US00PP35511P3

(12) **United States Plant Patent**
Nakagoshi et al.

(10) **Patent No.: US PP35,511 P3**
(45) **Date of Patent: Nov. 28, 2023**

(54) **BUNASHIMEJI MUSHROOM NAMED 'HKHM25'**

(50) Latin Name: ***Hypsizygus marmoreus***
Varietal Denomination: **HKHM25**

(71) Applicant: **HOKUTO CORPORATION**, Nagano (JP)

(72) Inventors: **Ayumi Nakagoshi**, Nagano (JP); **Aya Seki**, Nagano (JP); **Chiaki Goto**, Nagano (JP)

(73) Assignee: **HOKUTO CORPORATION**, Nagano (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 183 days.

(21) Appl. No.: **17/803,159**

(22) Filed: **Mar. 8, 2022**

(65) **Prior Publication Data**

US 2022/0304209 P1 Sep. 22, 2022
US 2023/0148465 P4 May 11, 2023

(51) **Int. Cl.**
A01H 15/00 (2006.01)

(52) **U.S. Cl.**
USPC **Plt./394**
CPC ***A01H 15/00*** (2013.01)

(58) **Field of Classification Search**
USPC Plt./394
See application file for complete search history.

Primary Examiner — Annette H Para
(74) *Attorney, Agent, or Firm* — Taro Yaguchi

(57) **ABSTRACT**

The present variety of Bunashimeji mushroom named 'HKHM25' was cultivated by the gathering and repeated breeding of Bunashimeji mushrooms having thick and elastic stems, dark cap color, strong cap roll, and a large mushroom size, has a high quality and enhanced cultivation stability. This edible mushroom is exquisite in stability, reproducibility and uniformity when being produced.

25 Drawing Sheets

BACKGROUND OF THE INVENTION

This invention relates to a new and distinct variety of Bunashimeji mushroom, *Hypsizygus marmoreus* (Peck) H.E.Bigelow. This new variety named 'HKHM25', cultivated by repeated breeding of Bunashimeji mushroom having thick and elastic stems, dark cap color, strong cap roll, and a large mushroom size, has a high quality and enhanced cultivation stability, and ensures presentable stability, reproducibility, and uniformity.

The annual production of Bunashimeji mushrooms is approximately 120,000 tons in 2020, making it the second most consumed edible mushroom after enokitake mushroom in Japan. However, since Bunashimeji mushrooms have become available in large quantities in the market, Bunashimeji mushrooms with improved flavor, quality and shelf life has been pursued, which led to the development of 'Hokuto 18gokin' by our company. Further, our company has been conducting a wide variety of studies for the more stable cultivation and better quality of Bunashimeji mushrooms. Our company has developed a 'marmo22go' mushroom which is thicker, whiter, and has larger mushroom than 'Hokuto 18gokin'.

Further, using 'marmo22go' as a parent, as a result of continuing breed improvement by cross breeding to improve stability and quality, 'HKHM25', which has thicker stems and stronger cap roll, as compared to 'marmo22go', was developed, confirming its stability, reproducibility, and uniformity.

SUMMARY OF THE INVENTION

The present invention is a new and distinct variety of mushroom plant characterized particularly by having thick and elastic stems, dark cap color, strong cap roll, and a large mushroom size, which can be cultivated by gathering and repeated breeding of fungal strains having a stability, reproducibility and uniformity when being produced. This novel and distinct variety of mushroom is identified as 'HKHM25'.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows a phylogenetic tree illustrating the antecedents of 'HKHM25'.

FIGS. **2**A and **2**B respectively show front and back images of a dual-culture of 'HKHM25'.

FIGS. **3**A and **3**B respectively show front and back images of a dual-culture of 'HKHM25' and 'Hokuto 18gokin'.

FIGS. **4**A and **4**B respectively show front and back images of a dual-culture of 'HKHM25' and 'marmo22go'.

FIGS. **5**A and **5**B respectively show front and back images of a fungal flora of 'HKHM25' grown in a petri dish.

FIGS. **6**A and **6**B respectively show front and back image of a fungal flora of 'Hokuto 18gokin' grown in a petri dish.

FIGS. **7**A and **7**B respectively show front and back images of fungal flora of 'marmo22go' grown in a petri dish.

FIG. **8** shows an image of cultivation area of 'HKHM25'.

FIG. **9** shows an image of cultivation area of 'Hokuto 18gokin'.

FIG. **10** shows an image of cultivation area of 'marmo22go'.

FIG. **11** shows an image of a fruit body of 'HKHM25'.

FIG. **12** shows an image of a fruit body of 'Hokuto 18gokin'.

FIG. **13**: shows an image of a fruit body of 'marmo22go'.

FIG. **14** shows an image of density and surface color of fungal flora in cultured hyphae of 'HKHM25'.

FIG. **15** shows an image of density and surface color of fungal flora in cultured hyphae of 'Hokuto 18gokin'.

FIG. **16** shows an image of density and surface color of fungal flora in cultured hyphae of 'marmo22go'.

FIG. **17** shows an image of development of aerial hypha of 'HKHM25'.

FIG. **18** shows an image of development of aerial hypha of 'Hokuto 18gokin'.

FIG. **19** shows an image of development of aerial hypha of 'marmo22go'.

FIG. **20** shows an image of size and distribution of mottle of 'HKHM25'.

FIG. **21** shows an image of size and distribution of mottle of 'Hokuto 18gokin'.

FIG. **22** shows an image of size and distribution of mottle of 'marmo22go'.

FIG. **23** shows an image of shape and thickness of stipe of 'HKHM25'.

FIG. **24** shows an image of shape and thickness of stipe of 'Hokuto 18gokin'.

FIG. **25** shows an image of shape and thickness of stipe of 'marmo22go'.

DETAILED DESCRIPTION OF THE INVENTION

'HKHM25' has been asexually reproduced by tissue culture, at HOKUTO CORPORATION in Japan. The history of the 'HKHM25' mushroom in terms of improvement period and the like are set forth in the following chronological list of each stage of variety improvement:

December 2014: Cultivation of 'MH025615' strain.

January 2017: Cultivation of 'M11025617' ('marmo22go') strain.

December 2019: 'MH025615' and 'MH025617' ('marmo22go') strains were crossed and a strain with thick stem, strong cap roll, and good quality among the obtained strains was selected as 'MH025633'. We then repeated the cultivation test to distinguish the strains.

March 2021: After repeated cultivation tests, since distinguishability, stability, and uniformity were confirmed, the strain was named 'HKHM25' and its cultivation was completed. Applied for registration of a new variety to the Ministry of Agriculture, Forestry and Fisheries of Japan.

The above crossing is summarized in the phylogenetic tree illustrated in FIG. **1**.

The 'HKHM25' mushroom has the following characteristics: thick and elastic stems, dark cap color, strong cap roll, and overall large size.

(1) Comparison with Existing Variety by Dual Culture

Dual culture was performed for the 'HKHM25' mushroom and a similar variety to examine whether or not a zone line is formed.

Study Method:

As an examination method, a potato dextrose agar medium was used, and the 'HKHM25' mushroom and the similar variety were inoculated thereon face to face at an interval of 3 cm, and then culture was performed at 25° C. for 28 days to examine whether or not a zone line was formed.

Strains used for the comparison between present variety, 'HKHM25' and other varieties:

'HKHM25': Present variety

'Hokuto 18gokin': Variety similar to the present variety

'marmo22go': Parent variety of the present variety

Results:

Zone lines were formed between 'HKHM25' and all other co-cultured varieties

(TABLE 1, FIGS. 2 to 4)

This clearly shows that the present mushroom is a new variety.

	Similar varieties	
	Hokuto 18gokin	marmo22go
HKHM25	+	+

+ is present and − is absent.

*Zone line formation was not observed in the dual culture between 'HKHM25' strains.

(2) Growth Characteristics of 'HKHM25'

Study Method:

After inoculating an agar piece of the 'HKHM25' having a diameter of 5 mm and an agar piece of the similar variety having a diameter of 5 mm on a potato dextrose agar medium, preculture was performed at 25° C. for 4 days so as to make the regeneration of hyphae equal (about 10 mm in diameter), and then culture was performed for 7 days at intervals of 5° C. between 5° C. and 30° C. An average daily hyphae growth rate was calculated based on a hyphae growth rate for 7 days of the culture. Also, the optimum culture period for 'HKHM25' is 80 days, and the number of growing days at that time is 23.1 days.

Results:

'HKHM25' had the fastest average daily hyphae growth rate at 20° C. In addition, HKHM25 had slower mycelial growth rate than 'Hokuto 18gokin' and 'marmo22go' at each temperature zone (Table 2).

(3) Morphological Characteristics of the 'HKHM25' Mushroom in a Cultivation Example

Cultivation Method:

Container: An 850 polypropylene bottle (Capacity: 850 ml, diameter 58 mm) was used.

Culture medium: Conifer sawdust, corn cob, rice bran and wheat bran were mixed at the dry weight ratio of 7:3:8:2, and the water content was adjusted to 65%. The culture medium was filled up to the brim of the bottle at the rate of 540±20 g per bottle, and was sterilized at high pressure.

Starter culture: About 20 ml of sawdust starter cultures per bottle was inoculated.

Culture: Culture was performed at 22° C. for 50 to 90 days at 70% moisture.

Growth: After completing the culture, the inoculum is removed, and shifted to a growing room. Development was conducted under a temperature of 15±1° C., at humidity of 95% or about 2,000 ppm CO_2 density. Also, light was not particularly irradiated until the first 14th day, then irradiated with about 500 to 1,000 Lx. The mushroom is harvested when the cap in the center of the stump has sufficiently opened.

Cultivation Results:

Table 2 shows the characteristics of the 'HKHM25' and specific difference in characteristics as compared with the similar variety when culture was performed under the abovementioned conditions.

In addition, according to R.H.S. Colour Chart, the color of the central part of cap: 199A, the color of the peripheral area of cap: 199C, the color of gill: 158C, the color of stripe: 155B. All color references are from The R.H.S. Colour Chart of The Royal Horticultural Society of London (R.H.S.), fifth edition published in 2007. Also, the descriptions are from mushrooms that are on the 24th day.

TABLE 2

Fungus characteristics Table of *Hypsizygus marmoreus* (Peck) H.E. Bigelow of Recording and Registration
(Please circle the applicable items for the characteristics.)

