# DESCRIPTION

Title of Invention: PLANT GERM FOR CELL-FREE PROTEIN SYNTHESIS

## Technical Field

[0001]

This invention relates to plant germ for cell-free protein synthesis; an extract and a reaction liquid for cell-free protein synthesis, obtained from the plant germ; and methods for cell-free protein synthesis and for improving protein synthesis capability, the methods including using the extract or reaction liquid.

## Background Art

[0002]

Artificial synthesis of protein is not limited to academic applications. The need therefor has recently increased in various situations. An ideal method of artificial protein synthesis would be the one that can produce the target protein inexpensively, stably, and in large quantities and can be carried out conveniently without requiring special equipment or complicated processes.

[0003]

Methods for synthesizing proteins artificially include introducing a target gene into cells originating from various organisms, including mammals, insects, plants, and *E. coli*. Apart from this, cell-free protein synthesis systems that use materials derived from wheat or rabbit reticulocytes are known (see patent literature 1). Cell-free protein synthesis is a technique that performs the process of protein synthesis *in vitro*, for example, in a test tube. This process involves transcribing genetic information from DNA into mRNA, and then having ribosomes translate the mRNA information to synthesize the target protein.

Cell-free protein synthesis systems have advantages, such as accurate translation, and disadvantages, such as poor synthesis efficiency and yield. Various studies have been conducted to overcome these disadvantages. Examples of reported techniques for wheat germ-based cell-free protein synthesis systems include preparing a formulation by lyophilization of a cell extract-containing liquid (patent literature 2), adding a polyhydric

alcohol for stabilization during frozen storage (patent literature 3), removing impurities originating from animals and microorganisms (patent literature 4), maintaining the initial substrate concentration (patent literature 1), and concentrating an extracted liquid for use in protein synthesis and maintaining the resulting protein concentration at or above a given level (patent literature 5).

Citation List

Patent literature:

[0004]

Patent literature 1: JP H1-503119A

Patent literature 2: JP 2000-316594A

Patent literature 3: US 2003/0199076A1

Patent literature 4: JP 2007-97438A

Patent literature 5: JP H6-225783A

Summary of Invention

[0005]

Using wheat, which is inexpensively available, as the material for cell-free protein synthesis systems is expected to ensure the large-volume synthesis of target proteins at low cost. However, the abovementioned conventional techniques relating to cell-free protein synthesis do not improve the yield as much as expected. Some of these techniques require special equipment. Furthermore, when an additive is used, the synthesized protein has limited applications.

[0006]

The invention relates to providing plant germ for cell-free protein synthesis that enables inexpensive, high-yield synthesis of desired proteins in cell-free protein synthesis systems without requiring complicated equipment or processes, and also products and methods related to the plant germ.

[0007]

As a result of intensive investigation, the inventors have found that the protein synthesis capability in cell-free protein synthesis systems can be enhanced without

requiring complicated apparatus or processes by using plant germ, e.g., wheat germ, with gibberellin (hereinafter abbreviated as GA), which is a plant hormone, added thereto. The invention has been accomplished based on such a finding.

[0008]

The invention provides plant germ for cell-free protein synthesis. The plant germ has GA added thereto.

The invention also provides an extract for cell-free protein synthesis, the extract being from the plant germ.

The invention also provides a reaction liquid for cell-free protein synthesis. The reaction liquid contains the extract.

The invention also provides a cell-free protein synthesis method that includes using the extract or reaction liquid.

[0009]

The invention also provides a method for improving protein synthesis capability. This method includes: adding GA to plant germ; leaving the germ to stand under a low temperature condition; and extracting the germ after standing, thereby obtaining a germ liquid extract having enhanced protein synthesis capability in cell-free protein synthesis.

[0010]

The invention also provides a method for improving protein synthesis capability in cell-free protein synthesis. The method includes the following steps: (1) adding a plant hormone to plant germ, (2) leaving the plant germ to stand under a low temperature condition, (3) grinding and homogenizing the plant germ after standing, (4) adding a solution for extracting germ components to the plant germ to dissolve the germ, and (5) purifying the dissolved germ.

