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(54) **MUSHROOM NAMED 'PLE-3'**

(50) Latin Name: ***Pleurotus eryngii***
Varietal Denomination: **PLE-3**

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(57) **ABSTRACT**

The present invention relates to new, distinct horticultural varieties of mushroom plant of the species *Pleurotus eryngii*. The new varieties, named 'PLE-3', was found by crossbreeding *eryngii* varieties having dominant traits. The varieties are featured by its presentable carpophore, delicacy, stability and uniformity and pronounced distinguishability.

10 Drawing Sheets

VARIETAL DENOMINATION

Latin name of the genus and species of the plant claimed: *Pleurotus Eryngii*.

Variety denomination: *Eryngii 'PLE*-3'.

BACKGROUND OF THE INVENTION

The present invention relates to a new and distinct cultivar of mushroom plant of the species *Pleurotus eryngii*. Particularly, this invention relates to a new cultivar of *eryngii*, which is produced by crossbreeding *eryngii* varieties having dominant traits and featured by its presentable carpophore, delicacy, stability and uniformity and pronounced distinguishability.

Bottle cultivation has started around 1993 in Japan to produce the *eryngii*. In 2008, 38000 tons of *eryngii* was produced in Japan. The *eryngii* grows wild in North America, but it has been sparsely cultivated by a cultivation method using a bag and is in slight demand as food. Recently, bottle cultivation for producing the *eryngii* on a factory scale has started. Since it has been learned that the *eryngii* is low in calories and has beneficial effects of preventing fatty liver, future increase in demand for *eryngii* is expected.

However, the modern cultivar of *eryngii* is good in quality, but it has little adaptability to cultivation conditions such as a culture medium type in the U.S. As an example, a cultivating cycle are somewhat lengthened, thus posing a problem to industrial yieldability.

SUMMARY OF THE INVENTION

In view of the foregoing disadvantages inherent in the known types of *eryngii* now present in the prior art, for the purpose of solving problems in cultivating the modern cultivar of *eryngii* and preserving the quality as well or better than the modern cultivar, the present invention provides mushroom named 'PLE-3' cultivated and produced by crossbreeding parent strains 'HOKUTO PLE-2' and 'MH006376' having superior properties of stability, uniformity and distinguishability.

The successfully cultivated mushroom named 'PLE-3' of the invention is the same strain created by crossbreeding the same parents, so to speak, brothers.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**(A) and **1**(B) are photographs showing the top and bottom of dual culture of two 'PLE-3' strains of the invention.

FIGS. **2**(A) and **2**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and parent strain 'HOKUTO PLE-2'.

FIGS. **3**(A) and **3**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'ATCC32047'.

FIGS. **4**(A) and **4**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'ATCC90212'.

FIGS. **5**(A) and **5**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'ATCC90787'.

FIGS. **6**(A) and **6**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'ATCC90887'.

FIGS. **7**(A) and **7**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'ATCC90888'.

FIGS. **8**(A) and **8**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'ATCC96054'.

FIGS. **9**(A) and **9**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006062'.

FIGS. **10**(A) and **10**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006200'.

FIGS. **11**(A) and **11**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006202'.

FIGS. **12**(A) and **12**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006207'.

FIGS. **13**(A) and **13**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006322'.

FIGS. **14**(A) and **14**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006219'.

FIGS. **15**(A) and **15**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006242'.

FIGS. **16**(A) and **16**(B) are photographs showing the top and bottom of dual culture of 'PLE-3' strain of the invention and strain 'MH006279'.

FIG. **17** is a photograph showing carpophore of 'PLE-3' strain of the invention in a culturing state in a characteristic assay.

FIG. **18** is a photograph showing carpophore of parent strain 'HOKUTO PLE-2' in a culturing state in a characteristic assay.

FIG. **19** is a photograph showing carpophore of 'PLE-3' strain of the invention under normal cultivation.