	Characteristic values of present variety (comparison with standard varieties									Remarks (Measured values,	Characteristic values of similar varieties	
Character	01	02	03	04	05	06	07	08	09	etc.)	(marmo22go)	(Hokuto 18gokin)
Physiological property												
Dual culture												
Zone line formation	none								observed	—	09	09
Dislike-touch reaction	none								observed	—	01	01
Density of cultured hyphae	low	medium	high							03	02	02
Development of aerial hypha	low	medium	high							03	03	02
Color of surface of fungal flora	white	light yellow	pale yellow						other	02	01	01
Color of back of fungal flora	white	light yellow	pale yellow						other	02	02	02
Accommodativeness for temperature												
Optimal temperature for hyphal growth			22° C.		24~26° C.		28~30° C.			03 21.9° C.	05 24.1° C.	04 22.5° C.
Hyphal growth rate 5° C./mm			low		medium		high			05 0.52 mm	06 0.70 mm	05 0.56 mm
Hyphal growth rate												
10° C./mm			low		medium					04 0.86 mm	05 1.16 mm	05 1.24 mm
15° C./mm			low		medium		high			05 1.65 mm	05 1.95 mm	05 1.80 mm
20° C./mm			low		medium		high			04 2.47 mm	05 2.79 mm	05 2.60 mm
25° C./mm			low		medium		high			04 2.01 mm	05 3.29 mm	04 2.47 mm
30° C./mm			low		medium		high			04 0.91 mm	07 2.17 mm	05 1.23 mm
Morphological property												
Cap												
Cross sectional shape	convex	round fungal	flat	concave					other	02	02	02

TABLE 2-continued

Fungus characteristics Table of *Hypsizygus marmoreus* (Peck) H.E. Bigelow of Recording and Registration
(Please circle the applicable items for the characteristics.)

Character	Characteristic values of present variety (comparison with standard varieties)									Remarks (Measured values, etc.)	Characteristic values of similar varieties	
	01	02	03	04	05	06	07	08	09		(marmo22go)	(Hokuto 18gokin)
Mottle of the surface	few	medium	many							02	02	02
Size of mottle	small	medium	large							03	01	03
Distribution of mottle	center part	whole part								01	02	02
Clarity of mottle	unclarity	moderate	clarity							03	03	03
Size			small		medium		large			03 21.2 mm	03 23.2 mm	02 20.4 mm
Color of central area	white	grey-yellow	light grey-brown	yellow-brown	[illegible]	dark tan	dark grey tan	dark grey brown	other	05 199A	05 199C	05 199B
Color of peripheral area	white	grey-yellow	light grey-brown	yellow-brown	[illegible]	dark tan	dark grey tan	dark grey brown	other	05 199C	05 199D	05 199D
Thickness			thin		medium		thick			08 9.8 mm	09 11.3 mm	0.7 8.1 mm
Fleshy			soft		medium		hard			05	05	07
Gill												
Color	white	[illegible]	light orange yellow	grey-yellow					other	02 158C	02 158C	02 158C
Alignment	normal	[illegible]							other	02	02	02
Width	narrow	medium	wide							02	02	02
Density	low	Medium	high							02	02	02
Stipe												
Shape	long and thin	short and thin	long and thick	short and thick	Medium					05	05	01
Length			short		Medium		long			05 56.0 mm	05 53.9 mm	04 52.8 mm
Thickness			thin		medium		thick			09 17.7 mm	08 16.5 mm	05 9.4 mm
Color	white	yellow-white	grey	grey-brown					other	01 155B	01 155B	01 155B
Hair	absent	few	medium	many						01	01	01
Fleshy	soft	medium	hard							03	02	02

TABLE 2-continued

Fungus characteristics Table of *Hypsizygus marmoreus* (Peck) H.E. Bigelow of Recording and Registration
(Please circle the applicable items for the characteristics.)

Character	Characteristic values of present variety (comparison with standard varieties) 01	02	03	04	05	06	07	08	09	Remarks (Measured values, etc.)	Characteristic values of similar varieties (marmo22go)	(Hokuto 18gokin)
Ratio of maximum diameter of stem to diameter just below cap			small		medium		(large)			07 2.24	06 1.91	04 1.52
Cultural property												
Develop-ment of fruit body												
Develop-ment	group	scattered	(stock)							03	03	03
Optimal culture period			short		(Med-ium)		long			05 80 days	05 80 days	06 90 days
Length of time from fruit body-formation promotion to fruit body harvesting			short		(Med-ium)		long			05 23.3 days	04 21.9 days	05 22.3 days
Optimal temperature for primordial development			low		medium		(high)			07 19° C.	07 19° C.	05 15° C.
Optimal temperature for fruit body growth			low		(Med-ium)		high			05 15° C.	05 15° C.	05 15° C.
Adaptivity for illuminance												
Adaptivity for culture												
Yield												
Yield of fruit body			few		(Med-ium)		many			05 125.4 g	06 137.0 g	02 98.4 g
The number of productive stems		(○)	few		medium		many			02 19.4 stems	03 21.7 stems	03 23.6 stems
Other property												
Bitter ingredient	none	weak	strong									
Disease resistance												
Ingredients												

Notes:
Circle shows the characteristics of 'HKHM25'.

The R.H.S. Colour Chart of The Royal Horticultural Society of London (R.H.S.), fifth edition published in 2007, The R.H.S. contact: R.H.S. Membership Department The Royal Horticultural Society PO Box 313 London, SW1P 2PE

What is claimed is:

1. A new, distinct variety of Bunashimeji Mushroom as substantially illustrated and described in the specification.

* * * * *

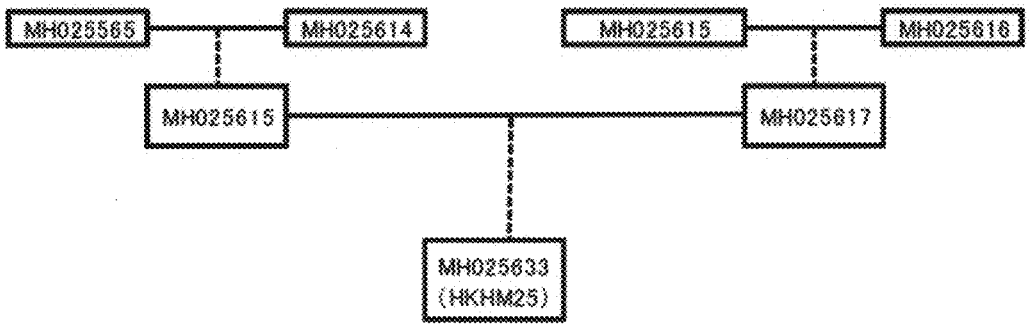
MH025565
MH025614
MH025615
MH025616
MH025615
MH025617
MH025633
(HKHM25)