Brief Description of Drawings

[0011]

[Fig. 1] Fig. 1 is a graph showing the evaluation results in Example and Comparative Example.

Description of Embodiments

[0012]

The invention (plant germ, an extract, and a reaction liquid for cell-free protein synthesis; and methods for cell-free protein synthesis and for improving protein synthesis capability) enables inexpensive, high-yield synthesis of desired proteins in cell-free protein synthesis systems without requiring complicated equipment or processes.

The invention provides an inexpensive, convenient way to supply desired proteins not only for research but also for a variety of applications, such as medical and diagnostic drugs, research reagents, and protein substitutes in animal feed and human food products, which will increase the industrial utilization of protein synthesis.

[0013]

The invention will be described on the basis of its preferred embodiments.

The invention relates to plant germ-based cell-free protein synthesis systems. As used herein, the term “cell-free protein synthesis systems” includes cell-free translation systems, where a protein or polypeptide is synthesized based on the information read from mRNA, and systems that include both a cell-free transcription system, where RNA is synthesized using DNA as a template, and a cell-free translation system.

Plant germ is germ of plant origin. Germs of various plants, such as wheat, barley, rice, corn, and spinach, can be used. From the standpoint of, for example, improvement in protein synthesis capability and/or ease of availability, germs from gramineous plant seeds are preferred, with wheat germ being more preferred. Examples of the proteins synthesized by the cell-free method in the invention include polypeptides and proteins with molecular sizes generally synthesizable through cell-free methods, and specifically, polypeptides and proteins with molecular weights ranging from 10 to 200 kD may be targeted. The invention will be described sometimes specifically with reference to wheat germ, but the description equally applies to other plant germ types.

[0014]

Ribosome-containing plant germ, which is useful for cell-free protein synthesis, can be prepared from plant seeds using commonly known techniques.

For example, the method for obtaining plant germ includes: a preparatory

process for preparing plant germ to which GA is to be added; and a grinding process, an extraction process, and a purification process on the plant germ. The preparatory process includes, for example, seed grinding, sieving, heavy liquid separation, removing seed coat and endosperm fractions through visual inspection or the like, and washing out endosperm contaminants. In the present invention, GA is preferably added to the plant germ after the preparatory process.

[0015]

For example, the plant germ to which GA is to be added is preferably obtained through (A1) a plant seed grinding process and (A2) a germ separation process, and more preferably further (A3) a moisture content adjustment process.

[0016]

(A1) Plant Seed Grinding Process:

To efficiently obtain the germ, it is preferable to remove as much of the parts other than germ as possible prior to grinding the plant seeds, because the germ is scarce in seeds.

The grinding process is generally performed by applying mechanical force to the plant seeds to obtain a ground mass of the plant seeds, the ground mass containing germ, crushed endosperm, and crushed seed coat. The grinding can be achieved using a known grinding apparatus. The grinding apparatus can be a device that applies an impact force to the material to be ground, such as a pin mill or a hammer mill. The degree of grinding may be appropriately selected according to the size of the germ of the plant seeds to be used. For example, wheat seeds are preferably ground to a maximum length of 4 mm or smaller, and more preferably to a maximum length of 2 mm or smaller. The grinding may be performed under dry or wet condition.

[0017]

(A2) Germ Separation Process:

The germ separation process preferably involves the separation of a crude germ fraction. The separation of the crude germ fraction is preferably followed by an enrichment treatment to increase the proportion of germ-derived components.

In the separation of the crude germ fraction, the crushed endosperm, crushed

seed coat, and the like are removed from the ground seeds resulting from the grinding process to yield a crude germ fraction. The separated crude germ fraction contains germ as a main component, along with the ground endosperm and ground seed coat. The crude germ fraction is collected from the ground mass of the plant seeds using known classifiers, such as sieves. For example, a crude germ fraction with a mesh size of 0.5 to 2.0 mm, preferably 0.7 to 1.4 mm can be typically obtained in cases of wheat seeds. If necessary, the resulting crude germ fraction may be freed of the seed coat, endosperm, dust, and the like by wind power or static electricity.

