On the Spore-Germination and the Pure-Culture of *Armillaria Matsutake* Ito et Imai, the most Important Edible Mushroom in Japan.

By

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Contents.

I. Introduction.

Armillaria Matsutake Ito et Imai is the most important edible mushroom in Japan. The mushroom grows in the forest land of "Akamatu", or *Pinus densiflora* Sieb. et Zucc., mostly in autumn and rarely in early summer. Sometimes the mushroom grows in the forest of *Tsuga Sieboldii* Carr. Some fruit bodies of this mushroom found in the *Tsaga* forest near Tanabe, Pref. Wakayama, were studied by the present writers personally, and noted that they were quite similar to those found in the pine forests. In Saghalien the mushroom is said to grow in the forest of *Picea jedoensis* Carr. But the yield in the latter two is nothing compared with that in the pine forests.

The Japanese name of this fungus, the "Matutake" means the mushroom growing in the pine forests, the "Matu" being pine, especially *Pinus densiflora* Sieb. et Zucc., and the "Take", mushroom.

According to ITO[4,5] and his collaborator the fungus was described for the first time by SCHROETER in 1886 with the name *Agaricus (Armillaria) edodes* BERKELEY. Since then the following names have been assigned to it by various authors:

Armillaria Matsutake ITO & IMAI, Bot. Mag. Tokyo, 39 : 327, 1925.

Syn. Agaricus (Armillaria) edodes SSHROET. (sub BERK.) Gartenfl. XXXV, p. 135, 1886.

? *Armillaria edodes* P. HENN. (sub SACC.), in ENGL. PRANTL, Natürl. Pflanzenfam. I, 1**, p. 270, 1900 (1897).

Armillaria edodes P. HENN. (sub BERK.), Not. Königl. Bot. Gart. Mus. Berlin, II, p. 385, 1899; in ENG. Bot. Jahrb. XXVIII, P. 270, 1901.

Cortinellus edodes P. HENN. Hedw. XXXIX, p. (156), 1900; MATS. Ind. Pl. Jap. I, p. 137, 1904; KAWAMURA, Ill. Jap. Fungi, pl. VIII, fig. 11—12, 1913.

Armillaria caligata HAR. et PAT. (sub VIVIANI), Bull. Mus. Hist. Natur. VIII, p. 132, 1902.

Table I.

The Annual Yield of *Armillaria Matsutake* Ito et Imai during Last Ten Years in Japan.

	Yield in kilograms (As fresh mushrooms)	Value in Yen
1924	4,160,540	3,144,404
1925	5,393,003	3,644,615
1926	5,512,960	3,648,109
1927	6,992,359	3,900,651
1928	6,722,294	3,799,906
1929	7,192,532	4,195,806
1930	4,864,436	2,910,087
1931	7,685,652	3,520,192
1932	7,304,565	3,486,804
1933	6,632,126	3,518,617

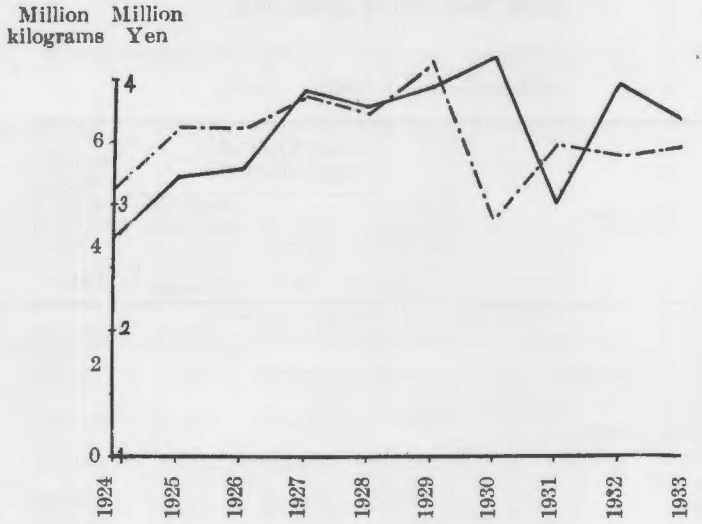
In Formosa a similar mushroom has been known to grow in the forests of *Pinus taiwanensis* HAY., or "Niitake-akamatu" in Japanese. The mushroom was described by K. SAWADA[14] in 1931, with the name *Armillaria Matsutake* ITO et IMAI, var. *formosana* SAWADA nov. var. (Japanese name = "Taiwan-matutake").