FIG. 1

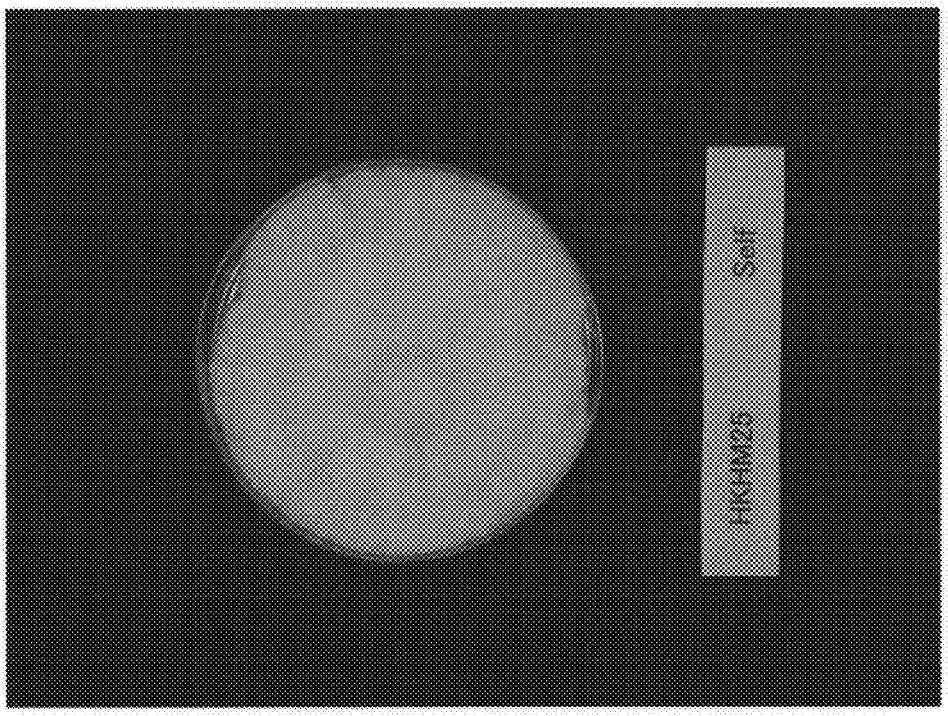

FIG. 2A

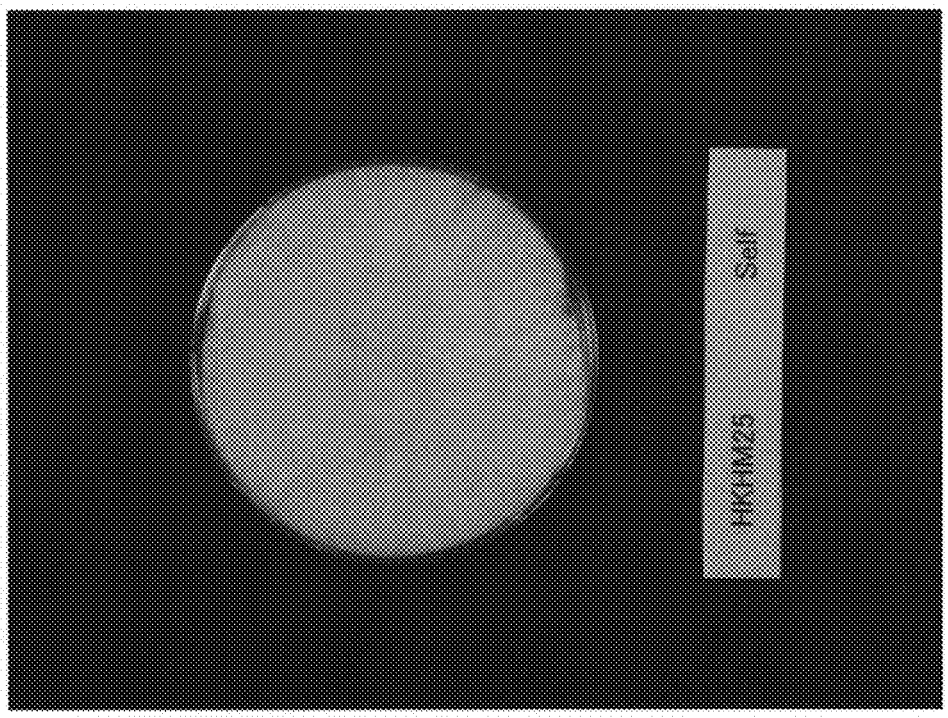

FIG. 2B

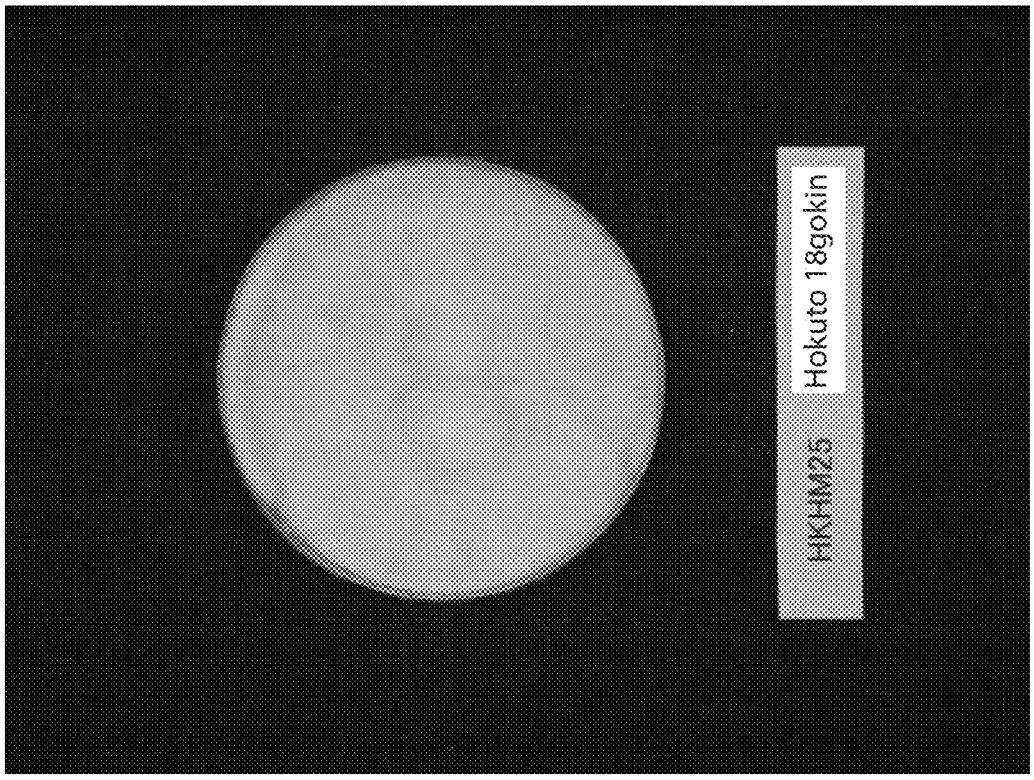

FIG. 3A

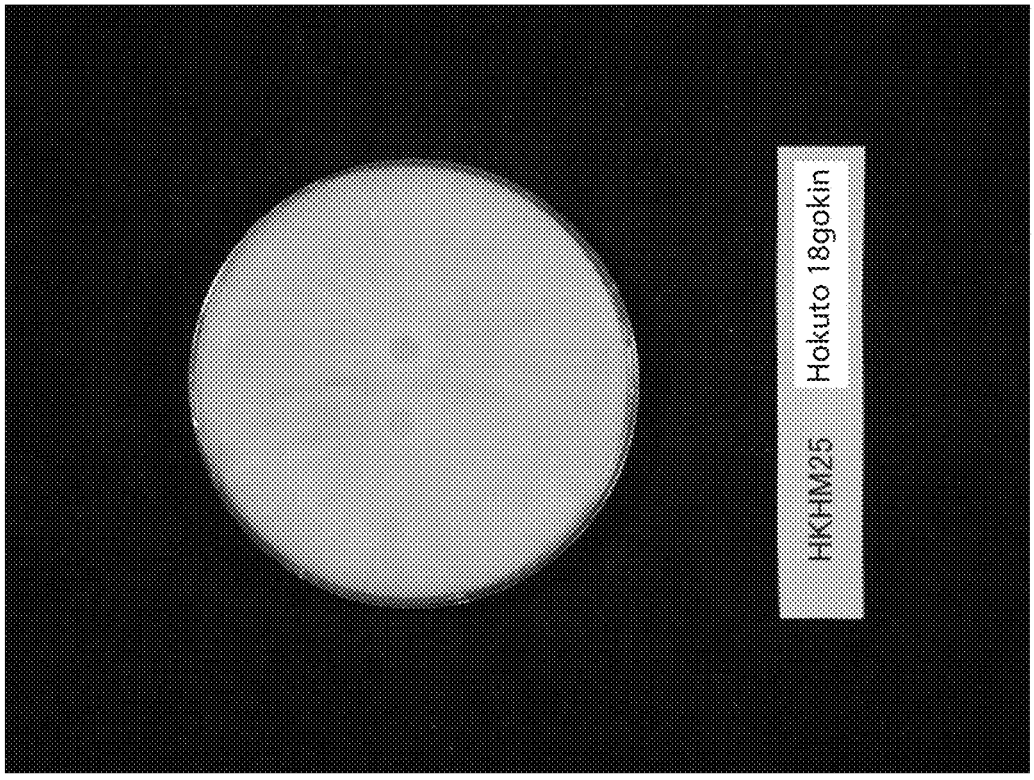

FIG. 3B

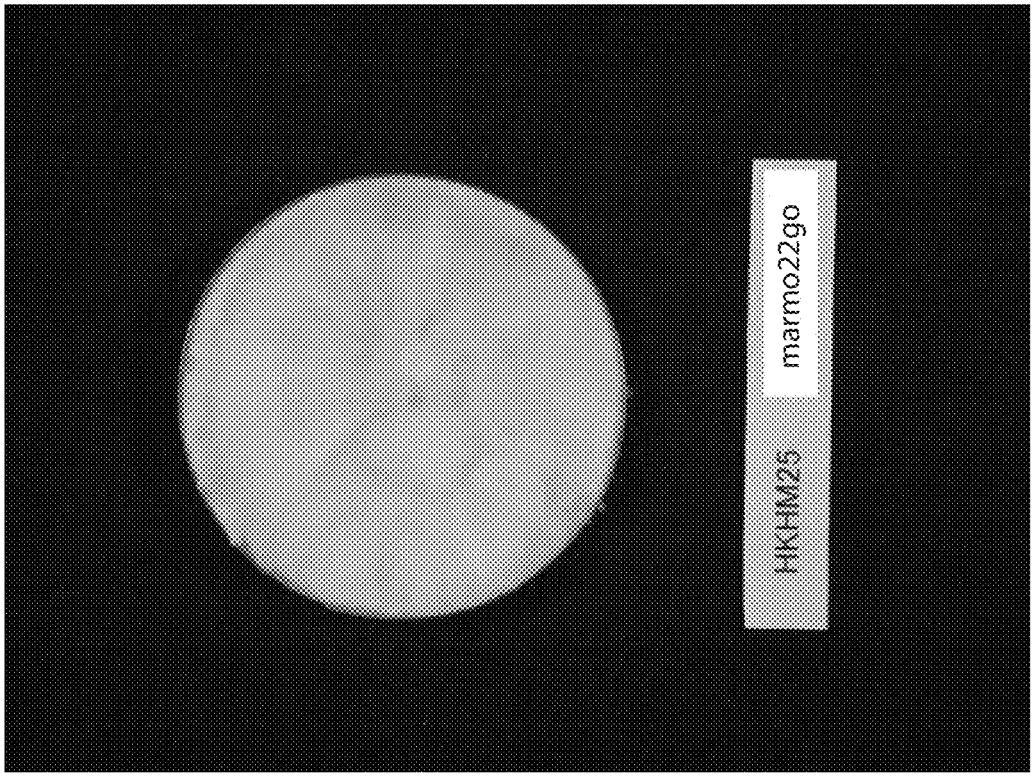

