

[DESCRIPTION]

[Title of Invention]

LACTIC ACID BACTERIUM, FERMENTATION PRODUCT, PYROGALLOL PRODUCTION METHOD AND METHOD FOR SELECTING PYROGALLOL-PRODUCING LACTIC ACID BACTERIUM

[Technical Field]

[0001] The present invention relates to a lactic acid bacterium producing pyrogallol from a substrate. The present invention also relates to a fermentation product produced from the lactic acid bacterium. The present invention also relates to a method for producing pyrogallol utilizing the lactic acid bacterium. The present invention further relates to a method for selecting such a lactic acid bacterium.

[Background Art]

[0002] "An antiallergic composition comprising a hot water extract of coarse tea of Awa and a hot water extract of mulberry tea" was previously proposed (for example, see Japanese Patent Application Laid-open No. 2012-153613).

[Citation List]

[Non Patent Literature]

[0003]

[PTL 1] Japanese Patent Application Laid-open No. 2012-153613

[Summary of Invention]

[Technical Problem]

[0004] In order to prepare the above antiallergic composition, it is required to place and retain tea leaves of coarse tea of Awa

and mulberry tea in hot water, and thus, naturally, consuming a relatively high amount of thermal energy.

[0005] Moreover, hot water extracts of tea leaves as above contain a large amount of components other than antiallergic active components. Therefore, in order to ingest a certain amount of active components, a user has to take a relatively high amount of the antiallergic composition. Therefore, such an antiallergic composition puts a relatively heavy burden on the user and has impaired portability when, for example, the user goes out.

[0006] An object of the present invention is to provide an antiallergic agent which requires no or relatively low thermal energy for preparation thereof and has a relatively low burden on a user for intake thereof and excellent portability when, for example, the user goes out.

[Solution to Problem]

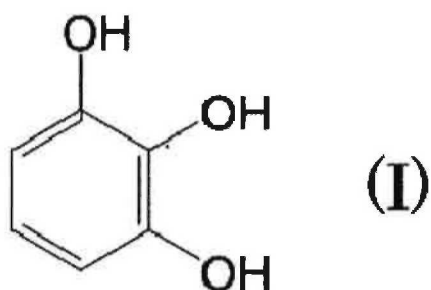
[0007] A lactic acid bacterium according to the first aspect of the present invention is a lactic acid bacterium *Lactobacillus pentosus* which produces pyrogallol (see the following chemical formula (I)) from a substrate. The lactic acid bacterium is preferably a lactic acid bacterium *Lactobacillus pentosus* which produces 10 $\mu\text{g/mL}$ or more pyrogallol from a substrate which is at least one of coarse tea of Awa and starting material tea leaves thereof or an extract thereof (for example, a mixture of a hot water extract (100°C, 3 minutes) of tea leaves (5 leaves) of coarse tea of Awa and ground tea leaves (5 leaves) of coarse tea of Awa).

Examples of the lactic acid bacterium include *Lactobacillus pentosus* OLL203969 (accession number: NITE BP-01986), *Lactobacillus pentosus* OLL203982 (accession number: NITE BP-01987) or *Lactobacillus pentosus* OLL203984 (accession number: NITE BP-01988). Pyrogallol is also referred to as benzene-1,2,3-triol, 1,2,3-trihydroxybenzene, 1,2,3-benzenetriol, 3-hydroxy pyrocatechol, trihydroxybenzene or pyrogallic acid. Examples of the substrate include coarse tea of Awa, starting material tea leaves thereof (tea leaves of coarse tea of Awa before fermentation) or a processed product or extract thereof. The "coarse tea of Awa" as used herein is fully-fermented tea produced in a very limited area around mountain areas in Tokushima prefecture, Japan and is recently also referred to as "Awa Bancha". The coarse tea of Awa is manufactured by macerating boiled starting material tea leaves in a wooden tub over 10 days to a half a month for allowing lactic acid fermentation followed by sun drying.

