the content of tyrosine in the culture was measured using folin phenol reagent. Specifically, 0.7 mL of 0.44 M TCA (trichloroacetic acid) was added to 0.7 mL of the culture and then incubated at 37°C for 30 minutes. The solution was centrifuged at 15,000 rpm for 10 minutes, and the precipitate was removed. 2.5 mL of 0.55 M Na₂CO₃ and 0.5 mL of phenol reagent were sequentially added to 1 mL of the collected supernatant and was allowed to react in a water bath at 37°C for 30 minutes. The reaction solution was cooled to room temperature, and then the absorbance at 660 nm was measured using a spectrophotometer (UNION, Kontron Instruments, France).

The titers of α-amylase and protease in the culture were measured based on α-amylase and protease activities. As a substrate for α-amylase, 1 mL of 1% soluble starch (0.02 M phosphate buffer, pH 7.0) was used. 1 mL of a pre-prepared enzyme solution was added to the substrate and allowed to react at 37°C for 30 minutes, and the reaction was stopped with 10 mL of 1 M acetic acid. 2 mL of an iodide solution (0.005% I₂ + 0.05% KI) was added to the reaction solution, and the absorbance at 660 nm was measured. A decrease in blank OD of 10% was taken as 1 unit, and the result was expressed in terms of 1 g of the sample. As a control blank, a deactivated solution obtained by boiling a pre-prepared enzyme solution at 100°C for 30 minutes was used.
To measure the activity of protease, 0.35 mL of 0.6% casein solution as a substrate and 0.35 mL of the enzyme solution were placed in an e-tube and allowed to react in a water bath 37°C for 10 minutes, and then 0.7 mL of 0.44 M TCA solution was added thereto to stop the reaction. The reaction solution was allowed to stand at 37°C for 30 minutes. The reaction solution was centrifuged at 15,000 rpm for 15 minutes, and then 2.5 mL of 0.55 M Na₂CO₃ and 0.5 mL of 3-fold-diluted folin reagent were added to 1 mL of the filtrate and then allowed to react at 37°C for 30 minutes. Then, the absorbance at 660 nm was measured. The enzyme amount that liberates 1 g of tyrosine for 1 minute under such reaction conditions was taken as 1 unit.

The activity of fibrinolytic enzyme was measured using the Astrup and Müller method that is a kind of fibrin plate method. Specifically, 10 mL of a solution of 0.5% fibrinogen in 0.067 M sodium phosphate buffer (pH 7.4) was added to a 9 cm-diameter Petri dish. To the solution, 0.1 mL of a solution of thrombin (100 units/mL) in 0.067 M sodium phosphate buffer (pH 7.4) was added and mixed rapidly, and the mixture was allowed to stand at room temperature for 30 minutes so as to be solidified. 20 L of the culture was dropped onto each marked position of the Fibrin plate and allowed to react at 37°C for 2 hours, and then the enzyme activity was determined by the dissolution area. The standard curve of standard plasmin enzyme activity was plotted, and the fibrinolytic enzyme activity (%) of the culture was expressed as plasmin units in comparison with the standard curve. As a control, the purified fibrinolytic enzyme plasmin (5 units/mL) was used.

2-1-5. Measurement of sugar and protein contents

The sugar content was measured by the phenol-sulfuric acid method. Specifically, the sugar content was determined by adding 25 µL of 80% phenol to 1 mL of a sample diluted at various concentrations, adding 2.5 mL of sulfuric acid thereto, cooling the mixture to room temperature, and measuring the absorbance at 425 nm. The protein content was measured by the BCA method using bovine serum albumin as a standard.
2-1-6. Measurement of molecular weight of exopolysaccharide (EPS) by GPC

The dry viscous substance was dissolved in 0.1 M Na₂SO₄/0.05 M NaN₃ solution (adjusted to pH 4 with glacial acetic acid) at a concentration of 1%, and the solution was centrifuged. The supernatant was filtered through a 0.45 μm syringe filter and analyzed by GPC (Gel Permeation Chromatography) under the following conditions: detector: RI; GPC column: Shodex SB 805 HQ (Japan); mobile phase: 0.1 M Na₂SO₄/0.05 M NaN₃ (adjusted to pH 4 with glacial acetic acid); and flow rate: 1.0 mL/min. A standard curve was plotted using dextran (American Polymer Corporation, USA) having different molecular weights (130, 400, 770 and 1,200 kDa), and the molecular weight of EPS was measured using a refractive index meter (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determination of Molecular weight</td>
</tr>
<tr>
<td>HPLC System</td>
</tr>
<tr>
<td>Column</td>
</tr>
<tr>
<td>Mobile phase</td>
</tr>
<tr>
<td>Flow rate</td>
</tr>
<tr>
<td>Detector</td>
</tr>
</tbody>
</table>

2-2. Experimental results

2-2-1. Sequencing of *Ceriporia lacerata* ITS-5.8S rDNA

ITS-5.8S rDNA sequencing of the *Ceriporia lacerata* strain showed that the strain has a sequence homology of 92% with *Ceriporia lacerata* FJ462746 (FIG. 1).