FIG. **20** is a photograph showing carpophore of parent strain 'HOKUTO PLE-2' under normal cultivation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a new and distinct cultivar of mushroom named 'PLE-3' belonging to *Pleurotus eryngii*, featured by its presentable carpophore, delicacy, stable and consistent productivity and pronounced distinguishability. The mushroom 'PLE-3' of the invention are cultivated in the following cultivation processes.

PROCESSES OF CULTIVATION

July 2004: Strain MH006367 having dominant trait was produced by crossbreeding parent strains HOKUTO PLE-2 and MH006220.

February 2007: Strain MH006376 was produced by crossbreeding an existing strain MH006322 and the aforementioned strain MH006367.

December 2007: Variety PLE-3 was produced by crossbreeding the aforementioned HOKUTO PLE-2 and MH006376, and then, a cultivation experiment and a characteristic assay were made about the variety PLE-3 thus obtained.

August 2008: The cultivation of PLE-3 was concluded after confirmation of distinguishability, stability and uniformity of the strains.

September 2008: With respect to the new variety PLE-3, applications for plant variety registration were filed with Agriculture, Forestry and Fisheries Ministry of Japan and accepted on 5 Sep. 2008.

The place where PLE-3 was asexually reproduced is Mushroom Multidiscipline Laboratory of Hokuto Corporation of 800-8, Shimokomazawa, Nagano-shi, Nagano, Japan.

The asexual reproduction was via 'successive subculture' or 'subculture', that is, a method in which the strain is repeatedly subcultured.

CHARACTERISTICS OF PLE-3

(1) Feature Comparison with Existing Varities

To briefly confirm a genetical new kind of variety, a method for investigating a reluctant reaction in a dual culture can be applied. That is, this method can clarify genetic difference of the new variety from the existing by observing the reluctant reaction in the dual culture. Consequently, PLE-3 of the invention could be identified as new varieties by the dual culture method using the existing varieties (FIGS. **1**(A) and **1**(B)).

[Verification Method]

To verify the reluctant reaction, the dual culture was prepared by placing fungal strains on potato dextrose agar medium at intervals of 3 cm in juxtaposition and cultivating the strains at 25 degrees C. for 28 days. The fungal strains used in the reluctant reaction experiment in the dual culture are shown in Table 1 below.

TABLE 1

Fungal strains used in the reluctant reaction experiment

No.	Strain Name
—	HOKUTO PLE-2
07E2232	PLE-3
1	ATCC32047
2	ATCC90212
3	ATCC90787
4	ATCC90887
5	ATCC90888
6	ATCC96054
7	MH006062 (commercial strain: Fujita's Eryngii)
8	MH006200 (commercial strain: JA Fukuoka)
9	MH006202 (commercial strain: Yukiguni Maitake)
10	MH006207 (commercial strain: Masuda-Kashiwagi Mushroom)
11	MH006322 (commercial strain: Chokin-15)
12	MH006219 (commercial strain: Wild strain #1 grown in Italy)
13	MH006242 (commercial strain: Wild strain #2 grown in Italy)
14	MH006279 (commercial strain: Wild strain #1 grown in Spain)

[Results:]

The results of the reluctant reaction experiment are shown in Table 2. In Table 2, the mark "+" denotes occurrence of reluctant reaction, and the mark "–" denotes no reluctant reaction.

All strains other than PLE-3 showed reluctant reaction relative to PLE-3 (07E2232) of the invention.

TABLE 2

Results of dual culture

Strain No.	PLE-3
07E2232 (PLE-3)	–
HOKUTO PLE-2	+
1 (ATCC32047)	+
2 (ATCC90212)	+
3 (ATCC90787)	+
4 (ATCC90887)	+
5 (ATCC90888)	+
6 (ATCC96054)	+
7 (MH006062)	+
8 (MH006200)	+
9 (MH006202)	+
10 (MH006207)	+

TABLE 2-continued

Results of dual culture	
Strain No.	PLE-3
11 (MH006322)	+
12 (MH006219)	+
13 (MH006242)	+
14 (MH006279)	+

+: Reluctant reaction occurred
−: No reluctant reaction occurred

(2) Cultural Characteristics of PLE-3

The strain PLE-3 of the invention was cultivated on the potato dextrose agar medium to be investigated in mycelial characteristics and so on. Consequently, the mycelia of all of the strains energetically grew. The growing rate of the mycelia at 20 degrees C. was about 3 mm a day (colony diameter). The strain having a flora surface of white (RHS: NN155C) could be obtained.