[0008] The lactic acid bacterium produces a highly effective antiallergic active component, pyrogallol, from a substrate under a temperature from room temperature (about 20°C) to about 30°C. Therefore, by using the lactic acid bacterium, an antiallergic active component can be obtained with no or relatively low usage (consumption) of thermal energy. The fermentation product from the lactic acid bacterium contains a relatively high level of pyrogallol. Therefore, by using the lactic acid bacterium, an antiallergic agent which puts a relatively low burden onto a user for intake thereof and has excellent portability when, for example,

the user goes out can be manufactured (produced) with high efficiency. Thus, use of the lactic acid bacterium allows preparation of pyrogallol with no or relatively low consumption of thermal energy and provision of an antiallergic agent which puts a relatively low burden onto a user for intake thereof and has excellent portability when, for example, the user goes out.

[C1]



[0009] A fermentation product according to the second aspect of the present invention is produced by inoculating the lactic acid bacterium according to the first aspect of the present invention on a food material to allow fermentation. The "food material" as used herein is preferably coarse tea of Awa and starting material tea leaves thereof (tea leaves of coarse tea of Awa before fermentation) or a processed product or extract thereof (particularly, hot water extract). The fermentation product is preferably produced by inoculating solely the lactic acid bacterium according to the first aspect of the present invention to allow fermentation.

[0010] The fermentation product contains a highly efficient antiallergic active component, pyrogallol. The fermentation product containing pyrogallol is produced at a temperature from

room temperature to about 30°C. Therefore, by using the fermentation product, an antiallergic active component can be obtained with no or relatively low usage (consumption) of thermal energy. The fermentation product contains a relatively high level of pyrogallol. Therefore, by using the fermentation product, an antiallergic agent which puts a relatively low burden onto a user for intake thereof and has excellent portability when, for example, the user goes out can be manufactured (produced) with high efficiency. Thus, use of the fermentation product allows preparation of an antiallergic agent with no or relatively low consumption of thermal energy and provision of an antiallergic agent which puts a relatively low burden onto a user for intake thereof and has excellent portability when, for example, the user goes out.

[0011] A method for producing pyrogallol according to the third aspect of the present invention can be carried out by providing a substrate to at least one lactic acid bacterium selected from *Lactobacillus pentosus* OLL203969, *Lactobacillus pentosus* OLL203982 and *Lactobacillus pentosus* OLL203984 and culturing the lactic acid bacterium at a predetermined temperature in an anaerobic environment for a predetermined period. The "substrate" as used herein is preferably a component contained in fermented tea such as coarse tea of Awa and starting material tea leaves thereof (tea leaves of coarse tea of Awa before fermentation) or a processed product or extract thereof (particularly, hot water extract).

[0012] According to the method for producing pyrogallol, a highly efficient antiallergic active component, pyrogallol, is produced from the substrate at a temperature from room temperature to about 30°C. Therefore, by using the method for producing pyrogallol, an antiallergic active component can be obtained with no or relatively low usage (consumption) of thermal energy. A fermentation product from the lactic acid bacterium contains a relatively high level of pyrogallol. Therefore, by using the method for producing pyrogallol, an antiallergic agent which puts a relatively low burden onto a user for intake thereof and has excellent portability when, for example, the user goes out can be manufactured (produced) with high efficiency. Thus, use of the method for producing pyrogallol allows preparation of an antiallergic agent with no or relatively low consumption of thermal energy and provision of an antiallergic agent which puts a relatively low burden onto a user for intake thereof and has excellent portability when, for example, the user goes out.