2-2-2. Evaluation of physiochemical characteristics depending on the kind and concentration of sugar

To evaluate physiochemical characteristics depending on the kind of sugar, each of five kinds of sugar (lactose, sucrose, glucose, fructose and galactose) was added to a culture
at a concentration of 3% and incubated for 7 days. As a result, it was found that there was no significant difference in pH among the sugars, and residual sugar was slightly low in the case of glucose. Also, it was shown that since the shape and size of mycelial pellets changed depending on the kind of carbon source, the influence of the carbon source on mycelial growth is very significant. Also, the mycelium and EPS contents were higher in the order of glucose, fructose and sucrose. Glucose was added at various concentrations up to 15%, and the mycelium and EPS content were measured. As a result, the contents increased in a concentration-dependent manner up to 3%, and did not significantly change over 3%. Thus, a glucose concentration of 3% was chosen as an optimal condition.

2-2-3. Evaluation of physiochemical characteristics depending on the kind and concentration of nitrogen sources

In order to evaluate physiochemical characteristics depending on the kind of nitrogen sources, each of 7 kinds of nitrogen sources (tryptone, yeast extract, soy flour, L-glutamic acid, ammonium persulfate, malt extract and peptone) was added at a concentration of 3% and incubated for 7 days. The mycelium content was the highest in the case of soy flour, and the EPS content was high in the cases of tryptone, yeast extract, soy flour and L-glutamic acid at similar levels. However, in economical and industrial terms, soy flour showing high mycelium and EPS contents was selected as a nitrogen source. When soy flour was added at a concentration of 0.25%, there was no great change in the pH of the culture, and the Brix of the culture increased in a manner dependent on the concentration of soy flour. The tyrosine content of the culture also increased in a manner dependent on the concentration of soy flour, and the protease and alpha-amylase activities were high at a soy flour concentration of 2-3% and slightly decreased at higher soy flour concentrations. However, the fibrinolytic enzyme activity increased in a manner dependent on the concentration of soy flour. The mycelium and EPS contents showed a tendency to increase up to a soy flour concentration of 3% and did not significantly change at a soy flour concentration of more than 3%, like the case of the carbon source concentration. Thus, the
optimal soy flour concentration was chosen to be 3%. The contents of sugar and protein in the EPS of the culture cultured in the presence of soy flour were measured. As a result, the sugar content was about 40% and the protein was about 33%, suggesting that the EPS is a polysaccharide composed of sugar bonded with protein. Table 2 below shows chemical characteristics and enzyme activities depending on the content of soy flour, and Table 3 below shows the composition of exopolysaccharide depending on the content of soy flour.

Table 2

<table>
<thead>
<tr>
<th>Soy flour</th>
<th>pH</th>
<th>Brix</th>
<th>Tyrosine content (mg%)</th>
<th>Protease activity (unit/mL)</th>
<th>α-amylase activity (unit/mL)</th>
<th>Fibrinolytic enzyme activity (unit/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>4.25</td>
<td>3.0</td>
<td>7.46</td>
<td>0.04</td>
<td>5.77</td>
<td>0.60</td>
</tr>
<tr>
<td>1</td>
<td>4.47</td>
<td>3.9</td>
<td>30.12</td>
<td>0.17</td>
<td>3.64</td>
<td>0.60</td>
</tr>
<tr>
<td>2</td>
<td>4.59</td>
<td>4.8</td>
<td>48.37</td>
<td>0.75</td>
<td>6.78</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>4.74</td>
<td>5.5</td>
<td>64.21</td>
<td>1.02</td>
<td>1.68</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>4.91</td>
<td>6.3</td>
<td>69.39</td>
<td>0.94</td>
<td>1.10</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>4.84</td>
<td>7.0</td>
<td>82.32</td>
<td>0.75</td>
<td>0.60</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Soy flour</th>
<th>Total sugar content (%)</th>
<th>Total protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>50.24±1.06</td>
<td>33.13±0.30</td>
</tr>
<tr>
<td>1</td>
<td>47.94±0.15</td>
<td>32.49±1.01</td>
</tr>
<tr>
<td>2</td>
<td>42.78±0.08</td>
<td>37.91±0.01</td>
</tr>
<tr>
<td>3</td>
<td>40.57±0.68</td>
<td>33.34±1.41</td>
</tr>
<tr>
<td>4</td>
<td>38.46±0.09</td>
<td>34.34±0.20</td>
</tr>
<tr>
<td>5</td>
<td>32.63±0.30</td>
<td>36.20±0.81</td>
</tr>
</tbody>
</table>

2-2-4. Evaluation of physicochemical characteristics depending on the kind and concentration of micronutrients
In order to evaluate physicochemical characteristics depending on the kind of micronutrients, each of five kinds of micronutrients (KH$_2$PO$_4$, MgSO$_4$, ZnSO$_4$, CuSO$_4$, FeSO$_4$, and CaCl$_2$) was added at a concentration of 0.5% and incubated for 7 days. As a result, the mycelium content was the highest in the case of CuSO$_4$, but the EPS production was the highest in the case of MgSO$_4$, and thus MgSO$_4$ was selected as a micronutrient. In addition, the micronutrient was added at various concentration of 0-0.25%, and the mycelium and EPS contents were measured. As a result, the contents increased up to a micronutrient concentration of 0.15%, but did not significantly change at a concentration of 0.22%. Thus, the optimal concentration of the micronutrient was chosen to be 0.15%.

2-2-5. Evaluation of physicochemical characteristics depending on culture time (5 L jar fermenter)

Culture was performed in a 5-L jar fermenter using the selected optimal medium while the mycelium and EPS contents were measured at various points of time. As a result, the mycelium content did not significantly change up to 8 days of culture, but showed a decrease after 10 days of culture, and the EPS content showed a tendency to increase after 8 days, but this increase was not significant. Thus, the optimal culture time was chosen to be 8 days.