One more mushroom resembling to the above stated Japanese Matu-take is the American Matu-take or Japanese mushroom as it is called along the Pacific Coast of Oregon and Washington. It is usually found in the pine barrens of

Pinus contorta Dougl. in the coastal sand dunes. The comparative study of this mushroom with Japanese Matu-take was undertaken by Zeller and Togashi[15,16] in 1934, and it was ascribed to *Armillaria ponderosa* (Peck) Sacc. [= *A. arenicola* Murrill, *A. magnivelaris* (Peck) Murrill.]

Graph I.

The Annual Yield of *Armillaria Matsutake* Ito et Imai during Last Ten Years in Japan.



Remarks: The heavy line shows the annual yield of *Armillaria Matsutake* as fresh mushrooms in million kilograms. The chain line shows the annual yield of *Armillaria Matsutake* in million Yen.

The annual yield of this important edible mushroom in Japan during last ten years is shown in Table I and Graph I. In 1933 it attained to 6.6 millon kilograms as fresh mushroom. The value of this crop in the year was about 3.5 million Yen. Although the appearance of this mushroom has been known all over our country, from Saghalien to Formosa, the greater parts are yielded in Western Japan. As shown in Table II, about ninety per cent. of the total yield in whole Japan are cropped within seventeen prefectures under the jurisdiction of the Osaka Local Forestry Bureau ; and over fifty five per cent. are produced in only seven prefectures in the vicinity of Osaka, or so-called 'Kinki District'.

Comparisons of the annual yield of *Armillaria Matsutake* to that of pine lumber in the chief mushroom growing districts are given in Table II and Graph II. In Kyôto prefecture the annual yield of *Armillaria Matsutake* is about three times of those of pine lumber, and even in the mean annual yield in seven

prefectures in the 'Kinki District', the former is 1.34 times of the latter. Therefore the artificial propagation of this important edible mushroom has been studied by the government authorities as well as the private persons[1, 2, 8, 9, 10, 11, 12].

The present writers set out their investigations on the pure culture of the mycelium to contribute to the performance of the artificial propagation of the

Table II.

The Annual Yield of *Armillaria Matsutake* Ito et Imai, in Comparison with that of Pine Lumber in Last Two Years (1932-33).

After the Tenth Statistical Report of the Japanese Department of Agriculture and Forestry. (1933).