[0013] A method for selecting a pyrogallol-producing lactic acid bacterium according to the fourth aspect of the present invention includes a culturing step, a measurement step and a selection step. In the culturing step, at least one of coarse tea of Awa and starting material tea leaves thereof or an extract thereof is provided to a lactic acid bacterium and the lactic acid bacterium is cultured at a predetermined temperature in an anaerobic environment for a predetermined period. The "predetermined

temperature" as used herein is, for example, 25 to 45°C. The "predetermined period" as used herein is, for example, 1 to 20 days. The "extract" as used herein includes a hot water extract and an ethyl acetate extract. In the measurement step, a production quantity of pyrogallol from the lactic acid bacterium in the culturing step is measured. In the selection step, the lactic acid bacterium is selected based on the production quantity of pyrogallol.

[0014] By using the method for selecting a pyrogallol-producing lactic acid bacterium, a lactic acid bacterium which produces a highly efficient antiallergic active component, pyrogallol, can be easily selected.

[Brief Description of Drawings]

[0015]

[Fig. 1]

Fig. 1 is a graph illustrating an inhibitory action of pyrogallol on interleukin-4 mRNA expression.

[Description of Embodiments]

[0016] The lactic acid bacterium of the present invention preferably produces 10 µg/mL or more, more preferably 30 µg/mL or more, still more preferably 50 µg/mL or more, still more preferably 100 µg/mL or more, still more preferably 130 µg/mL or more, still more preferably 150 µg/mL or more and particularly preferably 170 µg/mL or more pyrogallol from a substrate which is at least one of coarse tea of Awa and starting material tea leaves thereof or

an extract thereof (for example, a mixture of a hot water extract (90 to 100°C, 3 to 5 minutes) of tea leaves (4 to 6 leaves) of coarse tea of Awa and ground tea leaves (4 to 6 leaves) of coarse tea of Awa).

[0017] The lactic acid bacterium of the present invention is preferably at least one lactic acid bacterium selected from *Lactobacillus pentosus* OLL203969, *Lactobacillus pentosus* OLL203982 and *Lactobacillus pentosus* OLL203984.

[0018] In the method for producing pyrogallol of the present invention, a substrate which is at least one of coarse tea of Awa and starting material tea leaves thereof or an extract thereof (for example, a mixture of a hot water extract (90 to 100°C, 3 to 5 minutes) of tea leaves (4 to 6 leaves) of coarse tea of Awa and ground tea leaves (4 to 6 leaves) of coarse tea of Awa) is provided to the lactic acid bacterium of the present invention and the lactic acid bacterium is cultured at a predetermined temperature in an anaerobic environment for a predetermined period. The "predetermined temperature" as used herein is, for example, 25 to 45°C, preferably 26 to 40°C, more preferably 27 to 35°C, still more preferably 28 to 32°C and particularly preferably 30°C. The "predetermined period" as used herein is, for example, 1 to 20 days, preferably 3 to 15 days, more preferably 5 to 10 days, still more preferably 6 to 8 days and particularly preferably 7 days.

[0019] In the method for selecting a pyrogallol-producing lactic acid bacterium of the present invention, at least one of

coarse tea of Awa and starting material tea leaves thereof or an extract thereof is provided to the lactic acid bacterium of the present invention, the lactic acid bacterium is cultured at a predetermined temperature in an anaerobic environment for a predetermined period, a production quantity of pyrogallol from the lactic acid bacterium is measured and the lactic acid bacterium is selected based on the production quantity of pyrogallol. The "predetermined temperature" as used herein is, for example, 25 to 45°C, preferably 26 to 40°C, more preferably 27 to 35°C, still more preferably 28 to 32°C and particularly preferably 30°C. The "predetermined period" as used herein is, for example, 1 to 20 days, preferably 3 to 15 days, more preferably 5 to 10 days, still more preferably 6 to 8 days and particularly preferably 7 days.

Examples

[0020] The present invention is more specifically described hereinafter by way of Examples. The following Examples are merely illustrative and do not limit the present invention.

[0021] <Selection of pyrogallol-producing lactic acid bacteria>

Candidate pyrogallol-producing lactic acid bacteria (30 species) as listed in Table 1 were prepared (including lactic acid bacteria from human being and lactic acid bacteria from coarse tea of Awa). Each species of lactic acid bacteria was cultured twice in MRS medium for activation. The medium containing the lactic acid bacteria was placed in a sample tube and subjected to a centrifuge.