Subsequently, or in place of the abovementioned classification, for example, with sieves, a crude germ fraction may also be obtained using a separation technique that takes advantage of the difference in specific gravity between the germ and the seed coat and endosperm, for example, a heavy liquid separation process. The heavy liquid separation process involves placing the ground plant germ in a liquid and removing the seed coat and endosperm fraction by utilizing the difference in specific gravity, yielding the crude germ fraction. A mixture of carbon tetrachloride and cyclohexane is generally used as the liquid. Chlorinated organic solvents, such as carbon tetrachloride, are preferable, since the solvent may be selected with due consideration of environmental and health aspects, depending on the intended use of the protein to be synthesized. To obtain a crude germ fraction containing as much germ as possible, two or more of the above-described techniques can be used in combination.

[0018]

A germ fraction may be obtained by separating the germ from the resulting crude germ fraction, for example, through visual inspection or using a color sorter. To remove any endosperm components that may still be attached, it is preferable that the germ fraction be further enriched by washing. The washing is preferably carried out by, for example, dispersing and suspending the germ fraction in water or an aqueous solution typically cooled to 10°C or lower, preferably 4°C or lower, repeatedly until the washing no longer becomes cloudy. This treatment is more preferably performed by dispersing and suspending the germ fraction in an aqueous solution of a surfactant typically cooled to 10°C or lower, preferably 4°C or lower, repeatedly until the washing no longer becomes cloudy. The surfactant is preferably nonionic. A wide range of nonionic surfactants can be utilized, and preferable examples thereof include Brij, Triton, Nonidet® P40, and Tween, which are polyoxyethylene derivatives, with Nonidet® P40

being the most suitable. These nonionic surfactants can be used in concentrations, e.g., of approximately 0.5%.

The washing using water or an aqueous solution and the washing using a surfactant may be used individually or in combination. The washing may be combined with ultrasonication.

[0019]

(A3) Moisture Content Adjustment Process

In the present invention, to improve the protein yield when the germ prepared above is used for cell-free protein synthesis, the moisture content of the germ fraction may be adjusted. The germ's moisture content (% by mass) after the adjustment is preferably 30% or less, more preferably 28.6% or less, and even more preferably 24.8% or less. Although no particular lower limit is imposed, the moisture content may be, for example, 1.6% or more, and is preferably 18.8% or less.

The adjustment of the germ's moisture content can be accomplished in the following manner, for example. For instance, 1.6 to 8.0 mL of water is added to 20 g of wheat germ, and after storing the mixture under refrigeration at 4°C for one day, the moisture content of the germ is determined.

[0020]

The method for adjusting the moisture content of the plant germ is not particularly restricted as long as the final moisture content is as designed (e.g., 30% or less). The amount of water to be added and the way of adding water (conditions) may be selected as appropriate. Regarding the way of adding water, for example, the plant germ may be immersed in water, or atomized water may be applied to the germ using a spray. Following the addition of water, the germ may be refrigerated for approximately one day to ensure that the moisture content is evenly distributed. If necessary, a lyophilization process may be performed. The moisture content, whether excessive or insufficient, affects protein synthesis. In a preferred embodiment of the invention, therefore, the moisture content may be controlled appropriately by lyophilization, in addition to by adjusting the amount of water added. When lyophilization is carried out, the moisture content resulting from the lyophilization may be used as such, or may be adjusted to a desired level by adding a predetermined amount of water to the germ after

lyophilization.

The germ's moisture content after the moisture content adjustment can be calculated from formula:

Germ's moisture content after adjustment (mass%) = [(mass after moisture content adjustment – dry mass before moisture content adjustment)/(mass after moisture content adjustment)] × 100

The dry mass is obtained by weighing 2 g of wheat germ into a heat-resistant container, heating the wheat germ at 135°C for one hour, reweighing the heated germ, and subtracting the mass loss from the initial mass of the wheat germ. The weight is measured in increments of 0.001 g.