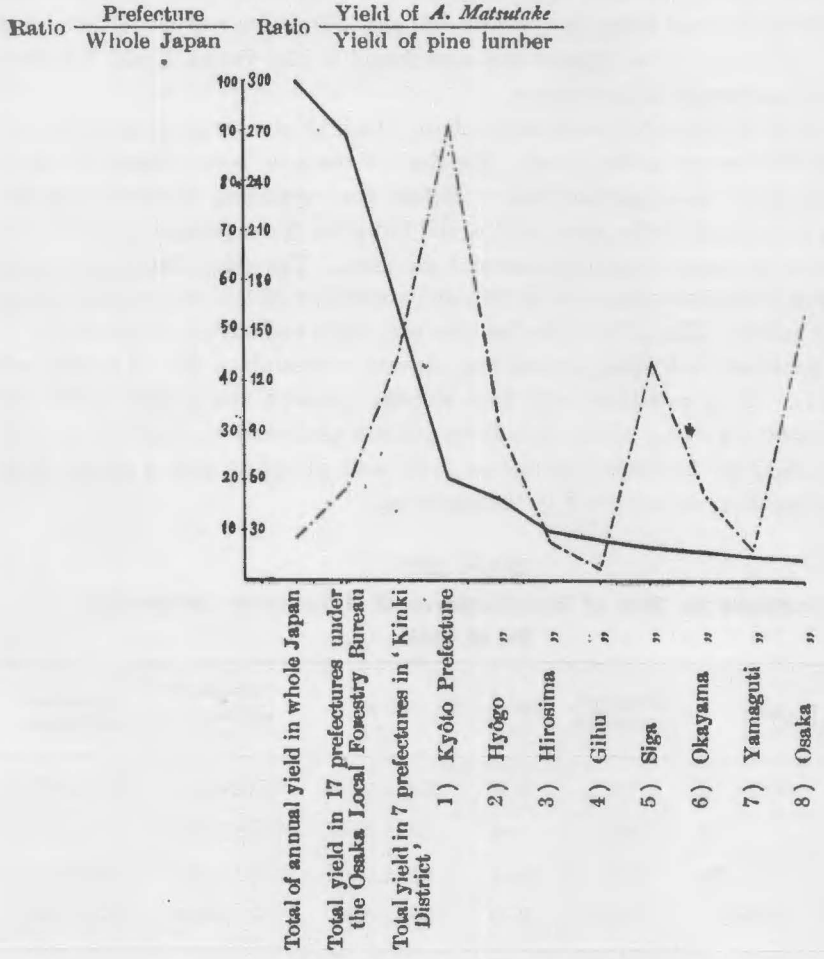
No.	Prefecture	Annual Yield of *Armillaria Matsutake*			Annual Yield of pine lumber in Yen (B)	Ratio (A)/(B)
		Yield in kilograms. (As fresh mushrooms)	Yield in Yen (A)	Ratio of the yield to the total of Japan		
1.	Total Yield in Japan	6,969,346	3,502,711	100.00	14,051,038	0.25
2.	Total Yield in 17 prefectures under the Osaka Local Foresty Bureau	6,351,822	3,125,730	89.30	5,932,210	0.53
3.	Total Yield in 7 prefectures in 'Kinki Distrit'	4,878,871	1,928,208	54.90	1,435,633	1.34
4.	Kyôto	1,449,971	701,529	20.50	258,433	2.72
5.	Hyôgo	1,168,645	568,374	16.20	596,332	0.95
6.	Siga	508,606	235,486	6.71	179,156	1.31
7.	Osaka	327,467	153,473	4.37	93,874	1.64
8.	Wakayama	201,921	99,130	2.82	76,556	1.31
9.	Nara	202,946	98,996	2.81	64,057	1.56
10.	Miye	147,119	71,221	2.03	167,237	0.43
11.	Hirosima	608,333	349,437	9.96	1,289,894	0.21
12.	Gihu	592,363	279,029	7.96	320,589	0.09
13.	Okayama	414,238	218,301	6.22	407,312	0.54
14.	Yamaguti	464,836	187,489	5.33	1,132,486	0.17
15.	Aiti	71,375	48,242	1.38	145,300	0.33
16.	Isikawa	67,054	40,294	1.15	244,300	0.17
17.	Simane	63,953	36,035	1.03	490,481	0.07
18.	Hukui	39,242	25,112	0.72	188,373	0.13
19.	Tottori	15,815	10,715	0.35	209,469	0.05
20.	Toyama	3,540	2,873	0.08	683,660	0.04

important mushroom in Japan[18]. This paper gives the results of their experiments and is the first report of the series.

The writers are obliged to Mr. H. Miyake, the director, Messrs. K. Onakado and S. Youkawa and other staffs of the Osaka Local Forestry Bureau for their help during the course of their investigation.

Graph II.

The Annual Yield of *Armillaria Matsutake* Ito et Imai, in Comparison with that of Pine Lumber in Last Two Years.



Remarks: In this graph the annual yield of chief mushroom growing prefectures within the jurisdiction of the Osaka Local Forestry Bureau is given. The heavy line shows the percentage of the yield of the prefectures in the comparison with total yield of Japan. The chain line shows the ratio of the annual yield of *Armillaria Matsutake* to that of pine lumber in Yen in each prefecture and in whole Japan.

II. Morphological Characteristics.

Morphological characteristics of *Armillaria Matsutake* ITO et IMAI are here given, although they were written by KAWAMURA[7], ITO IMAI[4], and ZELLER and TOGASHI[16].

The pileus is brown and spherical at first, but later expanded to plane. It is provided with slightly brownish, cottony scales. The flesh is white, compact and thick, when dried somewhat flexible and durable. The size of the pileus is very variable, but usually 10—20 cm. in diameter when fully expanded. The length of the stem is generally almost equall to the diameter of the pileus. The weight of a fresh fruit body lies between 50 and 200 grams and sometimes very big ones are found. The biggest one ever found in the Osaka Local Forestry Bureau weighed about 2.5 kilograms.