FIG. 4A

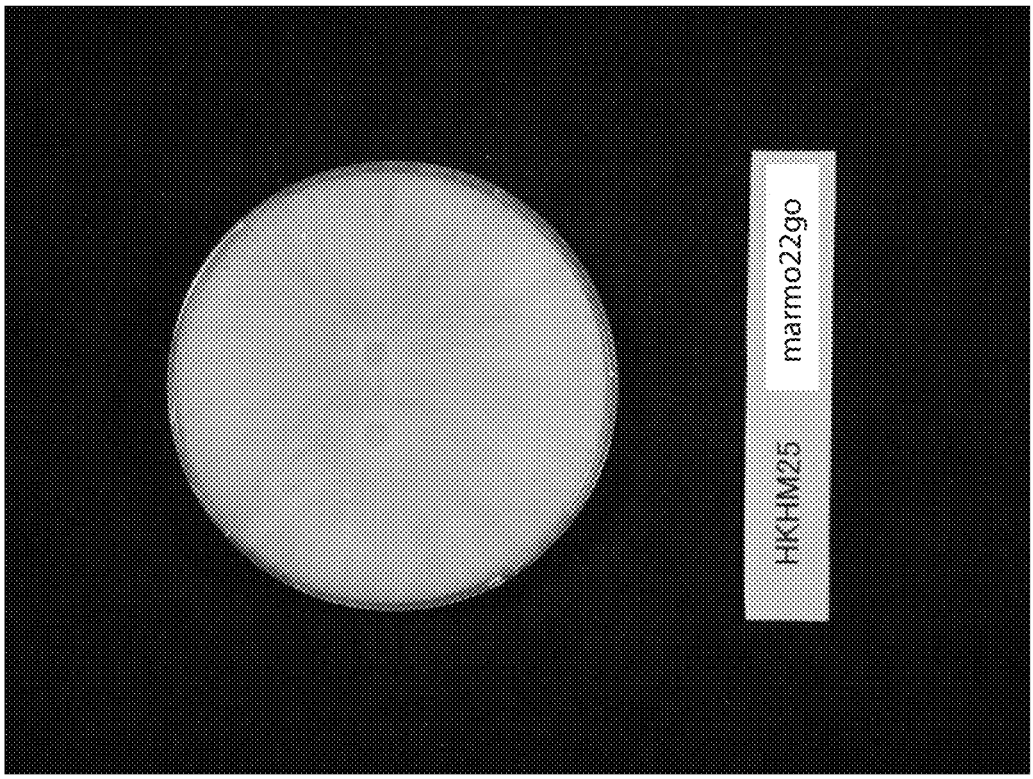

FIG. 4B

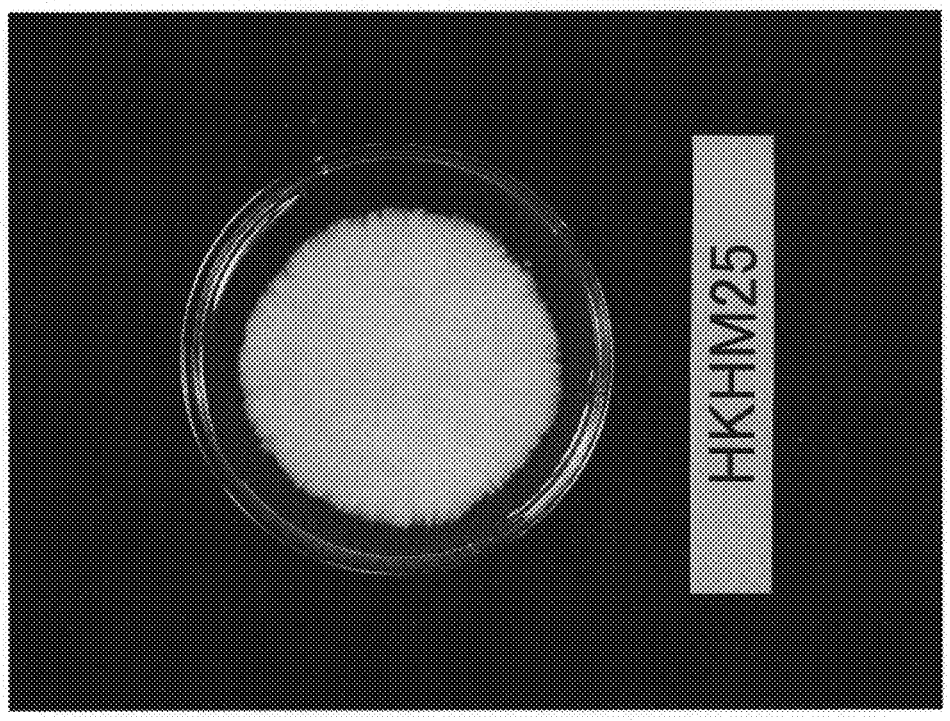

FIG. 5A

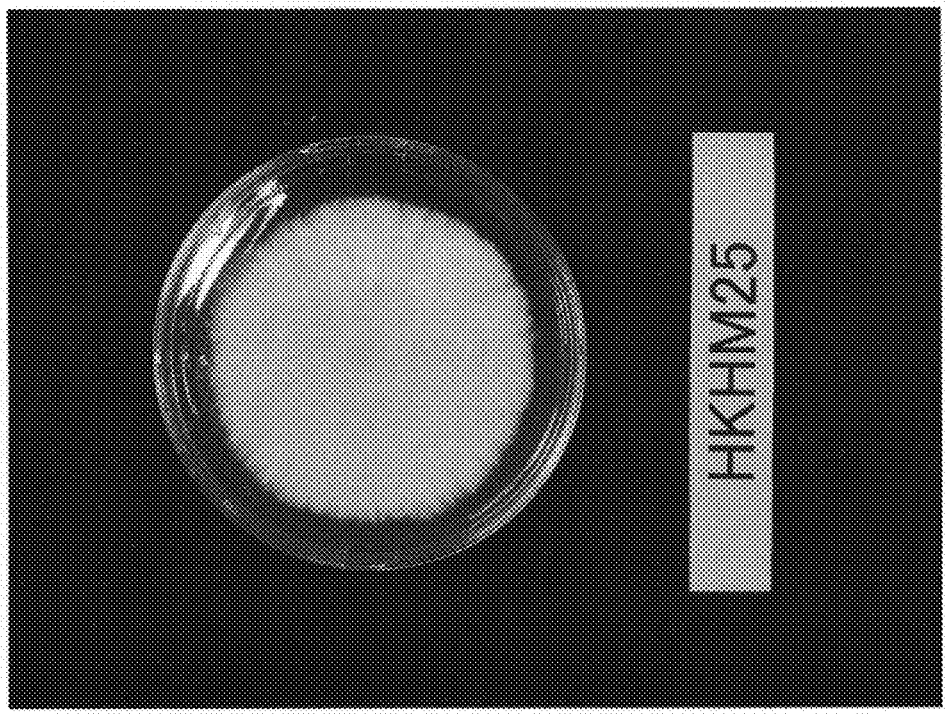

FIG. 5B

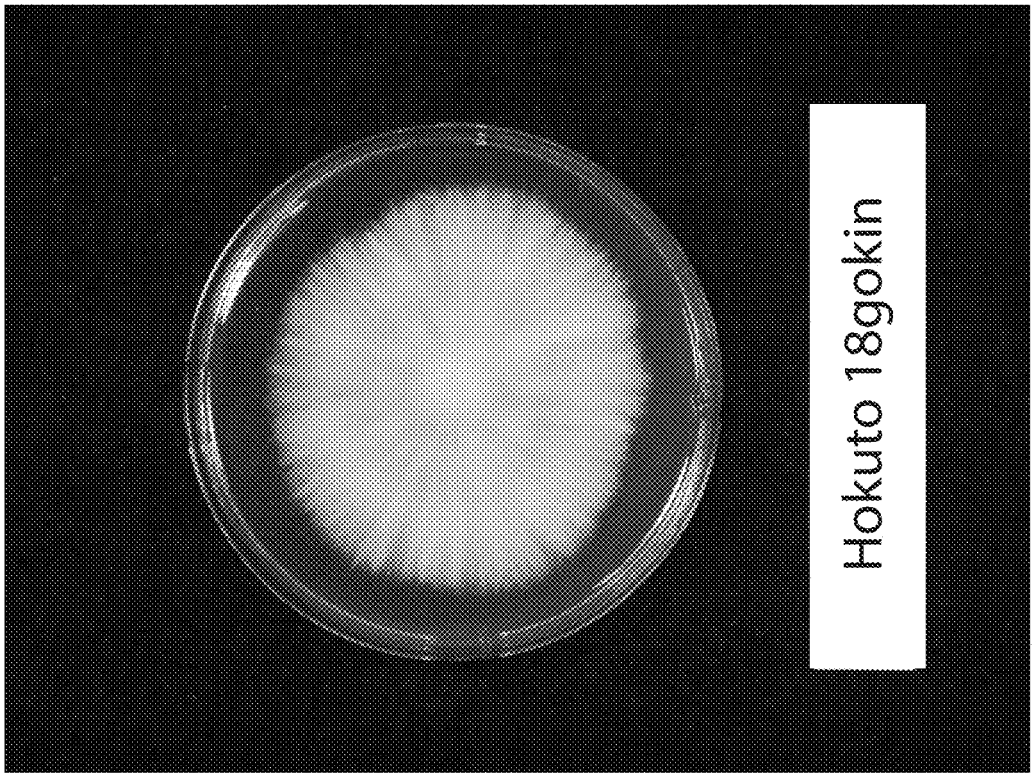

FIG. 6A