[0021]

The water added to the germ for moisture content adjustment includes, but is not limited to, pure water and ion-exchanged water. A solution containing a plant hormone described below (e.g., GA) may be used for the moisture content adjustment, either alone or in combination with another type of water mentioned above.

The amount of the plant germ to be used in a cell-free protein synthesis system can be controlled on the basis of the dry mass before water addition.

[0022]

(Gibberellin Treatment)

Gibberellin (GA), a plant hormone, is used in the invention to improve the capability in plant germ-based cell-free protein synthesis. Known gibberellins (GAs) include endogenous compounds in plants and compounds produced by microorganisms. They are largely classified into two types: carbon-20 GAs (e.g., A12, A13, and A14) and carbon-19 GAs (e.g., A1, A3, A4, A7, and A9). Any GA or GA analogs can be used. Those skilled in the art will properly select an appropriate substance to carry out the invention. As used herein, the term "gibberellin (GA)" includes GA analogs as well as GA.

For example, the amount of GA added to plant germ can range from 0.5 to 500 ppm (μg/mL; in terms of mass) relative to the germ dry mass.

GA is preferably added in the form of a GA solution. For instance, gibberellin A3 is dissolved in pure water at a concentration of 100 μg/mL to prepare a GA solution,

and the resulting GA solution is added in an amount of approximately 80 μL per 1 g of the wheat germ dry mass.

[0023]

The moisture content adjustment and GA treatment of the wheat germ may be performed separately or simultaneously. For instance, when the moisture content adjustment and GA treatment are conducted separately at the same time, 400 μL of a GA solution having an adjusted concentration as described later (e.g., 100 μg/mL) is added to 5 g of the wheat germ. When the adjustment of the moisture content of the wheat germ and GA addition are performed in combination under the condition that 800 μL of water is designed to be added for moisture adjustment, a GA solution with an adjusted concentration (e.g., 50 μg/mL) may be added to 5 g of germ. These processes can be carried out with appropriate adjustment by those skilled in the art depending on the concentration of the GA solution and the amount of water to be added.

[0024]

(Permeation Treatment in Low-Temperature Environment)

The plant germ after GA addition may be subjected to a permeation treatment where the plant germ is left in a low-temperature environment for a predetermined period of time. The germ is preferably left to stand during the treatment. This treatment allows the GA to permeate the plant germ, which leads to the improvement in cell-free protein synthesis capability. The permeation treatment is carried out by leaving the germ after the addition of a GA solution to stand at 4°C for 24 hours, for example.

[0025]

The plant germ having GA added thereto is a preferred embodiment of the plant germ of the invention for cell-free protein synthesis. When used for protein synthesis in cell-free systems, the plant germ of this embodiment enhances the protein synthesis capability as compared with plant germ with no GA added. Also, the plant germ after GA addition is preferably used in the form of its extract in protein synthesis by cell-free methods. For example, the plant germ after GA addition can be processed in the same manner as for plant germ with no GA added to prepare a germ extract, which can be used

in cell-free protein synthesis. Whether or not plant germ has GA added thereto can be determined through, for example, LC-MS of the liquid extract of the germ.

[0026]

A plant germ extract is obtained from the GA-treated plant germ thus obtained. The method for obtaining the plant germ extract may be any known method. For example, the plant germ may be frozen with liquid nitrogen and then pulverized, and an extraction solvent is added to the pulverized germ. After stirring, a liquid containing germ extract is collected from the mixture by centrifugation or other means. If necessary, the liquid can be purified by gel filtration, for example. The plant germ before or after GA treatment may be pulverized and the resulting pulverized germ may be used.

[0027]

The method for obtaining an extract or reaction liquid of the invention for cell-free protein synthesis from plant germ after GA treatment will be described based on its preferred embodiments.

The method for obtaining the extract from the plant germ preferably includes (B1) a grinding process and (B2) an extraction process and a purification process.