The stem is cylindrical with round base, straight or curved to one side and attaches to the center of the pileus. The flesh of the stem is also white, compact, fibrous and easily torn longitudinally. Before the expanding of the pileus, the margin is connected to the stem with a veil but after the expansion a part of the veil remains as a ring or annulus around the stem. The stem above the ring is white, while under the ring it is brown and resembles to the color of the upper side of the pileus. The gills or the lamellae are white and sinuate to the stem.

The basidium is hyaline, cylindrical, clavate or spatulate, 25—45 μ long and 5—8 μ wide. It is provided with four slender, pointed sterigmata which are 3.8—5.3 μ long 0.7—10 μ wide. Basidiospores are produced on the top of each sterigma ; they are hyaline, elliptical or ovate and provided with a hilum, with which the basidiospore attached to the sterigma.

Table III.

Constants for Size of Basidiospores of *Armillaria Matsutake* Ito et Imai.

		Locality	Number measured	Range (μ)	Mean (μ)	Standard deviation (μ)	Variation coefficient
Length	(A)	Saizyô I.	100	5—8	6.47±0.074	0.74±0.0523	11.45±0.80
		II.	100	5—8	6.42±0.057	0.57±0.0406	9.13±0.646
		III.	100	5—9	6.39±0.055	0.55±0.0386	8.55±0.620
		Average	300	5—9	6.43±0.060	0.62±0.0438	9.71±0.689
	(B)	Tanabe I.	100	6—8	6.82±0.043	0.43±0.0307	6.36±0.449
		II.	100	5—8	6.79±0.051	0.51±0.0358	7.46±0.527
		III.	100	5—8	6.78±0.048	0.48±0.034	7.10±0.520
		Average	300	6—8	6.80±0.049	0.48±0.035	6.97±0.498

(Table III Continued.)

	Locality		Number measured	Range (μ)	Mean (μ)	Standard deviation (μ)	Variation coefficient
Width	(A)	Saizyô I.	100	4—7	5.70±0.050	0.50±0.0354	8.77±0.62
		II.	100	5—7	5.66±0.049	0.49±0.0353	8.82±0.623
		III.	100	5—6	5.68±0.046	0.46±0.0328	8.17±0.577
		Average	300	4—7	5.68±0.048	0.48±0.0345	8.59±0.607
	(B)	Tanane I.	100	5—7	5.92±0.039	0.39±0.0278	6.63±0.469
		II.	100	5—7	5.84±0.043	0.43±0.0305	7.28±0.514
		III.	100	5—7	5.73±0.047	0.47±0.0329	8.13±0.575
		Average	300	5—7	5.83±0.043	0.43±0.0304	7.35±0.519

Remarks: A) Basidiospores of *Armillaria Matsutake* grown in a forest of *Pinus densiflora* Sieb. et Zucc. near Saizyô, Pref. Hirosima, measured on October 5, 1934. I, II and III show that they were measured from different fruit-bodies.

B) Basidospores of *Armillaria Matsutake* grown in a forest of *Tsuga Sieboldii* Carr. near Tanabe, Pref. Wakayama, measured on October 18, 1934.

As shown in Table III, the basidiospores of *Armillaria Matsutake* grown in a pine forest near Saizyô, Pref. Hirosima, were 5—9 μ long and 4—7 μ wide. The mean length of 300 measurements was 6.43±0.060 μ and the mean width, 5.68±0.048 μ. The basidiospores of a fruit body grown in a *Tsuge* forest near Tanabe, Pref. Wakayama, were almost the same with those stated sbove in shape and in size. They were 5—8 μ long and 5—7 μ wide, the mean value of 300 measurements being 6.80±0.049 μ in length and 5.83±0.043 μ in width.

III. Germination of Basidiospores.

The pure culture of *Armillaria Matsutake* was reported by K. Masui[7] in 192 in his elavorated work on the mycorrhyzal fungi on woody plants. But his pure culture were started from the mycelium around the base of the fruit bodies, and its identity with *Armillaria Matsutake* was not proved.

The pure culture of a mushroom must be started from the germination of the basidiospores. At least it is the most reliable method of isolation. As to the germination of the basidiospores of *Armillaria Matsutake* Ito et Imai, Mimura[10] reported the results of his experiments already in 1909, of which the essential points are follows:

The basidiospores of *Armillaria Matsutake* secured from a fresh fruit body, fully grown in a pine forest, may keep their germinating power over five months.