2-2-6. Evaluation of exopolysaccharide (EPS) characteristics

EPS was subjected to secondary purification and treated with protease, and the protein and sugar contents thereof were measured. As a result, purification of EPS showed a decrease in the sugar content and an increase in the protein. In addition, the molecular weight of EPS was measured by GPC, and as a result, the molecular weight of EPS was about 120 kDa and was slightly decreased by treatment with protease.

3. Verification of anti-diabetic effects of CLD and EPS in Type 2 diabetes model

3-1. Experimental method

17
3-1-1. *Ceriporia lacerata* culture extract

The *Ceriporia lacerata* culture extract used in this experiment was one prepared in the above Example 1.

5  3-1-2. Method for measurement of mycelium and exopolysaccharide contents

The freeze-dried culture was prepared into a 10% solution which was then centrifuged at 8,000 rpm for 20 minutes, and the supernatant was isolated. A 4-fold volume of cold isopropyl alcohol was added to the isolated supernatant and incubated at 4°C overnight. Then, the solution was centrifuged again at 10,000 rpm for 20 minutes, and the precipitate was collected and weighed to determine the content of the marker substance exopolysaccharide. It was shown that the major component of the *Ceriporia lacerata* culture was a carbohydrate. The crude carbohydrate content was about 79% and the crude protein content was about 15%.

15  3-1-3. Experimental animal and experimental design

In order to examine the blood glucose lowering effects of the *Ceriporia lacerata* culture and exopolysaccharide, C57BL/KsJ (BL/Js) homozygous diabetic (db/db) mice (SPF) as a typical Type 2 diabetes animal model were used in this experiment. *db/db* mice are animals in which diabetes is caused by a point mutation in the leptin receptor gene *Lepr* of chromosome 4. In the animals, as the leptin receptor decreases, the signal transduction ability decreases, and thus the blood glucose level increases. The animals are recognized as an insulin-independent diabetes model and suitable for the evaluation and comparison of experimental results on the basis of raw data. The *db/db* mice used in this study were 6-week-old male mice weighing about 30-40 g, and these mice were produced in Japan SLC Inc. and obtained from Central Laboratory Animal (Korea). The mice were acclimated for about 7 days, and the weight and blood glucose level thereof were measured. 30 healthy animals suitable for experimentation and having no abnormal general conditions were selected.
The experimental animals were divided according to a randomized block design into a negative control group, an exopolysaccharide low-dose group (150 mg/kg), an exopolysaccharide high-dose group (300 mg/kg) and a positive control group (metformin-300 mg/kg) such that the blood glucose level and weight are equal among the groups. These mice were reared for 6 weeks. In addition, normal and control groups, each consisting of 6 animals, were kept for 6 weeks under the same conditions. All test substances and positive control substances were orally administered at the same point of time every day, and the normal and negative control groups were orally administered with water (FIG. 11). During the rearing period of 6 week, a change in the weight was measured once weekly. In order to examine a change in the blood glucose level, the tail vein blood glucose level was measured using ACCU-CHEK Sensor (Germany) once weekly after 12-hr fasting. The experimental animals were fed with a commercially available animal solid feed (Samtaco Co. Ltd., Korea) and allowed to access water ad libitum. The animals were reared under the conditions of temperature of 23 ± 3°C and relative humidity of 50 ± 10% with a 12-hr light/12-hr dark cycle (lighting: 8 a.m. to 8 p.m.).

3-1-4. Oral glucose tolerance test (OGTT)

The animals were fasted for 12 hours or more, and the fasting blood glucose level was measured. Each of samples obtained by dissolving each of the Ceriporia lacerata powder and EPS in distilled water at various concentrations was administered orally to five animals of each group. The control group was administered with the same amount of saline. Next, the groups other than the control group were orally administered with 40% glucose at a dose of 2 g/kg bw, and blood was collected from the tail vein at 30, 60, 90 and 120 minutes after glucose administration to observe a change in the blood glucose level. The increase in the blood glucose level at each point of time was calculated to plot a blood glucose curve.

3-1-5. Sacrifice of experimental animals and sampling
Blood glucose levels should be measured in tail veins after 12-hr fasting, and blood for biochemical analysis should be collected after fasting of 12 hours or more. Thus, the sacrifice of the experimental animals was performed 2 days after 6th blood glucose level measurement at 6 weeks after the start of rearing.

For sacrifice of the experimental animals, all the animals were fasted for 12 hours and anesthetized with ether. Blood was taken from the saphenous vein and placed in a tube for serum separation. Then, the blood was centrifuged at 3,000 rpm for 20 minutes to obtain serum which was to be used as a sample for analysis of biochemical markers. Immediately after sacrifice, the liver, abdominal fat and the like were extracted from all the animals, weighed, fast-frozen at -80°C and stored until use. Portions of the extracted liver and pancreas were fixed in 4% paraformaldehyde solution and subjected to histological analysis.

3-1-6. Serum c-peptide, insulin and leptin levels

The serum c-peptide, insulin and leptin levels that are blood glucose-related functional indices were measured from the serum (collected from the saphenous vein) using double antibody C-peptide (DPC, USA), an insulin RIA kit (DPC, USA), and a mouse leptin RIA kit (LINCO, USA), respectively, by a radioimmunoassay.