(B1) Grinding Process

The grinding process is preferably a grinding and homogenizing process, in which a raw feed is stirred during grinding thereby to accomplish homogenization of the ground product.

In the grinding and homogenizing process, the plant germ is ground in a grinding apparatus, such as a mill. Preferably, the plant germ after addition of a GA solution is frozen with, for example, liquid nitrogen, and the frozen germ is ground in a grinding apparatus. Homogenization can be achieved by employing a grinding method that involves stirring.

Homogenization by stirring during grinding or grinding followed by a separate stirring process is preferred for reliably improving the protein synthesis capability in a cell-free protein synthesis method. Grinding the germ in a frozen state is preferred for suppressing the decomposition of GA.

When the plant germ after GA treatment is stored until extraction, the storage

temperature is preferably 25°C or lower, more preferably 10°C or lower, for suppressing the decomposition of GA. It is preferable that the plant germ be stored in a frozen state in a centrifuge tube, as described later.

(B2) Extraction Process and Purification Process

The extraction process is carried out, for example, as follows. An adequate amount of the ground germ is weighed out and placed in a centrifuge tube, a flask, or another suitable container. A solution for extracting germ components is added, and the mixture is shaken. After shaking, the mixture is subjected to centrifugation, either directly as it is in the centrifuge tube or after being transferred to a centrifuge tube, and the resulting supernatant is collected. The collected supernatant is preferably sterilized and/or subjected to gel filtration. A desired germ liquid extract is thus obtained.

An extraction buffer can be used as the solution for extracting germ components. Examples of the extraction buffer include Extraction buffers 1 and 2 used in Examples given later. The solution for extracting germ components preferably contains at least potassium acetate, magnesium acetate, and a reducing agent, such as dithiothreitol (hereinafter DTT), and more preferably further contains calcium chloride, etc. The sterilization process is preferably a treatment that can be performed at low temperatures for preventing denaturation of the components contained in the resulting wheat germ extract. The gel filtration process is designed to purify the plant germ dissolved in the solution for extracting germ components.

[0028]

The germ liquid extract obtained from plant germ usually contains at least ribosomes and translation factors. The translation factors include initiation factors, elongation factors, and release factors. The liquid extract obtained from the plant germ with added GA or a liquid or solid obtained from the germ liquid extract by, for example, lyophilization, spray drying, or concentration is a preferred embodiment of the extract of the invention, which is preferably used in cell-free protein synthesis systems.

The synthesis of a target protein by a cell-free protein synthesis system using the extract of the invention for cell-free protein synthesis can be carried out with the resulting germ extract using any known method. The extract may be used in a cell-free protein synthesis reaction with a commercially available kit for the synthesis. The cell-free

protein synthesis reaction can be performed using any known techniques. Commercially available kits for synthesis reactions may be used.

[0029]

In a cell-free translation synthesis system, where a protein or polypeptide is synthesized based on the information read from mRNA, mRNA carrying the information of a target protein is added to the extract that is obtained from the plant germ with added GA and contains ribosomes, translation factors, etc. to cause a reaction, whereby the target protein can be produced efficiently in an increased yield.

When a cell-free protein synthesis system includes a cell-free transcription system for RNA synthesis using DNA as a template and a cell-free translation system, the synthesis reaction kit preferably includes components for the cell-free translation system, such as RNA polymerase, deoxynucleotide triphosphate, an amino acid solution, and creatine kinase; and those for the cell-free transcription system, such as DNA polymerase, magnesium sulfate, and various proteins participating in transcription. The extract that is obtained from the plant germ with added GA and contains ribosomes, translation factors, etc. and DNA carrying the information about a target protein are added to such a synthesis reaction kit to cause a reaction, whereby the target protein can be produced efficiently in an increased yield.

[0030]

The protein synthesis efficiency in a cell-free protein synthesis method using the extract of the invention can be determined using, for example, the method described below. Determining the synthesis efficiency in advance enables estimating the yield of the protein to be synthesized. While “the extract of the invention” is preferably the liquid extract prepared from plant germ with added GA using the method described above, the method of extraction, the properties of the extract, and other conditions are not particularly limited, as long as the extract is obtained from plant germ with added GA.