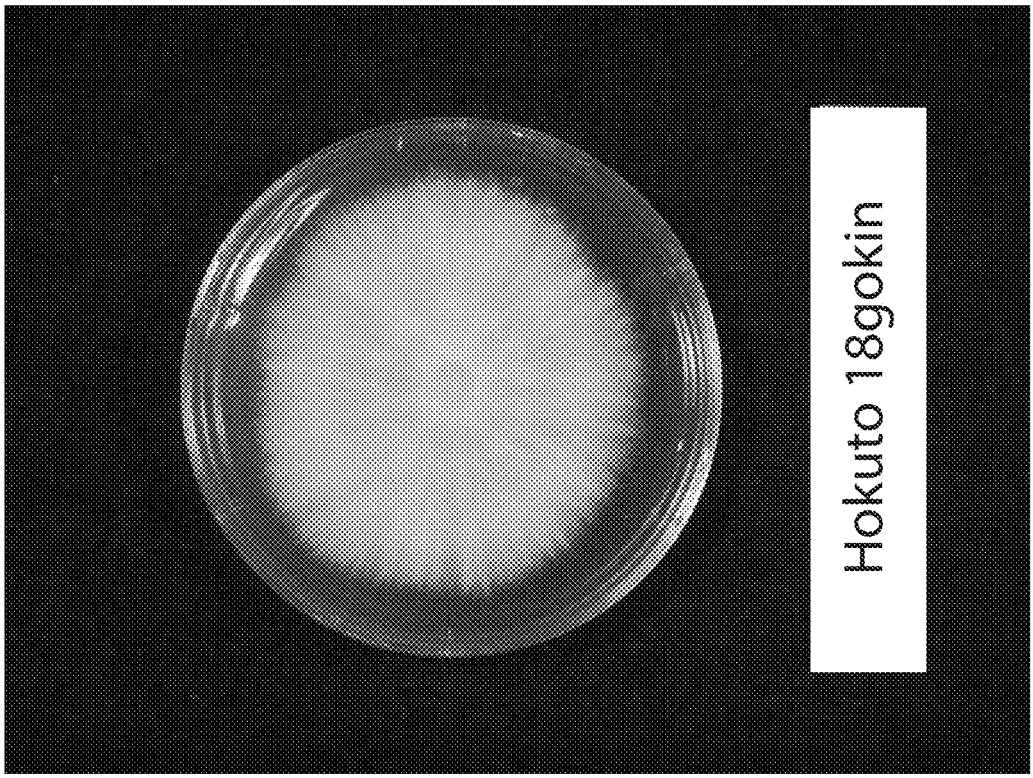

FIG. 6B

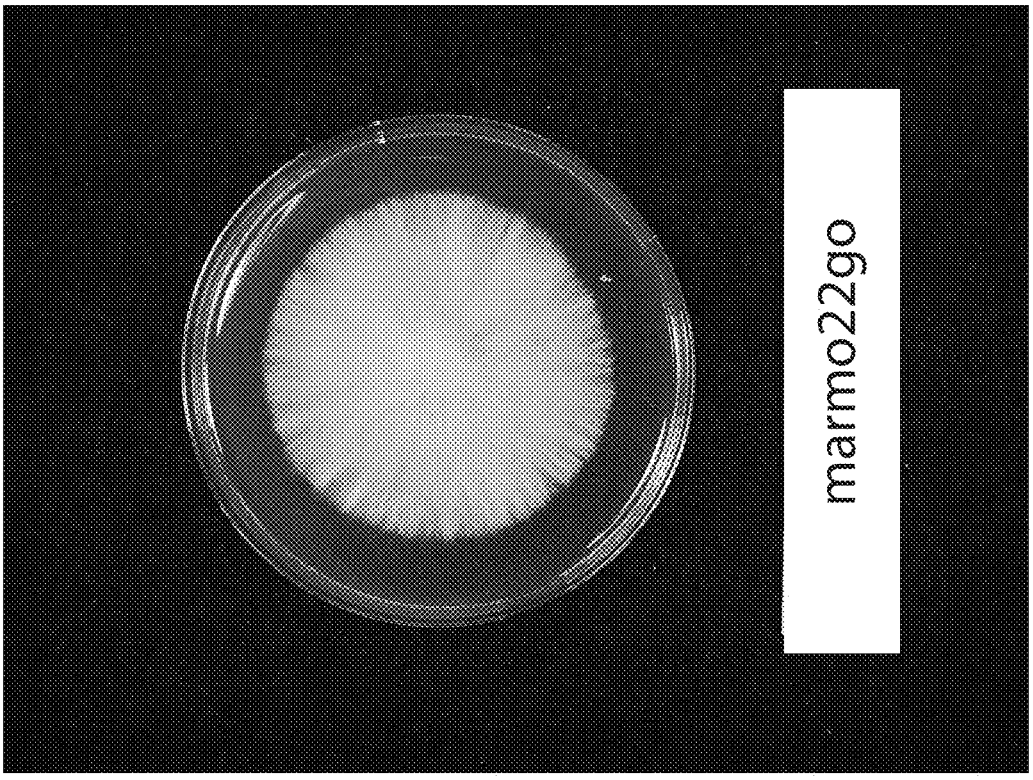

FIG. 7A

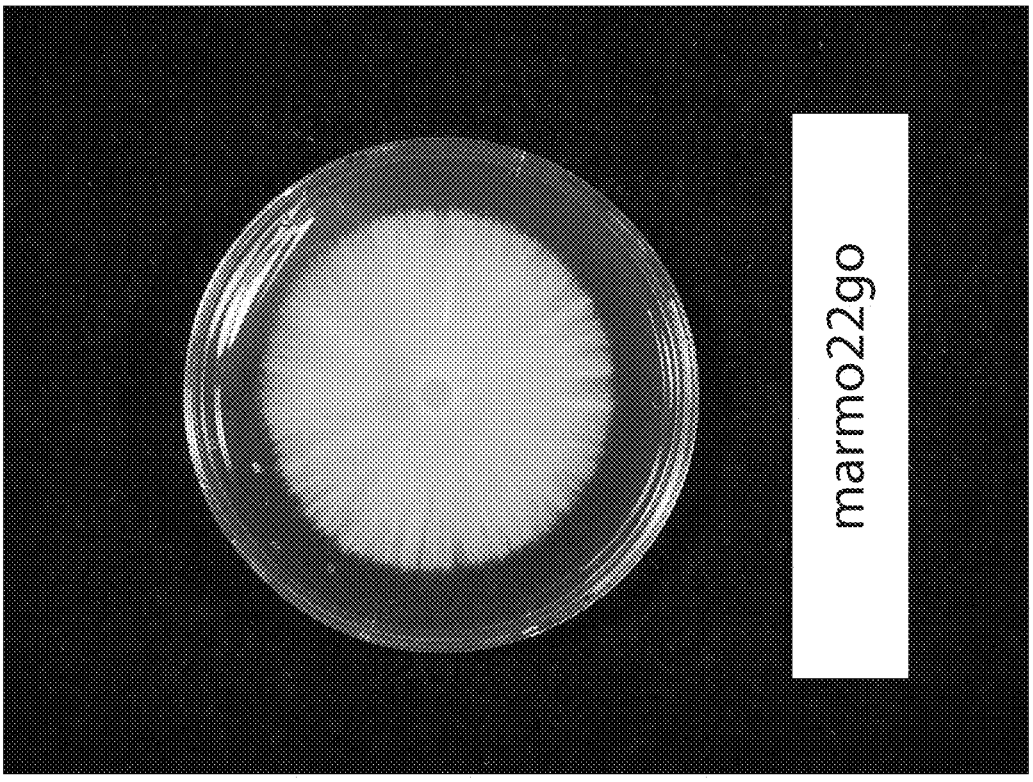

FIG. 7B

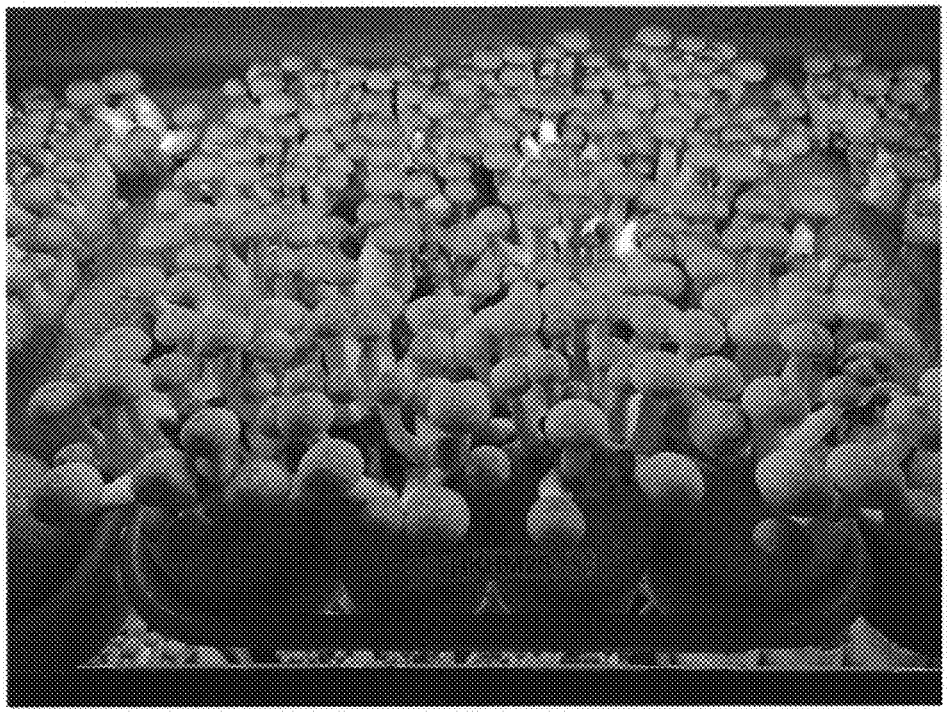

FIG. 8

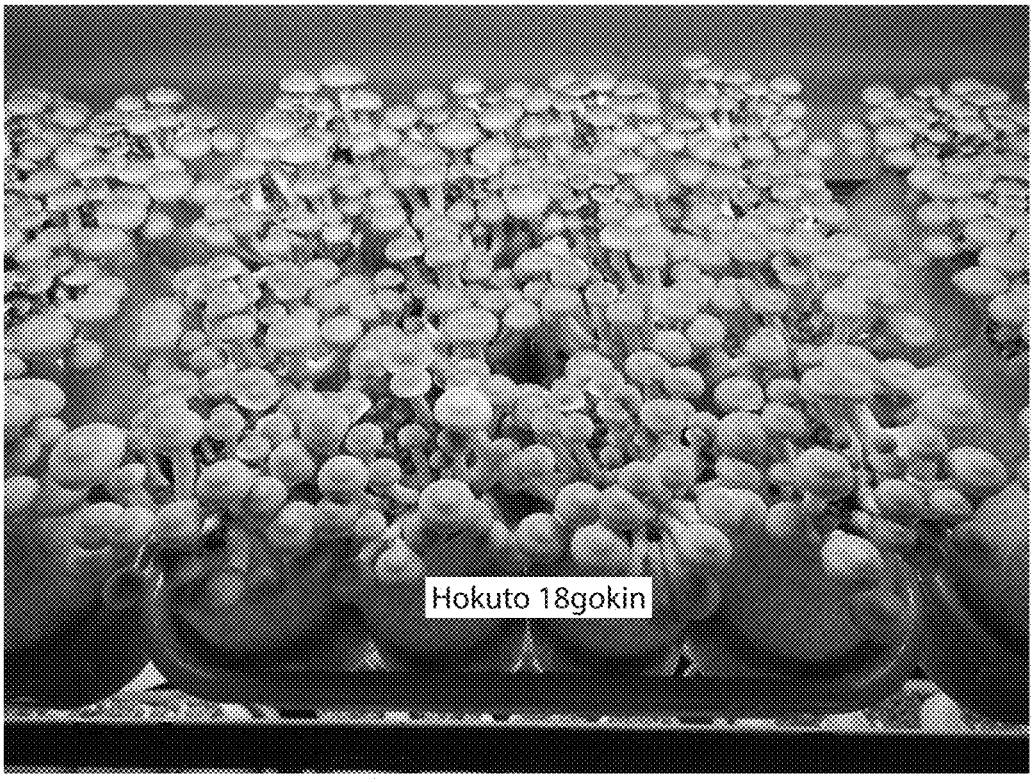