After discarding the supernatant from the sample tube, the equivalent amount of saline as the supernatant was added to the sample tube to prepare a suspension of each species of the lactic acid bacteria.

[0022]

[Table 1]

Species	Strain Number	Pyrogallol ($\mu\text{g/mL}$)
Enterococcus sp.	ME P15009 01	0.20
Enterococcus sp.	ME P15009 02	2.70
Enterococcus sp.	ME P15009 03	0.00
Leuconostoc sp.	ME P15009 04	0.00
Lactobacillus pentosus	ME P15009 05	39.50
Lactobacillus pentosus	ME P15009 06	67.10
Lactobacillus pentosus	OLL 203969	196.30
Lactobacillus pentosus	ME P15009 07	44.50
Lactobacillus pentosus	OLL 203982	181.90
Lactobacillus pentosus	OLL 203984	176.00
Lactobacillus plantarum	ME P15009 08	5.33
Lactobacillus plantarum	ME P15009 09	14.39
Lactobacillus plantarum	ME P15009 10	5.96
Lactobacillus vacciostercus	ME P15009 11	2.10
Lactobacillus vacciostercus	ME P15009 12	3.40
Lactobacillus vacciostercus	ME P15009 13	3.64
Lactobacillus coryniformis	ME P15009 14	1.74
Lactobacillus coryniformis	ME P15009 15	2.14
Lactobacillus fermentum	ME P15009 16	1.77
Lactobacillus acidophilus	ME P15009 17	6.0

Lactobacillus crispatus	ME P15009 18	0.0
Lactobacillus gasseri	ME P15009 19	2.9
Lactobacillus brevis	ME P15009 20	0.1
Lactobacillus buchneri	ME P15009 21	5.4
Lactobacillus fermentum	ME P15009 22	0.0
Lactobacillus mucosae	ME P15009 23	0.2
Lactobacillus reuteri	ME P15009 24	0.0
Lactobacillus rhamnosus	ME P15009 25	0.0
Lactobacillus salivarius	ME P15009 26	0.0
Lactobacillus casei	ME P15009 27	0.0

[0023] Meanwhile, a few tea leaves of coarse tea of Awa were added to distilled water which was then boiled at 100°C for 3 minutes. The tea leaves after boiling were gently ground in a mortar to prepare tea leaf pieces.

[0024] To the liquor (4 mL) of tea leaves were added the above tea leaf pieces (from about 5 tea leaves) and a suspension (80 µL) of each species of the lactic acid bacteria was then inoculated thereto. The lactic acid bacteria were cultured at 30°C in an anaerobic environment for 7 days. Thereafter, the culture solution was subjected to high-performance liquid chromatography to measure the production quantity of pyrogallol. Pyrogallol is eluted at 14.015 minutes in high-performance liquid chromatography under the conditions indicated below.

[0025]

Column: COSMOSIL (Registered trademark)
5C₁₈-MS II (diameter: 4.6 mm, length: 250 mm) (manufactured by Nacalai Tesque, Inc.)

Mobile phase: Solvent A (water : acetic acid = 99:1), solvent B (methanol : acetone : acetic acid = 95:4:1)

Elution conditions:

0 to 15 min.: solvent A 95% by mass / solvent B 5% by mass

15 to 30 min.: solvent A 45% to 50% by mass / solvent B 55-50% by mass

30 to 40 min.: solvent A 50% to 0% by mass / solvent B 50-100% by mass

40 to 50 min.: solvent A 0% by mass / solvent B 100% by mass

45 to 55 min.: solvent A 0% to 95% by mass / solvent B 100-5% by mass

Column temperature: room temperature

Detection wavelength: 270 nm

Sample injected: 10 μ L

Flow rate: 0.5 mL/min.