[0031]

Using a cell-free protein synthesis kit, an mRNA sequence is synthesized from the DNA sequence of a protein whose properties resemble the properties of a target protein. Examples of the proteins used to determine the synthesis efficiency include, but

are not limited to, fluorescent proteins, such as GFP, and any protein can be used to determine the synthesis efficiency. A fluorescent protein, such as GFP, is preferable since a value reflecting the protein quantity can be readily obtained from the intensity of the fluorescence generated upon irradiation with specific excitation light. To determine the synthesis efficiency, protein synthesis is performed by, for example, using the synthesized mRNA as a template, and the yield is measured after a predetermined time has elapsed. For instance, after a predetermined period of time has elapsed since translation initiation, the translation reaction solution placed in a plate reader is irradiated with excitation light at 485 nm, and the fluorescence intensity is measured.

[0032]

The cell-free protein synthesis method of the invention encompasses a broad range of protein synthesis methods in cell-free systems using the above-described plant germ or its extract for cell-free protein synthesis or a reaction liquid for cell-free protein synthesis containing the extract, and is not particularly limited by the type of proteins to be synthesized, type of template DNA used, production method, etc.

A typical reaction liquid of the invention for cell-free protein synthesis is a mixture including the liquid extract obtained by the aforementioned method from plant germ with added GA, and other components necessary for cell-free protein synthesis. The liquid extract typically contains ribosomes and translation factors. Examples of the other components include an amino acid solution, creatine kinase, and an mRNA solution obtained by transcription from the template DNA.

[0033]

A method of the invention for improving protein synthesis capability (first method) includes: adding GA to plant germ; leaving the plant germ to stand under a low temperature condition; and extracting the germ after standing, thereby obtaining a germ liquid extract having enhanced protein synthesis capability in cell-free protein synthesis. In a preferred embodiment of the first method, the addition of GA to plant germ is carried out in the same manner as for the GA treatment described above, and the extraction process after standing under a low temperature condition is carried out in the same manner as for the permeation treatment in a low-temperature environment described above. According to the method of the invention for improving protein synthesis

capability (first method), the protein synthesis capability of cell-free protein synthesis systems can easily be enhanced without requiring complicated equipment or processes.

[0034]

A method of the invention for improving protein synthesis capability (second method) is for enhancing protein synthesis capability in cell-free protein synthesis, and the method includes:

(1) adding GA to plant germ,

(2) leaving the plant germ to stand under a low temperature condition,

(3) grinding and homogenizing the germ after standing,

(4) adding a solution for extracting germ components to the plant germ to dissolve the plant germ, and

(5) purifying the dissolved germ.

[0035]

In a preferred embodiment of the second method, Step (1) corresponds to the GA treatment; Step (2) to the permeation treatment in a low-temperature environment; Step (3) to the grinding process (B1); and Steps (4) and (5) to the extraction and purification processes (B2), respectively. The description of each process applies to the corresponding step.

For example, the leaving under a low temperature condition in Step (2) is preferably carried out for at least 1 hour, more preferably 6 hours or longer. There is no upper limit to the leaving time; for example, it may be as long as 3 months.

In Step (4), the phrase “to dissolve the plant germ” means to dissolve the ribosomes, translation factors, and other components that are necessary to translate protein from mRNA into the solution for extracting the germ components.

In Step (5), the phrase “purifying the dissolved plant germ” means to purify and recover the components dissolved in Step (3).

The method of the invention for improving protein synthesis capability (second method) easily improves the protein synthesis capability of cell-free protein synthesis systems without requiring complicated equipment and processes.

Examples

[0036]

The invention will now be illustrated in greater detail by way of Examples, but it should be understood that the invention is not limited thereto.

[Comparative Example 1]

A wheat germ extract was prepared from the wheat germ available from Nisshin Flour Milling, Inc. according to the following procedure, which is a partial modification of the method described in Methods in Enzymology (1983), Vol. 96, pp. 38-50.