FIG. 9

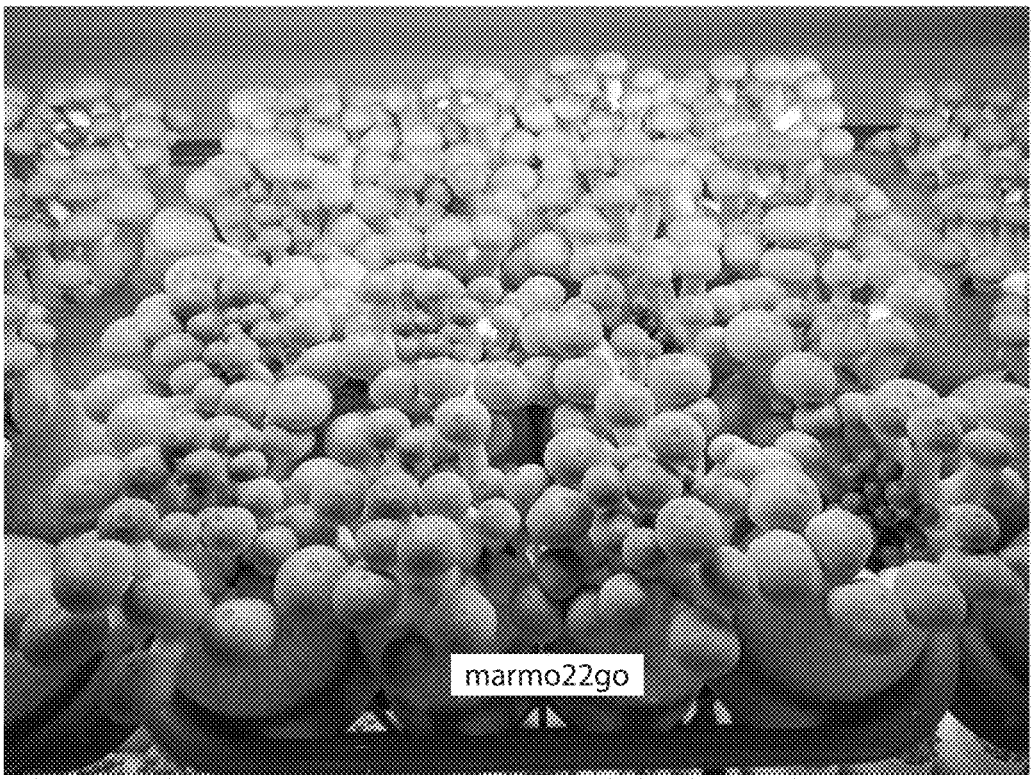

FIG. 10

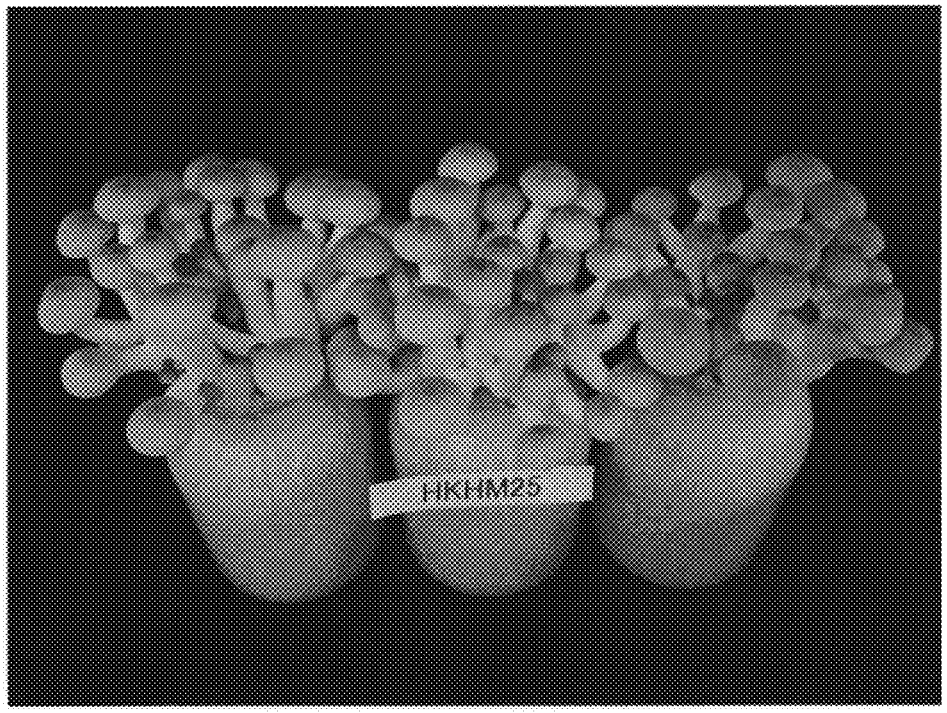

FIG. 11

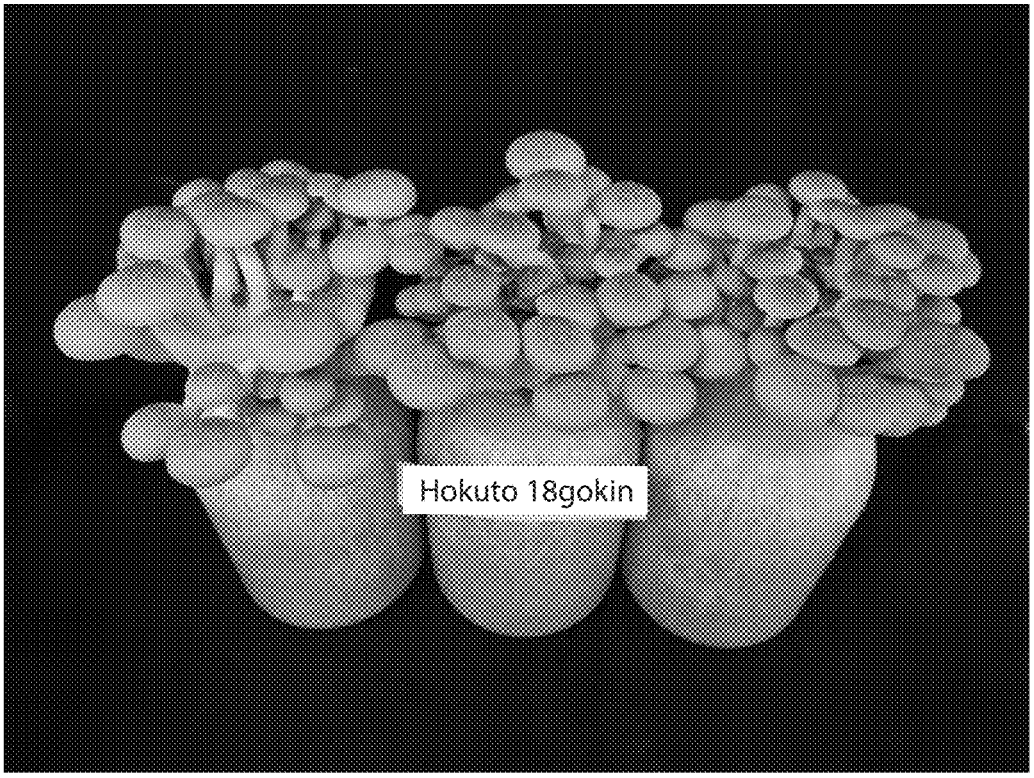

FIG. 12

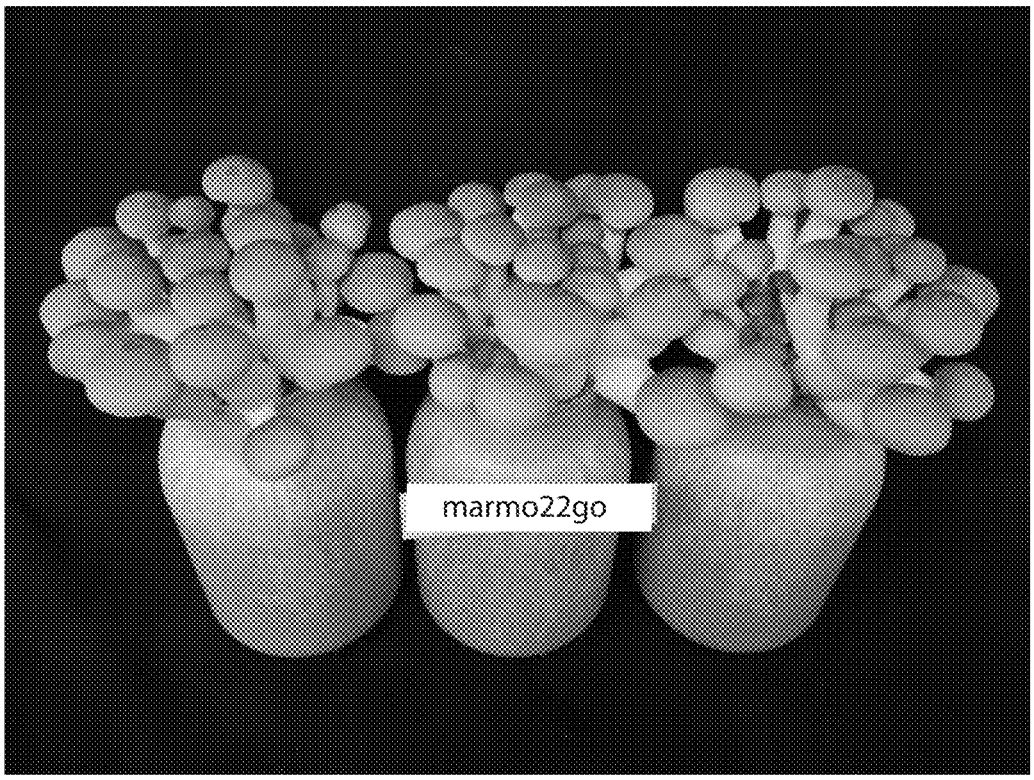

FIG. 13