3-2. Experimental results

3-2-1. Changes in food intake and weight

FIGs. 14, 15 and 16 show the changes in weight, food intake and water intake of the experimental animals for 6 weeks. The initial bodyweights of the diabetes control group and the EPS-administered group were similar (about 32 g). Also, there was no significant difference in bodyweight after 6 weeks between the diabetes control group and the test groups, and the bodyweight showed a tendency to increase throughout the experimental period. The feed and water intakes were higher in the diabetes control group than in the
normal control group, and the water and feed intakes of the positive control group MET300 were significantly lower than those of other groups.

3-2-2. Organ weight

The weights of liver, kidney, spleen, kidney fat and abdominal fat of the experimental animals were measured, and the results of the measurement are shown in Table 4 below.

<table>
<thead>
<tr>
<th></th>
<th>Liver (W/BW)</th>
<th>Kidney (W/BW)</th>
<th>Spleen (W/BW)</th>
<th>Kidney fat (W/BW)</th>
<th>Abdominal fat (W/BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.94 ± 0.14</td>
<td>0.29 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>0.12 ± 0.04</td>
<td>0.43 ± 0.17</td>
</tr>
<tr>
<td>DM</td>
<td>2.89 ± 0.04</td>
<td>0.41 ± 0.06</td>
<td>0.04 ± 0.02</td>
<td>0.82 ± 0.17</td>
<td>2.34 ± 0.28</td>
</tr>
<tr>
<td>DM-EX0150</td>
<td>2.50 ± 0.29</td>
<td>0.39 ± 0.05</td>
<td>0.06 ± 0.03</td>
<td>0.63 ± 0.12</td>
<td>2.48 ± 0.14</td>
</tr>
<tr>
<td>DM-EX0300</td>
<td>2.65 ± 0.05</td>
<td>0.40 ± 0.04</td>
<td>0.05 ± 0.02</td>
<td>0.55 ± 0.17</td>
<td>2.55 ± 0.15</td>
</tr>
<tr>
<td>DM-MET300</td>
<td>2.60 ± 0.19</td>
<td>0.39 ± 0.03</td>
<td>0.03 ± 0.02</td>
<td>0.89 ± 0.13</td>
<td>2.564 ± 0.33</td>
</tr>
<tr>
<td>DM-ALL300</td>
<td>2.50 ± 0.27</td>
<td>0.40 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>0.48 ± 0.12</td>
<td>2.41 ± 0.21</td>
</tr>
</tbody>
</table>

The liver weight showed a tendency to increase rapidly in the diabetes-induced group and showed a tendency to significantly decrease in the groups administered with the sample. These results are consistent with the report that fat is accumulated in the liver upon the induction of diabetes to increase the liver volume. With respect to the kidney, it is known that the kidney volume increases with an increase in glomerular filtration rate in the initial stage of development of diabetes. In this study, the weight of the kidney also showed a tendency to increase, but this increase was not significant. In addition, the weight of the spleen did not significantly differ among the groups. The weight of the kidney fat increased rapidly in the diabetes-induced group, but was significantly decreased by administration of
EPS and *Ceriporia lacerata* powder. In addition, the weight of the abdominal fat did not significantly differ among the groups.

3-2-3. Effect on change in blood glucose level

FIG. 16 shows the results of measuring the change in blood glucose levels after 12-hr fasting. The initial blood glucose level was similar (about 150 mg/dL) among the groups, but started to increase slightly from one week after administration of the sample. After 3 weeks, the blood glucose level increased rapidly so that the diabetes control group showed a blood glucose level of about 400 mg/dL, whereas the metformin group showed no increase in the blood glucose level. After that, the blood glucose level continued to increase, but the groups administered with EPS and *Ceriporia lacerata* powder showed a significant decrease in the blood glucose level compared to the diabetes control group.

3-2-4. Oral glucose tolerance

The oral glucose tolerance of EPS and *Ceriporia lacerata* powder was measured at 6 week of sample administration, and the results of the measurement are shown in FIG. 17. The diabetes control group showed a blood glucose level of 600 mg/dL which is the highest value measurable by the blood glucose meter, and it maintained high blood glucose levels throughout the glucose tolerance test. On the other hand, the group administered with EPS and *Ceriporia lacerata* powder showed an initial fasting blood glucose level of 500 mg/dL which was significantly lower than that of the diabetes control group. However, the blood glucose level of the group administered with EPS and *Ceriporia lacerata* powder increased gradually with the passage of time and was 600 mg/dL (the highest value measurable by the blood glucose meter) after 30 minutes. Thereafter, the blood glucose level gradually decreased, and after 180 minutes, the blood glucose level was restored to 520 mg/dL which was similar to the initial blood glucose level.

3-2-5. Blood glucose level
The blood glucose levels of the animals sacrificed after 6-week oral administration of EPS were measured. As a result, it was shown that the blood glucose level of the DM group increased to about 900 mg/dL, whereas the blood glucose levels of the groups administered with the sample decreased in a manner dependent on the concentration of the sample. Specifically, the blood glucose level of the EPS 300 group decreased to about 700 mg/dL, suggesting that EPS plays a positive role in lowering blood glucose levels.

3-2-6. Effect on serum lipid levels

The serum lipid levels of the animals sacrificed after 6-week oral administration of EPS were measured. As a result, it was shown that the total cholesterol and triglyceride levels were about two times higher in the DM group than in the NC group, but were significantly lowered in the group administered with EPS. In addition, the group administered with EPS showed a significant increase in the HDL cholesterol and a significant decrease in the LDL-cholesterol level, indicating that EPS is a substance that reduces serum lipid levels in Type 2 diabetes models without changing the bodyweight.