(1) The wheat germ was ground and homogenized using a blender (Silent Millser from Iwatani Corp.).

(2) The moisture content of the wheat germ was measured as described below. The wheat germ was weighed out to have a dry weight of 3 g based on the measured moisture content, and placed in a 50-mL centrifuge tube. Ten milliliters of Extraction buffer 1, which had been prepared according to the composition of Table 1 below, was added thereto.

[Measurement of Moisture Content]

Two grams of the wheat germ was weighed out and placed in a heat-resistant container, heated at 135°C for one hour, and then reweighed to calculate the mass loss. The ratio of the mass loss to the initial mass of the wheat germ before heating was calculated and used as the moisture content. The weight was measured in increments of 0.001 g.

(3) The centrifuge tube was shaken for 5 minutes using a shaker (Utility Shaker US-S100 from AS ONE Corp.; preset speed 200 rpm).

(4) The centrifuge tube was subjected to centrifugation (23,000G, 10 mins.) using a centrifuge (Avanti JXN-30 from Beckman Coulter, Inc.).

(5) The aqueous phase of the supernatant was collected and sterilized using a 0.22-μm filter.

(6) After removing the storage solution from a PD-10 column (from Cytiva), Extraction buffer 1 shown in Table 1 was poured into the column to equilibrate the gel packed therein.

(7) A 2.5 mL portion of the impurity-free solution collected in (5) above was loaded onto the PD-10 column, and the flow-through was discarded.

(8) Through the column, 3.5 mL of Extraction buffer 2 with the composition of Table 2 was passed, and the collected flow-through was used as a wheat germ extract for the test.

[0037]

[Table 1]

| Extraction Buffer 1 | |
|---|---|
| 800 mM HEPES-KOH, pH 7.8 | 5 mL |
| 100 mM Magnesium Acetate | 1 mL |
| 1 M Potassium Acetate | 10 mL |
| 40 mM Calcium Chloride | 5 mL |
| 2 M DTT (Dithiothreitol) | 0.2 mL |
| Pure Water | 78.8 mL |
| Total | 100 mL |

[0038]

[Table 2]

| Extraction Buffer 2 | |
|---|---|
| 800 mM HEPES-KOH, pH 7.8 | 5 mL |
| 100 mM Magnesium Acetate | 5 mL |
| 1 M Potassium Acetate | 10 mL |
| 2 M DTT (Dithiothreitol) | 0.2 mL |
| Pure Water | 79.8 mL |
| Total | 100 mL |

[0039]

[Example 1] (Preparation of wheat germ for cell-free protein synthesis)

Gibberellin A3 (from Tokyo Chemical Ind., CO., Ltd.) was dissolved in pure water at a concentration of 100 μg/mL to prepare a GA solution. To 5 g of the same wheat germ as used in Comparative Example 1 was added 400 μL of the resulting GA solution, and then the mixture was left to stand at 4°C for 24 hours.

[Example 2] (Preparation of extract for cell-free protein synthesis)

A wheat germ extract was obtained from the GA-treated wheat germ in the same manner as in Comparative Example 1 (procedure (1) through (8)).

[Example 3] (Cell-free protein synthesis method)

mRNA was synthesized from plasmid DNA having the base sequence derived from GFP, described in Table 3 below, using a cell-free protein synthesis kit (from NUProtein Co., Ltd.) in accordance with the protocol of the kit. GFP was translated using the synthesized mRNA and Amino Acid Mix from the kit, and each of the wheat germ extracts obtained in Comparative Example 1 and Example 2 in accordance with the kit protocol. Sixteen hours after the initiation of the translation, the translation reaction solution was irradiated with excitation light at 485 nm, and the fluorescence intensity of GFP was measured at a measuring wavelength of 535 nm, using a plate reader (ARVO-X4, from Perkin-Elmer). The results obtained are shown in Fig. 1.