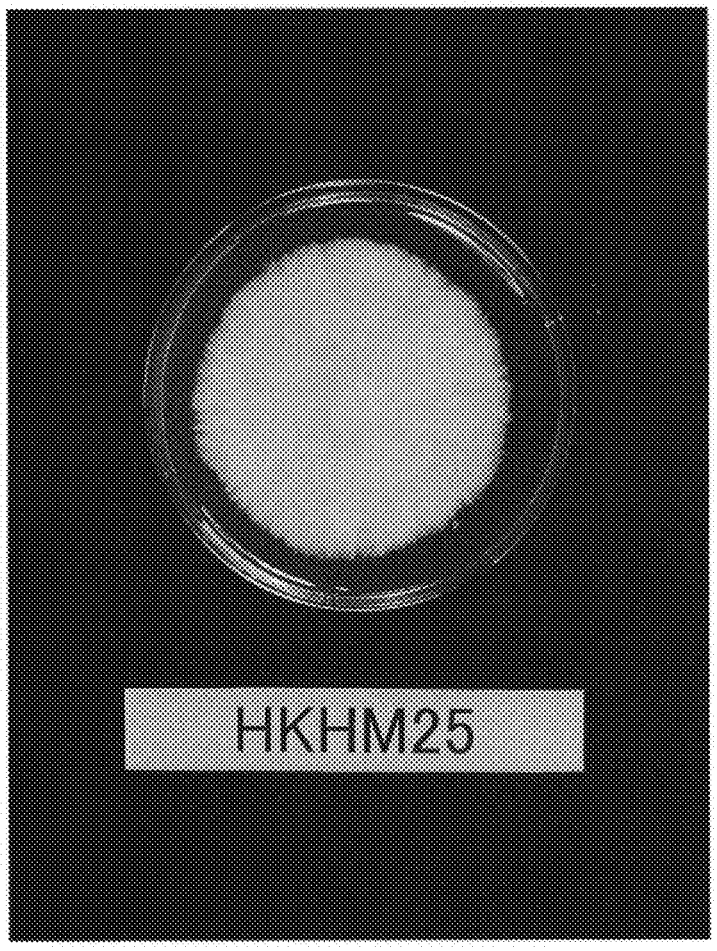

FIG. 14

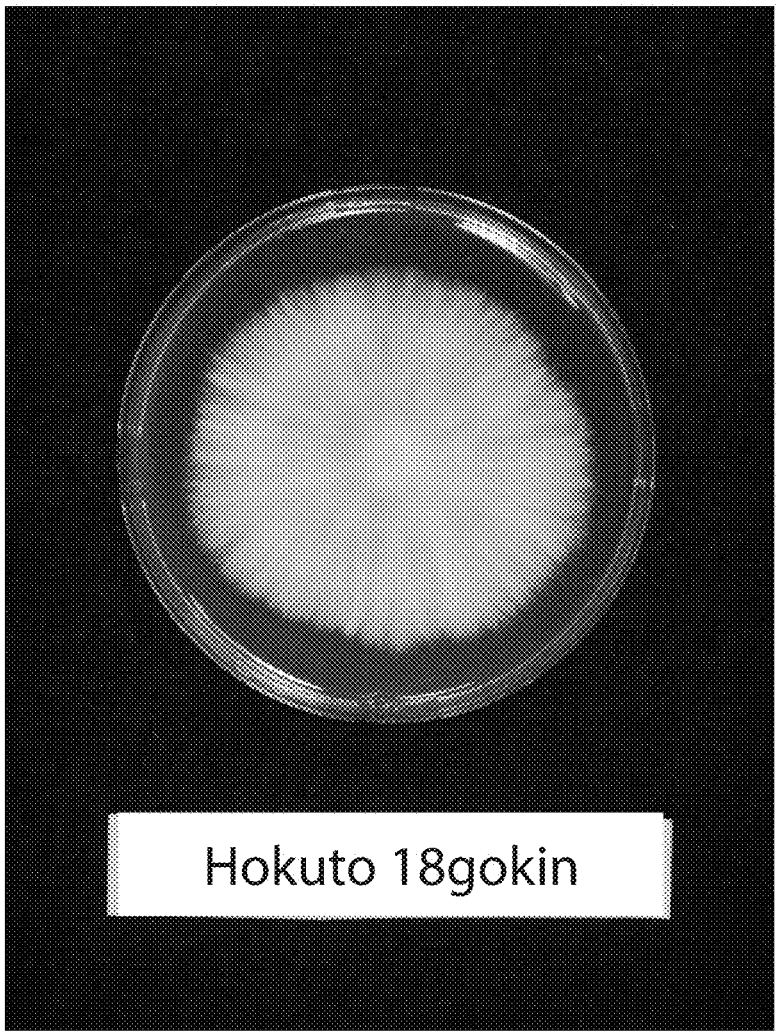

FIG. 15

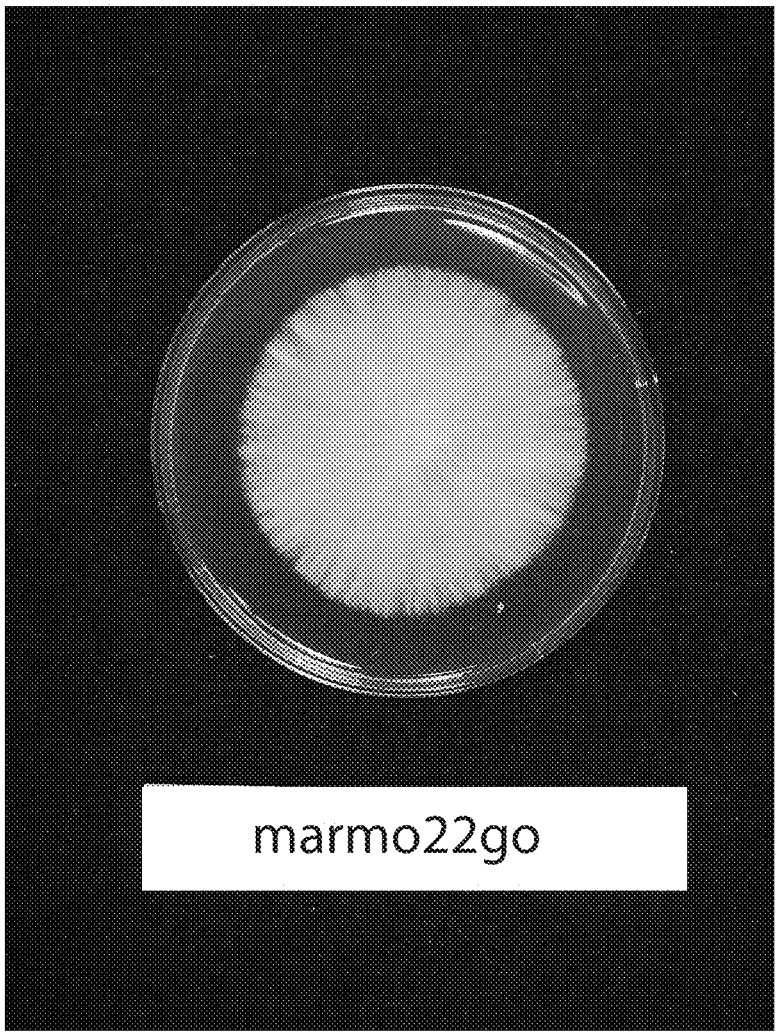

FIG. 16

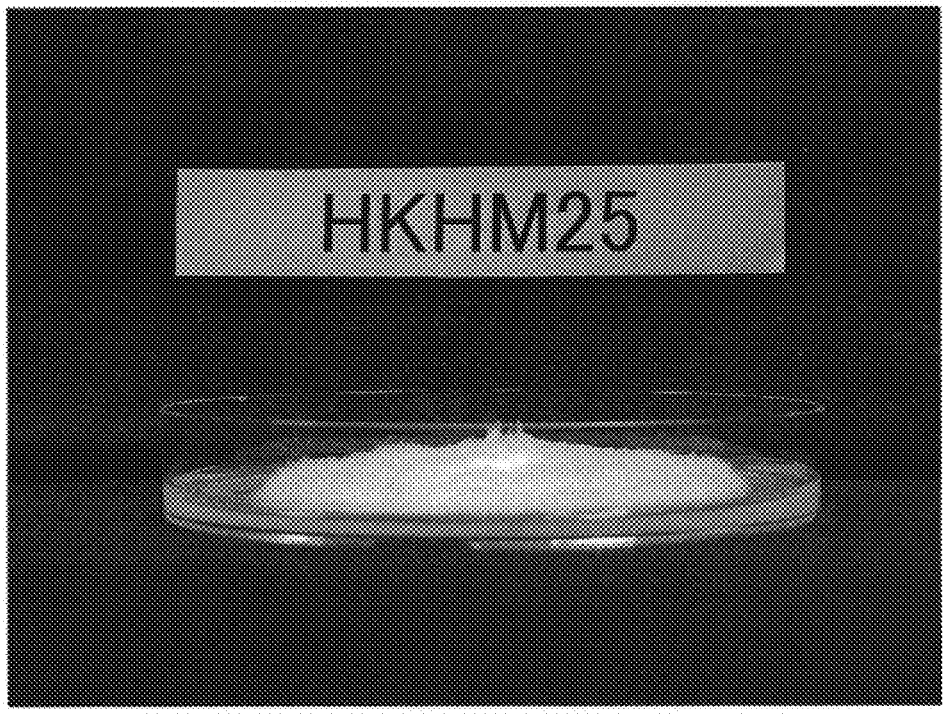

FIG. 17

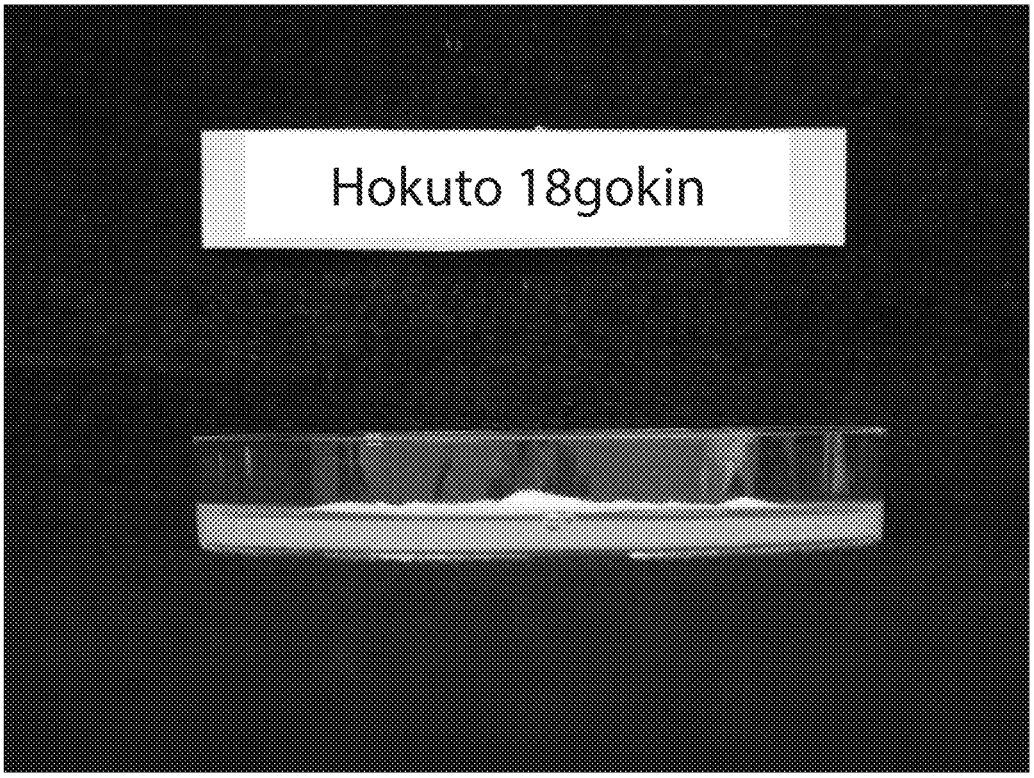