3-2-7. Effects on insulin, C-peptide and leptin levels

The serum insulin, C-peptide and leptin levels of the animals sacrificed after 6-week oral administration of EPS were measured. As a result, it was shown that the insulin level did not significantly differ among the groups, and the C-peptide level was higher in the sample-administered groups than in the DM group, suggesting that EPS activates insulin secretion in the pancreas. In addition, the leptin level also increased in the sample-administered groups, suggesting that EPS plays a positive role in insulin resistance.

4. Effect of exopolysaccharide on insulin signal transduction in 3T3-L1 cells

4-1. Experimental method

4-1-1. Cell culture
3T3-L1 fibroblasts that are preadipocytes have well established biological properties and differentiate into adipocytes when they are cultured under suitable conditions. These cells are used in studies on lipolysis inhibition or lipogenesis in the metabolic process of adipocytes. In addition, adipocytes that are insulin target cells are frequently used in studies on insulin signal transduction. The 3T3-L1 fibroblasts used in the experiment were obtained from the Korean Cell Line Bank and were cultured in Dulbecco's Modified Eagle's Medium (DMEM, GibcoBRL) (containing 10% fetal bovine serum (FBS, GibcoBRL), 200 mM glutaMAX (GibcoBRL), penicillin (10,000 units/mL, Sigma), streptomycin (10 mg/mL, Sigma) under the conditions of 37°C and 10% CO₂ while the medium was replaced with high-glucose medium at 3-day intervals. When the 3T3-L1 fibroblasts reached a confluence of 60-80%, the cultured cells were washed with Dulbecco's phosphate buffered saline (PBS, GibcoBRL). These cells were subcultured in a 75 cm² flask containing 500 µL of 2.5% trypsin (GibcoBRL) at 37°C for 5 minutes, after which the cells were detached from the flask. The detached cells were transferred into a fresh flask containing 15 mL of a high-glucose DMEM medium supplemented with 10% FBS.

4-1-2. Glucose uptake into adipocytes

3T3-L1 fibroblasts were cultured to confluence in the same manner as the above cell culture. 2 days after confluence, the cells were cultured for 3 days in a high-glucose DMEM medium containing 0.5 mM 3-isobutyl-1-methyl-xanthine (IBMX, Sigma), 25 µM dexamethasone (DEX, Sigma) and insulin (Sigma) which are differentiation inducers, and the medium was replaced with a fresh medium at 2-day intervals, whereby the cells were converted to adipocytes. Between 10 days and 15 days during which the preadipocytes were completely converted to adipocytes, a glucose uptake test was performed. In the glucose uptake test that reflects insulin sensitivity, the 3T3-L1 cells that had been completely converted to adipocytes were treated with 2.5% trypsin, and then seeded into a 24-well plate at a concentration of 20×10⁴ cells/mL as counted by a hemacytometer. The medium was replaced with a low-glucose DMEM medium to starve the cells. The adipocytes in the well
plate were washed with PBS and then incubated with a HEPES solution containing 0.1% bovine serum albumin (BSA, Roche), each of 1 µg/mL and 10 µg/mL of the *Ceriporia lacerata* culture extract and insulin at 37°C for 1.5 hours. Then, 10 Ci/mL of the glucose analogue, 2-deoxy-D-[3H] glucose (2-DG), was added to the cells which were then incubated at 37°C for 10 minutes. After 10 minutes, the cells were washed five times with PBS, lysed with 1N NaOH and neutralized with 1N HCl. The amount of 3H uptake into the cells was measured with a beta-counter (Tri-Carb 2100TR, Packard Bioscince, IL) for 5 minutes. In order to eliminate non-specific glucose uptake, cells incubated with cytochalasin B (Sigma) that inhibits the activity of glucose transporter 4 (GLUT4) was also measured. In the process of examining whether an insulin-sensitive component is contained in the *Ceriporia lacerata* culture, low-concentration insulin was chosen to be 1 ng/mL and high-concentration insulin was chosen to be 25 ng/mL, because insulin uptake into the cells was the highest at an insulin concentration of 50 ng/mL. In this experiment, 1 ng/mL of insulin together with a *Cordyceps militaris* fraction was added to the 3T3-L1 adipocytes which were then incubated for 1.5 hours. And then, the glucose uptake level into the cells was compared to that in 1 ng/mL of insulin addition. All the experiments were repeated three times. In order to eliminate the case in which an isolated substance acts as a detergent regardless of the action of insulin to destroy the cell membrane so as to increase glucose uptake, the glucose uptake of the isolated substance was measured together with a substance isolated at an insulin level of 0 ng/mL, and when the measured value was higher than the basal value, it was considered not to act as an insulin-sensitive component, even it increased glucose uptake.

4-1-3. Examination of expression of protein involved in insulin signaling system

For this experiment, 3T3-L1 adipocytes were treated with 2.5% trypsin and transferred into a 24-well plate. 24 hours before the experiment, the cells were starved by replacing the medium with a low-glucose Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Then, the cells were washed with PBS and incubated with HEPES containing the *Ceriporia lacerata* culture extract and 1 ng/mL of insulin at 37°C.
for 1 hour. Then, the cells were detached from the well plate on ice using an RIPA buffer containing 50 unit aprotinin, 1 mM Na<sub>4</sub>VO<sub>4</sub>, and 1 mM PMSF. The detached cells were centrifuged at 10,000 rpm for 20 minutes at 4°C. After centrifugation, the lower layer solution was diluted with an Laemmli sample buffer, electrophoresed by SDS-PAGE and then transferred to a nitrocellulose membrane. The membrane was analyzed by Western blot using rabbit GLUT4 antibody (Chemicon, Temecula, CA), IR, PI3-Kinase, Akt, MAPK, AMPK antibody, and then the optical density was determined by a laser densitometer.