(3) Morphologically Characteristics of PLE-3

[Cultivation Method] (Examination Standard for *Eryngii* prescribed for plant variety registration based on Japanese Seeds and Seedlings Law was adopted.)

Bottle cultivation was performed using a polypropylene bottle having a bore diameter of 58 mm and a capacity of 850 ml. A culture medium was prepared by mixing cedar sawdust, bran and corn bran at the mixture ratio of 10:3:0.5 and regulating the water content to 65%. The cultivating bottle was filled up to the bottle shoulder with 510±10 grams of the culture media, and then, sterilized at high pressure. After the culture medium cooled, about 15 ml of sawdust seed fungus was implanted in the culture medium. The culture medium was controlled at 23 degrees C. and at a humidity of about 70%.

The culture medium was subjected to fungus scratching treatment after being cultivated for 40 days, and then, moved to an emergence room without dilution by water. The emergence room was controlled at a temperature of 17±1 degrees C., a humidity of 90%, an illumination intensity of 200 LUX and CO_2 concentration of approximately 2000 ppm. Mushrooms thus grown were harvested three days after the mushroom cap at the center of the carpophore spreads horizontally. The carpophore thus harvested was observed to determine the shape of its pileus. The results of verification based on Examination standard for *Eryngii* prescribed for plant variety registration based on Japanese Seeds and Seedlings Law will be described below.

[Results]

Morphological Characteristics of PLE-3

The pileus of PLE-3 has a concave shape. There was a sharp difference in that PLE-3 has a thickness of 15.38 mm. With respect to the shape of hymenophorum, PLE-3 has a mushroom stem moderately attached to the pileus. The pileus assumes an "emarginatd" shape, i.e. is shaped in "upturn". The mushroom stem of PLE-3 has a relatively thick lower part. The mushroom stem of PLE-3 is 79.41 mm in length. The mushroom stem of PLE-3 is 26.7 mm in diameter. With respect to the other features than those noted above, PLE-3 has 3.14 carpophore stems on an effective average. The gill attachment of 'PLE-3' is essentially subdecurrent. The characteristic values of the mushrooms 'PLE-3' shown in Table 3 express the attaching intensity of the subdecurrent to the stem. The attaching intensity of the subdecurrent to the stem of the mushroom 'PLE-3' is "moderate" as shown in the table.

These characteristic features of PLE-3 are shown in Table 3 below. The distinctions between the new variety of the invention and the parent varieties "PLE-2" and "MH006376" are apparent from Table 3. MH006376 is an intermediate variety derived from the process of growing PLE-3, i.e., the variety obtained through mating experiments carried out in the laboratory. That is, the variety of PLE-3 and MH006376 are different in that the growing of the variety of PLE-3 has been completed, but the growing of MH006376 was insufficient. Furthermore, MH006376 having a small yielding ability (90.2 grams) and a small number of stems (two or three stems) is further distinguished from the variety of the present invention.