FIG. 18

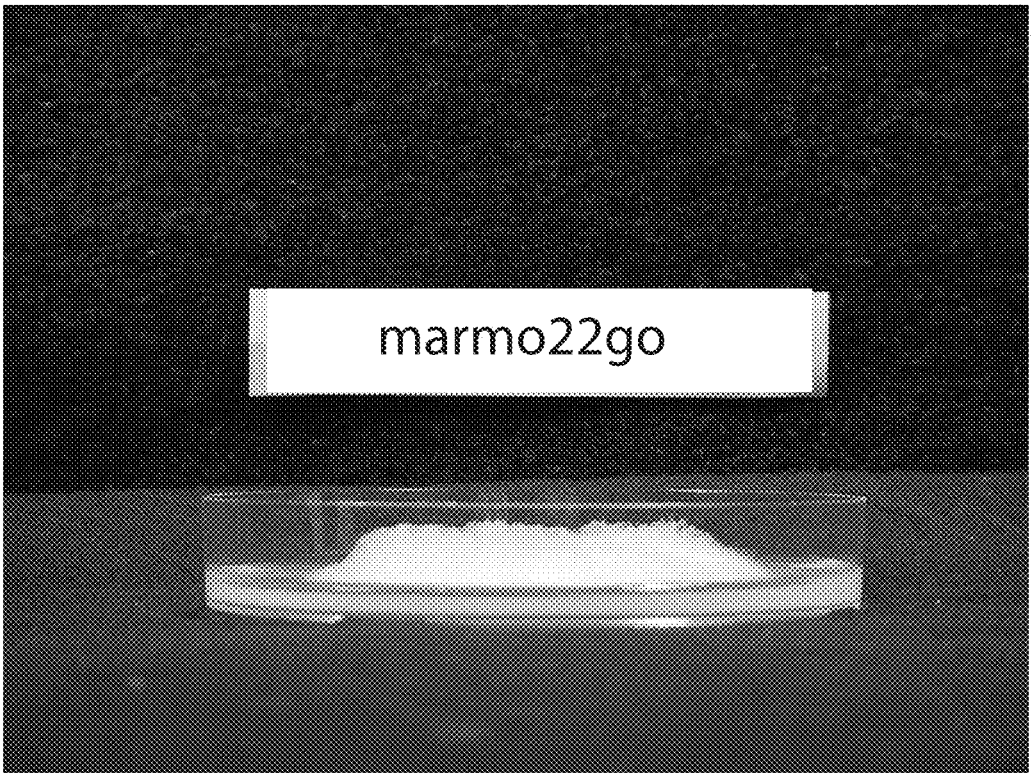

FIG. 19

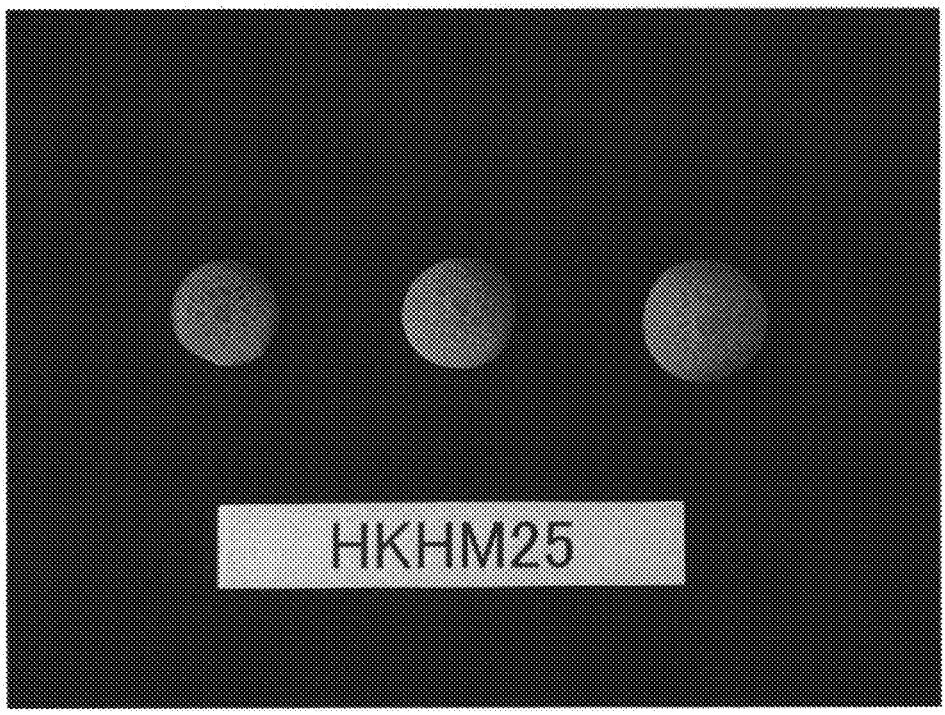

FIG. 20

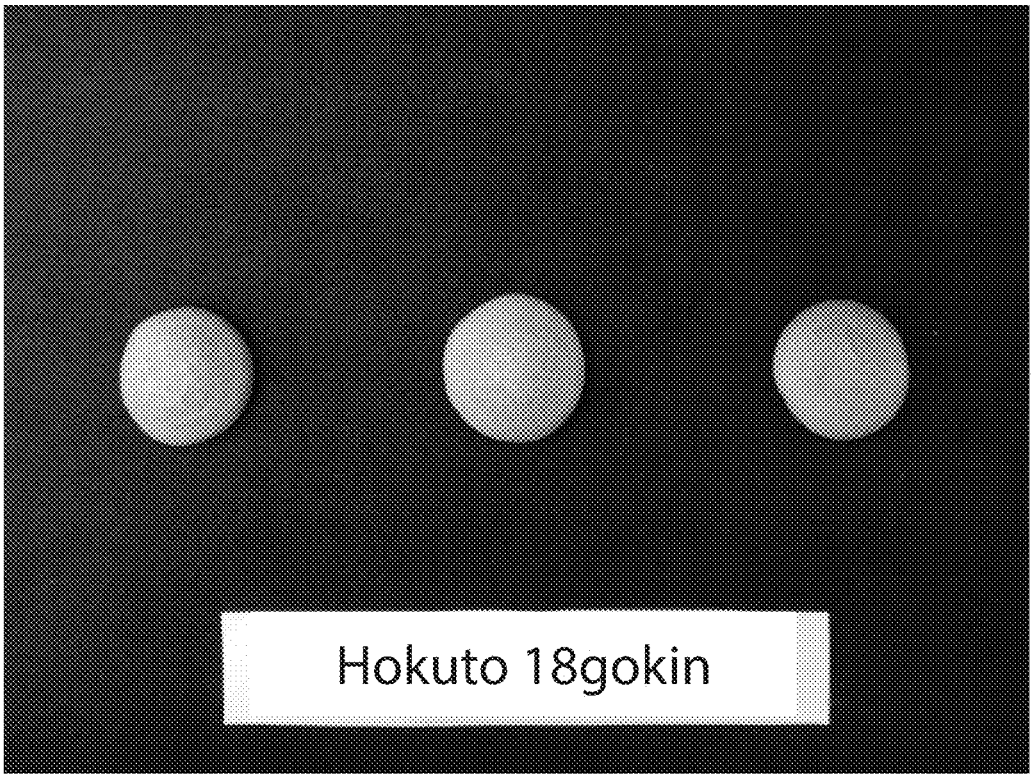

FIG. 21

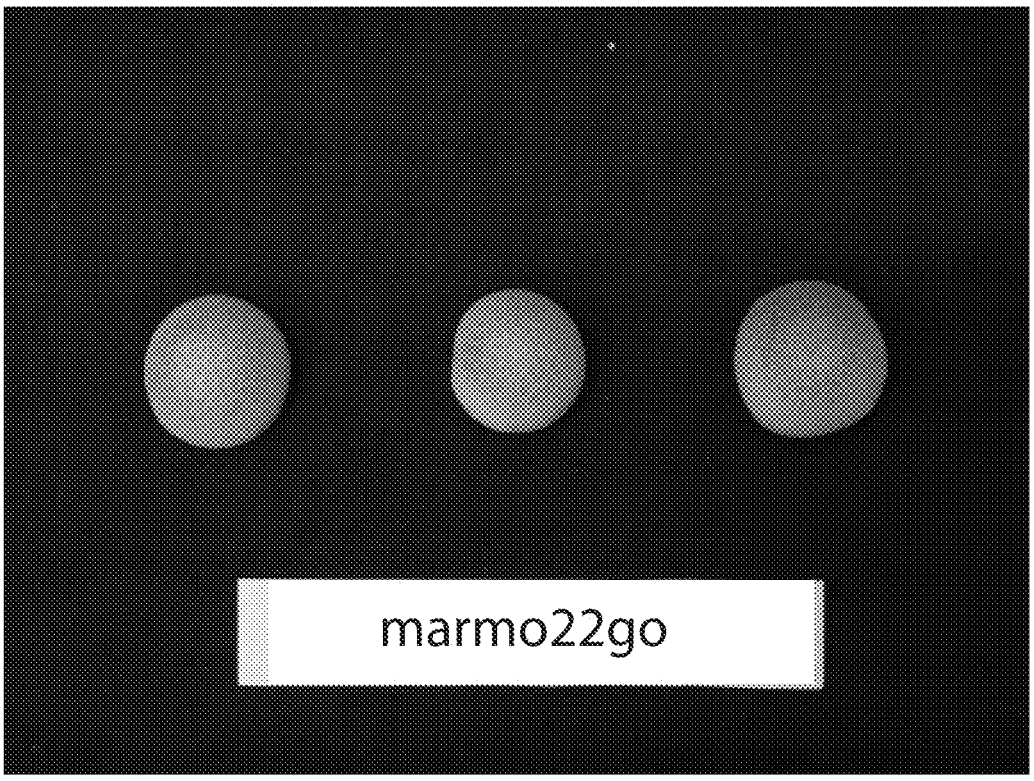

FIG. 22

FIG. 23

FIG. 24

FIG. 25