4-2. Experimental results

4-2-1. Insulin sensitivity

Insulin is a therapeutic agent which is most frequently used for treatment of Type 1 diabetes and Type 2 diabetes. Insulin is a factor that promotes adipocyte differentiation, and large amounts of adipocytes are produced by action of insulin. It was shown that the EPS of the Ceriporia lacerata culture extract promoted adipocyte differentiation in a concentration-dependent manner. It was found that the EPS is a natural material that can substitute for insulin.

4-2-2. Glucose uptake

3T3-L1 fibroblasts that are preadipocytes have well established biological properties and differentiate into adipocytes when they are cultured under suitable conditions. Thus, these cells are used in studies on lipolysis inhibition or lipogenesis in the metabolic process of adipocytes. In this experiment, based on the fact that inducers such as insulin rapidly increase enzyme activity to promote differentiation, whether an insulin-sensitive component is present was examined. Specifically, 3T3-L1 adipocytes converted from 3T3-L1 fibroblasts by adding differentiation inducers such as insulin, IBMX and dexamethasone thereto were used in the experiment. Glucose uptake into the cells was determined by measuring the amount of the glucose analogue, 2-deoxy-D-[3H]-glucose, transferred into the cells by the glucose transporter GLUT4. For the glucose uptake test, 3T3-L1 adipocytes
were starved with low-glucose DMEM 24 hours before the experiment and treated with HEPES and EPS. As a result, the glucose uptake into the cells by EPS could be seen. It was found that EPS acts as an insulin-sensitive component to increase glucose uptake in a concentration-dependent manner, and this increase in glucose uptake was higher than the control basal value.

4-2-3. Expression of protein involved on insulin signal transduction

The intracellular signal transduction of insulin is involved in various complex processes. In the mechanism in which insulin acts on target cells, insulin shows various actions by binding to insulin receptor in the plasma membrane. Specifically, the insulin receptor is composed of an α-subunit and a β-subunit, and the action of insulin begins when insulin in blood binds to the α-subunit of the insulin receptor of target cells. The activated α-subunit activates tyrosine kinase of the β-subunit in the cell membrane. It is generally thought that the tyrosine kinase activity of the β-subunit is the initial stage of insulin action, which is necessary for many physiological actions of insulin.

When the tyrosine kinase of the β-subunit is activated, it phosphorylates IRS-1, IRS-2, IRS-3, IRS-4, Shc, p60 and the like which are proteins involved in the insulin signaling process, and then insulin signal transduction occurs through several down-signaling pathways with complex interactions. Among them, the tyrosine phosphorylation of IRS results in the activation of phosphatidylinositol 3-kinase (PI3-Kinase).

PI3-kinase is a heterodimer composed of a 110-kDa catalytic subunit and an 85-kDa regulatory subunit. IRS-1 and IRS-2 bind to p85 subunits of PI3-Kinase, and then activate p110 subunits to convert phosphatidylinositol-4,5-biphosphate to phosphatidylinositol-3,4,5-triphosphate. These phosphoinositides are thought to be signaling substances that play important roles in the biological action of various growth-stimulating factors, the exact function of each of the phosphoinositides in hormone signaling processes is not yet known. Through this PI3-Kinase pathway, p70 S6 kinase downstream thereof is activated, and kinases and various proteins undergo phosphorylation and dephosphorylation signaling
processes. The activation of PI3-Kinase is important in many actions following insulin stimulation, including glucose movement, lipolysis inhibition, glycogen synthesis, protein synthesis and mitogenesis, but the relationship of PI3-Kinase with the occurrence of these responses is yet unclear.

Meanwhile, it is not thought that tyrosine kinase activation and tyrosine phosphorylation are always necessary for the action of insulin in all cells in all cases. It is known that there are signaling processes occurring regardless of tyrosine phosphorylation, and one of these signaling processes is a G protein signaling process. Among various G proteins, the GTP-binding protein Ras that induces various biological signals has been most actively studied. Ras is regulated by SOS and GTPase activating protein (GAP), and the activation of Ras sequentially induces the activation of MAP kinase kinase (MAPKK), Raf-1 MAPK/E kinase (MAPKK or MEK), p90 ribosomal S6 kinase and the like. Also, among G proteins, ARF and Rho proteins appear to play an important role in the recirculation of a glucose carrier by activating phospholipase D, and Rab 4 protein is thought to play an important role in pathways related to the secretion of GLUT4. Some of such signaling pathways act independently or together to induce the expression of biological effects of insulin, including glucose transfer, enzyme activation, and synthesis of protein and nucleic acid. Insulin signaling processes include many signaling mechanisms and many protein substrates, and among them, IRS-1 plays a pivotal role in transferring the signal of insulin receptor to signaling substances such as PI3-kinase, GRB-2, SOS, Ras, Rab 4, ARF, SYP, Nck and the like. In this experiment, the effects of the extract from the mycelial culture medium of Ceriporia lacerata that increases the expression of the glucose transporter GLUT4 on the insulin signal transduction were examined. Specifically, the intracellular level of IRS-1 that plays a pivotal role in insulin signal transduction was examined in the presence of the extract from the mycelial culture medium of Ceriporia lacerata, and the intracellular level of PI3-kinase that binds to IRS-1 to transfer the signal of insulin into cells was also examined. In addition, the intracellular level of GLUT4 that receives the signals of IRS-1 and PI3-Kinase to transfer glucose was also examined. As a result, it was found that
all the proteins were phosphorylated by insulin and the expression levels thereof were increased by treatment with EPS. This suggests that EPS can promote glucose uptake through the IR, PI3K and Akt pathways and increase the expression of AMPK protein to promote glucose uptake, thereby improving insulin resistance.