But the germination percentage decreases with the elapse of preserving period. Mature basidiospores may germinate in distilled water, the extract of pine roots, in acidified or alkalized water. The germination takes place within 20 hours at 16°C., 18 hours at 20°C., and 12 hours at 28°C. Mature spores in culture solution do not lose their germinating power even after the keeping at 0°F. for 4 hours or at 0°C. for 24 hours.

Since then no reports have been published on this point, so far as the writers aware. Although the above cited MIMURA's results showed that the basidiospores of *Armillaria Matsutake* germinated very easily, the present writers' preliminary experiments gave entirely different results. The germination of the basidiospores was tedious and the germination percentage was very small.

1. Preliminary experiment on the germination.

At the beginning of the present experiment, the basidiospores of *Armillaria Matsutake* were cast on a sterilized glass plate from a fresh, fully expanded fruit body, and they were sown in the hanging drops in Van Tieghem Cells. For the hanging drops not only sterilized tap water, and distilled water but also various culture solutions were used. These preparations were kept at various temperatures for various durations, and then inspected microscopically. But the spore germination was not observed at all.

Then the basidiospores were sown after the method shown by H. KNIEP on a plate culture of various agar media. A bit of the pileus with gills was cut from a fresh, fully expanded fruit body, and it was attached to the cover of a sterilized Petri-dish containing agar medium. Thus the basidiospores were cast on the surface of the medium. These plate cultures were inspected after various durations at various temperatures. In some of the plates the spore germination was observed, although it was comparatively rare.

2. Effect of culture medium on the germination.

To determine the factors affecting the germination of the basidiospores of *Armillaria Matsutake* ITO et IMAI, the effects of the constituents of culture media to the germination were studied first. The basidiospores were sown from the gills of a fresh, fully expanded fruit body on agar plates of different composition. The cultures were kept at 24°C., and the spore germination was inspected at different intervals. The results are shown as follows:

a) Pine rootlets decoction agar. For the preparation of the medium 100 g. of fresh rootlets of *Pinus densiflora* secured from a forest, where *Armillaria Matsutake* was grown, were soaked to 1000 cc. of tap water and then boiled for one hour. To the filtrate 20 g. of agar were added. On this medium the basidiospores did not germinate even after 3 weeks incubation.

b) Mushroom decoction agar. (Agar medium of mushroom decoction of *Armillaria Matsutake*.) The medium consisted of 100 g. of fresh fruit bodies of *Armillaria Matsutake*, 1000 cc. of tap water and 20 g. of agar. On this medium also

no spore germination was observed even at the end of 3 weeks incubation.

c) Potato agar. This was composed of 200 g of fresh potato tuber, 1000 cc. of tap water and 20 g. of agar. Even after 3 weeks no spore germination was observed.

d) Malt extract agar. The constituents were 30 g. of malt extract, commercial) 1000 cc. of tap weter and 20 g. of agar. On this agar plate a few of the basidiospores germinated after 6 days' culture. With the elapse of the culture duration the spore germination increased in number, but the elongation of the germ-tubes was comparatively slight.

e) Apricot decoction agar. The medium consisted of 25 g. of dried apricot, 1000 cc. of tap water and 20 g. of agar. Even after 3 weeks no spore germination was observed.

f) Soil decoction agar. The constituents were 200 g. of soil of pine forest, where fruit bodies of *Armillaria Matsutake* grew, 1000 cc. of tap water and 20 g. of agar. The spore germination was found after 6 days culture. Subsequent growth of the germ tubes were better on this medium than the above stated malt extract agar.

g) Soil decoction agar plus 2 per cent. glucose. To 1000 cc. of the above stated soil decoction agar (f) 20 g. of glucose was added. On this medium the spore germination was so good as on the above medium, the development of the germ-tubes was slightly better than the above medium.

In short, the germination of the basidiospores of *Armillaria Matsutake* was observed on some culture media, but the germination percentage was very small and the subsequent growth of the germ tubes was very slow. (cf. Fig. 5—8).

In the pine forest, leaves of pine-trees, Rhododendrons, etc., fallen under or near the fully expanded fruit bodies, are generally covered with white powder. According to the writers' study the white powder was the deposit of basidiospores of *Armillaria Matsutake*. And none of these spores have germinated so far. The spore deposit on these fallen leaves were taken to the writers' laboratory and kept at various temperatures and air-humidities. Even after a long incubation period almost all the basidiospores did not germinate. The above study shows that the spore germination is not easy even in nature.