[0026] Table 1 shows the production quantities of pyrogallol from various lactic acid bacteria. It was found from the results that each species of the lactic acid bacteria had different pyrogallol producing ability. It was also found that among the lactic acid bacteria indicated in Table 1, *Lactobacillus pentosus* have high pyrogallol producing ability and particularly strains OLL203969, OLL203982 and OLL203984 have prominent pyrogallol producing ability.

Accession Numbers

[0027]

NITE BP-01986

NITE BP-01987

NITE BP-01988

[0028]

0-1	Form PCT/RO/134	
	The indications relating to the deposited microorganism	
0-1-1	or other biological material (PCT Rule 13bis) was made by the authorities indicated on right.	JPO-PAS i250
0-2	International application No.	
0-3	Applicant's or agent's file reference	P150011

1	The following description relates to the microorganism or biological material	
1-1	referred in the detailed description of the invention. Paragraph	0007
1-3	Identification of deposit	
1-3-1	Name of depositary institution	NPMD, National Institute of Technology and Evaluation,

1-3-2	Address of depositary institution	Patent Microorganisms Depositary (NPMD) #122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan
1-3-3		
1-3-4	Date of deposit	05. 01. 2015
	Accession Number	NPMD NITE BP-01986
1-5	Designated states for which indications are made	All designated states
2	The following description relates to the microorganism or biological material referred in the detailed description of the invention. Paragraph	0007
2-3	Identification of deposit	
2-3-1	Name of depositary institution	NPMD, National Institute of Technology and Evaluation, Patent Microorganisms Depositary (NPMD)
2-3-2	Address of depositary institution	#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan
2-3-3		
2-3-4	Date of deposit	05. 01. 2015

	Accession Number	NPMD NITE BP-01987
2-5	Designated states for which indications are made	All designated states
3	The following description relates to the microorganism or biological material referred in the detailed description of the invention. Paragraph	0007
3-3	Identification of deposit	
3-3-1	Name of depositary institution	NPMD, National Institute of Technology and Evaluation, Patent Microorganisms Depositary (NPMD)
3-3-2	Address of depositary institution	#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba
3-3-3		292-0818, Japan
3-3-4	Date of deposit	05. 01. 2015
	Accession Number	NPMD NITE BP-01988
3-5	Designated states for which indications are made	All designated states

[CLAIMS]

[Claim 1] A lactic acid bacterium of *Lactobacillus pentosus*, producing pyrogallol from a substrate.

[Claim 2] The lactic acid bacterium according to claim 1, wherein the lactic acid bacterium is *Lactobacillus pentosus* OLL203969 (accession number: NITE BP-01986), *Lactobacillus pentosus* OLL203982 (accession number: NITE BP-01987) or *Lactobacillus pentosus* OLL203984 (accession number: NITE-BP-01988).

[Claim 3] A fermentation product produced by inoculating the lactic acid bacterium according to claim 1 or 2 to a food material to allow fermentation.

[Claim 4] A method for producing pyrogallol comprising providing a substrate to at least one lactic acid bacterium selected from *Lactobacillus pentosus* OLL203969 (accession number: NITE BP-01986), *Lactobacillus pentosus* OLL203982 (accession number: NITE BP-01987) and *Lactobacillus pentosus* OLL203984 (accession number: NITE BP-01988).

[Claim 5] The method for producing pyrogallol according to claim 4, wherein the substrate is a component contained in starting material tea leaves of coarse tea of Awa.

[Claim 6] A method for selecting a pyrogallol-producing lactic

acid bacterium, comprising:

a culturing step of providing at least one of coarse tea of Awa and starting material tea leaves thereof or an extract thereof to a lactic acid bacterium and culturing the lactic acid bacterium at a predetermined temperature in an anaerobic environment for a predetermined period;

a measurement step of measuring a production quantity of pyrogallol from the lactic acid bacterium in the culturing step; and

a selection step of selecting the lactic acid bacterium based on the production quantity of pyrogallol.