5. Analysis of marker substance and anti-diabetic functional substance

5-1. Experimental method

100 g of CLD was suspended in 1.5 L of water, and 1.5 L of hexane was added thereto. The suspension was placed in a separatory funnel and fractionated into a hexane-insoluble layer and a hexane-soluble layer, and the upper layer was collected. Fractionation and drying was repeated twice in the same manner as above using the same volume of hexane as the lower layer until the color of the solution became light so that the hexane-soluble substance was obtained in the largest possible amount. Subsequently, fractionation and drying was carried out in the same manner as above except for using methylene chloride, ethyl acetate and butanol. As a result, 15 g of a hexane-soluble extract, 25 g of a methylene chloride-soluble extract, 30 g of an ethyl acetate-soluble extract and 15 g of a butanol-soluble extract were obtained.

30 g of the ethyl acetate-soluble extract was subjected to silica gel column chromatography (12×60 cm, ASTM7734, Merck, Germany) using an eluent (hexane: ethyl acetate: methanol =10:3:1), and then fractionated by TLC, thereby obtaining 15 fractions.

5-2. Separation of 2,5-dihydroxybenzoic acid

Among the fractions, the 6th fraction was separated by silica gel column chromatography (1.5×12 cm, ASTM7734) using an eluent (toluene: ethyl acetate: acetic acid=5:3:1), and then purified by TLC (developing solvent/toluene: ethyl acetate: acetic acid =5:4:1), thereby obtaining 7 mg of a compound having an Rf of 0.58. The compound was analyzed by EI-MASS and 1H-NMR, and as a result, the compound was identified to be 2,5-dihydroxybenzoic acid having the following formula:
5-3. Separation of protocatechualdehyde

Among the above fractions, the 7th fraction was separated by silica gel column chromatography (1.5×15 cm, ASTM7734) using an eluent (toluene: ethyl acetate: acetic acid =2.5:1:0.5), and then purified by TLC (developing solvent/toluene: ethyl acetate: acetic acid =5:4:1), thereby obtaining 2 g of a compound having an Rf of 0.52. The compound was identified to be protocatechualdehyde having the following formula by EI-MASS and 1H-NMR analysis:

In order to measure the contents of the above two glucose tolerance compounds, LC/MS/MS analysis was performed using Agilent 6410 (Agilent Technologies) and a negative ion source at a fragmentor of 150 under the following conditions: gas temperature: 320°C; gas flow rate: 35 mL/min; and capillary volt: 4000. The HPLC column used was Epic C18, and the temperature of the column was maintained at 40°C. The mobile phase used was composed of 0.1% formic acid-containing distilled water and 0.1% formic acid-containing acetonitrile.
As a result, 2,5-dihydroxybenzoic acid and protocatechualdehyde, known to have anti-diabetic effects, were detected in trace amounts. The results of the analysis are shown in Table 5 below.

Table 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protocatechualdehyde (μg/g)</th>
<th>2,5-dihydroxybenzoic acid (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example 5</td>
<td>18.79 ± 0.87</td>
<td>37.65 ± 1.32</td>
</tr>
</tbody>
</table>

5-3. Separation of Exopolysaccharide

In order to measure the content of exopolysaccharide having excellent blood glucose lowering effects, 5 g of the extract from the mycelial culture medium of Ceriporia lacerata was sufficiently suspended in 100 mL of distilled water, and the suspension was centrifuged at 8,000 rpm for 20 minutes. A 2-3-fold amount of cold alcohol was added to the supernatant which was then placed in a refrigerator at 4°C and allowed to stand for 12 hours. The supernatant in the solution which had been allowed to stand was centrifuged again at 8,000 rpm for 20 minutes, and the precipitate was collected, thereby extracting crude exopolysaccharide. The crude exopolysaccharide was freeze-dried, and as a result, it was shown to have an exopolysaccharide content of 5.5±0.5%/100 mg. The sugar and protein contents of the EPS were measured, and as a result, it was shown that the sugar content was about 40% and the protein content was about 33%, suggesting that the EPS is a polysaccharide composed of sugar bonded with protein.

As described above, according to the present invention, there can be provided an extract from the mycelial culture medium of Ceriporia lacerata containing 5.5±0.5%/100 mg of exopolysaccharide having excellent blood glucose lowering effects, 18.79±0.87 μg/g of protocatechualdehyde and 37.65±1.32 μg/g of 2,5-dihydroxybenzoic acid.

6. Toxicity and efficacy tests in rodents
Tests for the safety and efficacy of the extract from the mycelial culture medium of *Ceriporia lacerata* were performed by K company (received a GLP certificate) in accordance with Good Laboratory Practice (GLP). As a result, it was found that the extract from the mycelial culture medium of *Ceriporia lacerata* exhibited statistically significant increase of pancreatic beta-cells in Type 2 diabetes rats (increased the weight of pancreas, spleen and thymus) and was safer and more effective than Metformin (Pfizer) which was used as a positive control drug (Table 6).