TABLE 3

Characteristic Features of PLE-3 (Characteristics of PLE-3 and characteristics of commercial similar variety HOKUTO PLE-2) Investigation place: Mushroom Laboratory, Hokuto Corporation of Shimokomazawa, Nagano-shi, Nagano, Japan Investigation Period: 1st May, 2008 to 20th August, 2008		
Trait	PLE-3	HOKUTO PLE-2
(1) Fungal Property		
Fungal density	Medium	Medium
Growth status of aerial mycelia	Normal	Normal
Peripheral shape of flora	Concentric	Concentric
Color of upper surface of flora	White (RHS: N155C)	White (RHS: N155C)
Color of lower surface of flora	Cream (RHS: 11D)	Cream (RHS: 11D)
(2) Temperature Adaptability		
Optimum temperature of mycelial growth (° C.)	27	28
Growth rate at respective temperatures (mm/day)		
5° C.	0.45	0.35
10° C.	1.18	1.46
15° C.	1.8	1.96
20° C.	2.95	3.73
25° C.	6.04	6.05
30° C.	5.22	5.96
(3) Morphological Features		
Pileus		
Shape	Concave	Flat
Pattern	Streaky	Streaky
Surfice protrusion	None	None
Size (mm)	51.01	54.89
Color of center part	(RHS: 199D)	(RHS: 199D)
Color of periphery	(RHS: 199D)	(RHS: 199D)
Thickness (mm)	15.38	15.51
Tenderness	Tough	Tough
Hymenophorum		
Alignment	Linear	Linear
Attaching intensity to stem	Moderate	Moderate
Width (mm)	3.17	3.07
Color	(RHS: 161D)	(RHS: 161D)
Stem		
Shape	Thick lower part	Thick lower part
Attaching to pileus	Center part	Center part
Length (mm)	79.41	86.87
Ratio of pileus diameter to stem length	0.66	0.64
Diameter (mm)	26.7	28.04
Color	Pale yellow (RHS: 158D)	Pale yellow (RHS: 158D)

TABLE 3-continued

Characteristic Features of PLE-3 (Characteristics of PLE-3 and characteristics of commercial similar variety HOKUTO PLE-2) Investigation place: Mushroom Laboratory, Hokuto Corporation of Shimokomazawa, Nagano-shi, Nagano, Japan Investigation Period: 1st May, 2008 to 20th August, 2008		
Trait	PLE-3	HOKUTO PLE-2
Tenderness	Tough	Tough
Growing development figure of carpophore		
Development figure	Monogenic	Monogenic
Effective stem number	3.14	3.07
Period up to development peak (day)	64.15	64.71
Period from fungus scratching to development (day)	24.15	24.71
Temperature Adaptability		
Optimum temperature of development (° C.)	17	17
Optimum temperature of growing (° C.)	17	17
Yield (g)	134.23	121.1

*"RHS" in the items related to color is short for Royal Horticultural Society, and the color codes found above are referred to the color charts stipulated by RHS (Fifth Edition, 2007).

(4) Embodiments in Case of Cultivation in a Culture Medium for Commercial Production

The culture media prescribed as above were used herein to evaluate the varieties cultivated according to the invention and identify the morphological features of the varieties, but the culture media applicable for cultivating the object varieties have been improved to increase productivity of the subject mushrooms in an actual commercial production. Accordingly, the quality of the mushrooms actually produced may possibly not be consistent with the resultant data obtained from the cultivation experiment as described above. Thus, the mushrooms produced commercially in actuality according to the invention are demonstrated in FIGS. **19** and **20**.

What is claimed is:

1. A new and distinct variety of mushroom as substantially illustrated or described in the specification.

* * * * *

FIG. 1(A)

FIG. 1(B)

FIG. 2(A)

FIG. 2(B)

FIG. 3(A)

FIG. 3(B)

FIG. 4(A)

FIG. 4(B)

FIG. 5(A)

FIG. 5(B)

FIG. 6(A)

FIG. 6(B)

FIG. 7(A)

FIG. 7(B)

FIG. 8(A)

FIG. 8(B)

FIG. 9(A)

FIG. 9(B)

FIG. 10(A)

FIG. 10(B)

FIG. 11(A)

FIG. 11(B)

FIG. 12(A)

FIG. 12(B)

FIG. 13(A)

FIG. 13(B)

FIG. 14(A)

FIG. 14(B)

FIG. 15(A)

FIG. 15(B)

FIG. 16(A)

FIG. 16(B)

FIG. 17

FIG. 18

FIG. 19

FIG. 20