[0040]

[Table 3]

Base Sequence of GFP

| |
|---|
| A T G A G T A A A G G A G A A G A A C T T T T C A C T G G A G T T G T C C C A |
| A T T C T T G T T G A A T T A G A T G G T G A T G T T A A T G G G C A C A A A |
| T T T T C T G T C A G T G G A G A G G G T G A A G G T G A T G C A A C A T A C |
| G G A A A A C T T A C C C T T A A A T T T A T T T G C A C T A C T G G A A A A |
| C T A C C T G T T C C A T G G C C A A C A C T T G T C A C T A C T T T C T C T |
| T A T G G T G T T C A A T G C T T T T C A A G A T A C C C A G A T C A T A T G |
| A A A C G G C A T G A C T T T T T C A A G A G T G C C A T G C C C G A A G G T |
| T A T G T A C A G G A A A G A A C T A T A T T T T T C A A A G A T G A C G G G |
| A A C T A C A A G A C A C G T G C T G A A G T C A A G T T T G A A G G T G A T |
| A C C C T T G T T A A T A G A A T C G A G T T A A A A G G T A T T G A T T T T |
| A A A G A A G A T G G A A A C A T T C T T G G A C A C A A A T T G G A A T A C |
| A A C T A T A A C T C A C A C A A T G T A T A C A T C A T G G C A G A C A A A |
| C A A A A G A A T G G A A T C A A A G T T A A C T T C A A A A T T A G A C A C |
| A A C A T T G A A G A T G G A A G C G T T C A A C T A G C A G A C C A T T A T |
| C A A C A A A A T A C T C C A A T T G G C G A T G G C C C T G T C C T T T T A |
| C C A G A C A A C C A T T A C C T G T C C A C A C A A T C T G C C C T T T C G |
| A A A G A T C C C A A C G A A A A G A G A G A C C A C A T G G T C C T T C T T |
| G A G T T T G T A A C A G C T G C T G G G A T T A C A C A T G G C A T G G A T |
| G A A C T A T A C A A A |

[0041]

As demonstrated in Fig. 1, when the wheat germ extracts obtained from GA-treated plant germ (Example 2) are used, the fluorescence intensity of GFP significantly increases as compared with wheat germ extracts from GA-untreated plant germ (Comparative Example 1). It is seen from the result that using GA-treated plant germ improves the protein synthesis capability and the protein yield in cell-free protein synthesis systems without requiring complicated equipment or processes.

Industrial Applicability

[0042]

According to the invention, a material necessary for plant germ-based cell-free protein synthesis is efficiently extracted from plant germ so that a germ liquid extract with reduced impurities can be provided. Thus, the invention stably and efficiently provides a germ liquid extract and a solution for cell-free protein synthesis. Using the germ liquid extract or solution of the invention for protein synthesis enables stable and efficient cell-free protein synthesis. Therefore, the invention contributes to large-volume

production of proteins for use in not only research but also various fields, including medical drugs, food products, and industrial products.

# CLAIMS

[Claim 1] Plant germ for cell-free protein synthesis, having gibberellin added thereto.

[Claim 2] An extract for cell-free protein synthesis, from the plant germ of claim 1.

[Claim 3] A reaction liquid for cell-free protein synthesis, comprising the extract of claim 2.

[Claim 4] A cell-free protein synthesis method comprising using the extract of claim 2 or the reaction liquid of claim 3.

[Claim 5] A method for improving protein synthesis capability, comprising:
adding gibberellin to plant germ;
leaving the plant germ to stand under a low temperature condition; and
extracting the germ after standing, thereby obtaining a germ liquid extract having enhanced protein synthesis capability in cell-free protein synthesis.

[Claim 6] A method for improving protein synthesis capability in cell-free protein synthesis, comprising the steps (1) to (5):

(1) adding gibberellin to plant germ,

(2) leaving the plant germ to stand under a low temperature condition,

(3) grinding and homogenizing the plant germ after standing,

(4) adding a solution for extracting germ components to the plant germ to dissolve the plant germ, and

(5) purifying the dissolved germ.