**Table 6**

<table>
<thead>
<tr>
<th>General toxicity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A single dose toxicity test on rodents</td>
<td>7 weeks</td>
</tr>
<tr>
<td>Toxicity test on rodents for 4 weeks repeated oral administration-DRF</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Toxicity test on rodents for 13 weeks repeated oral administration (including recovered group)</td>
<td>27 weeks</td>
</tr>
<tr>
<td>A single dose toxicity test on non-rodents</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Result</td>
<td>Nontoxic reaction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heredity toxicity test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Back mutation test (including preliminary test)</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Chromosomal anomaly test (including preliminary test)</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Micro nucleus test (including preliminary test)</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Result</td>
<td>Negative reaction</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effect test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral glucose tolerance test</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Effect test on blood sugar dropping after being caused Type 1 diabetes by STZ</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Curing diabetes using db/db mice</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Result</td>
<td>B-cell in pancreas with Type 2 diabetes rats increased meaningfully in statistics, and it is judged that it is a fundamental diabetes medicine being recorded with safe and excellent effect compared to Metformin by Pfizer used as a comparison medicine.</td>
</tr>
</tbody>
</table>
INDUSTRIAL APPLICABILITY

The present invention relates to a method for preparing an extract from the mycelial culture medium of Ceriporia lacera and a pharmaceutical composition for preventing or treating diabetic diseases and diabetic complications prepared by the preparation method, which has high industrial applicability.
WHAT IS CLAIMED IS:

1. A method for preparing an extract from the mycelial culture medium of *Ceriporia lacerata*, which comprises the steps of: culturing the mycelia of *Ceriporia lacerata* in a liquid, drying the culture to form powder and preparing a solvent extract from the powder,

   wherein a medium for culturing the mycelia of *Ceriporia lacerata* comprises 1-2 wt% of sugar, 0.2-1 wt% of glucose, 0.2-1 wt% of starch, 0.1-0.5 wt% of sorghum powder, 0.1-0.5 wt% of barley powder, 0.2-2 wt% of soy flour, 0.05-0.1 wt% of magnesium sulfate (MgSO₄), 0.05-0.1 wt% of monopotassium phosphate (KH₂PO₄), 0.05-0.1 wt% of dipotassium phosphate (K₂HPO₄) and 92-98 wt% of water and has a pH of 4.5-6.0.

2. The method of claim 1, wherein the culturing is carried out under a blue LED light source.

3. The method of claim 1, wherein the culturing is carried out at a carbon dioxide concentration of 1,000-2,000 ppm.

4. A pharmaceutical composition for preventing or treating diabetic diseases and diabetic complications, which contains, as an active ingredient, an extract from the mycelial culture medium of *Ceriporia lacerata* prepared by the method of any one of claims 1 to 3.

5. The pharmaceutical composition of claim 4, wherein the diabetic diseases is Type 2 diabetes.
6. The pharmaceutical composition of claim 4, wherein the diabetic complications are selected from the group consisting of hyperglycemia, atherosclerosis, microangiopathy, diabetic retinopathy and kidney disease.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

A61K 36/06(2006.01)i, C12N 1/14(2006.01)i, C12P 1/02(2006.01)i, A61P 3/10(2006.01)i, A61P 3/00(2006.01)i

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)

A61K 36/06; A01M 17/00; C12Q 1/02; C12P 1/02; A61K 35/66; B09B 3/00; C12N 1/14; A61P 3/10; A61P 3/00

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

Korean Utility models and applications for Utility models: IPC as above

Japanese Utility models and applications for Utility models: IPC as above

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

eKOMPASS (KIPO internal) & Keywords: Ceriporia lacerata (Ceriporia lacerata), diabetes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X KR 10-1031605 B1 (KIM, Byoung Cheon et al.) 27 April 2011</td>
<td>1-6</td>
</tr>
<tr>
<td>See claims 1, 4, 9 and 12; paragraphs [0003]-[0007].</td>
<td></td>
</tr>
<tr>
<td>A KIM, Ji-Eun et al., &quot;Hyperglycemic effect of submerged culture extract of Ceriporia lacerata in Streptozotocin-induced Diabetic Rats&quot;, Food Science and Biotechnology, 31 December 2012 (published online), vol. 21, no. 6, pages 1685-1693</td>
<td>1-6</td>
</tr>
<tr>
<td>See abstract.</td>
<td></td>
</tr>
<tr>
<td>A JP 2011-167073 A (TOTTORI UNIV.) 01 September 2011</td>
<td>1-6</td>
</tr>
<tr>
<td>See abstract and claim 1.</td>
<td></td>
</tr>
<tr>
<td>A JP 2008-245629 A (KYUSHU UNIV.) 16 October 2008</td>
<td>1-6</td>
</tr>
<tr>
<td>See abstract and claim 1.</td>
<td></td>
</tr>
<tr>
<td>A KR 10-2006-0003982 A (WHAN IN PHARMACEUTICAL COMPANY) 12 January 2006</td>
<td>1-6</td>
</tr>
<tr>
<td>See abstract and claim 1.</td>
<td></td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. 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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Document member of the same patent family

Date of the actual completion of the international search

18 OCTOBER 2013 (18.10.2013)

Date of mailing of the international search report

18 OCTOBER 2013 (18.10.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 109 Seomsa-ro, Daejeon 302-701,
Republic of Korea

Facsimile No. 82.42.472-7140

Authorized officer

Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR 10-1031605 B1</td>
<td>27/04/2011</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td>JP 2011-167073 A</td>
<td>01/09/2011</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td>JP 2008-245629 A</td>
<td>16/10/2008</td>
<td>NONE</td>
<td></td>
</tr>
</tbody>
</table>