3. Effect of temperature on the germination.

As stated above the basidiospores of *Armillaria Matsutake* are able to germinate on such culture media as soil decoction agar, of which the soil was obtained from a pine forest where the fruit bodies of this mushroom were found, or the soil decoction agar plus 2 per cent. glucose. Therefore the effects of temperature on the spore germination were tested on the last stated medium. The basidiospores from a fresh fruit body were sown on the agar plates by the above stated method after Kniep. The agar cultures thus prepared were kept at 10°, 15°, 2?°, 24°, 26°, 29° and 32°C. and the spore germination was tested microscopically after 4, 6, 10 and 14 days interval respectively. The results are given in Table IV.

Table IV.

Effect of Temperature on the Germination of Basidiospores of *Armillaria Matsutake* Ito et Imai.

Temperature C.	Results after				Length of germ-tubes after 14 days' culture (μ)	
	4 days	6 days	10 days	14 days	Range	Average
10°	− [1]	−	+	−		
15°	−	−	+	+	20—170	70
20°	−	+	+	+	20—280	120
24°	−	+	++	++	40—420	210
26°	−	+	++	++	30—420	210
29°	−	−	−	−		
32°	−	−	−	−		
Room temperature [2]	−	+	+	+	20—300	80

Remarks: 1) In this table + sign means the germination of the basidiospores and − sign, no germination.

2) The room temperature is that of the writers' laboratory during 14 days from October 24, to November 7, 1934. The temperature range was from 6° to 25°C. according to the thermograph.

According to the figures in the above table the basidiospores did not germinate at 10°C., but began to germinate at 10°—15°C. The optimum temperature for the germination was 24°—23°C. and the maximum, 26°—29°C. This result seems to show that the basidiospores are able to germinate at the temperatures in the autumn season, when the fruit bodies of *Armillaria Matsutake* ripe in nature, but not at a temperature in the summer or the winter season.

4. Effect of hydrogen-ion concentration on the germination.

The effect of hydrogen-ion concentration of culture media on the germination of the basidiospores of this mushroom was determined with malt extract agar as well as the above stated soil decoction agar. The pH value of the former culture medium before adjustment was about pH 6.0, while that of the latter medium, about pH 4.0. To these media various amounts of N/5 solution of hydrochloric acid and causatic soda were added and agar plates with the following various degrees of pH value were prepared. On these agar plates fresh basidiospores were sown as stated above and then the cultures were kept at about 24°C. The spore germination on the culture media of various pH values was studied after 4 days, 15 days and 20 days' incubation. The results are given in Table V.

Table V.

Effect of Hydrogen-Ion Concentration of Cultre Medium on the Germination of Basidiospores of *Armillaria Matsutake* Ito et Imai.

(I)

Soil extract agar, secured from a pine forest, on which *A. Matsutake* was grown. pH value before adjustment was 4.0.		
pH	Results after	
	4 days	20 days
2.4	−	−
2.7	−	−
2.9	−	−
3.3	−	−
4.0	+	++
6.0	±	±
6.7	−	−
8.6	−	−
9.4	−	−

(II)

Malt extract agar (3%). pH value before adjustment was 6.0.		
pH	Results after	
	4 days	15 days
3.2	−	−
3.5	−	−
4.4	−	±
6.0	+	+
8.4	−	−
9.0	−	−
9.5	−	−

Remarks: In this table + sign means the germination of the basidiospores and − sign, no germination.

The result given in Table V shows that the germination of the basidiospores takes place at the hydrogen-ion concentration of pH 4.0—6.0 on the soil extract agar as well as malt extract agar. The same experiments were repeated, but the results were entirely similar.

IV. Pure Culture of mycelium.

1. Method of isolation.

For the isolation of *Armillaria Matsutake*, agar medium of soil decoction secured from a pine forest, the soil decoction agar with addition of 2% of glucose, or malt extract agar was used, since they were proved to be suitable for the spore germination. The media were cleared through filter paper after boiling with the white of eggs and poured into Petri-dishes. As in the above stated germination test, the basidiospores were shed from a fulluy expanded, fresh fruit body on the agar plates. They were kept in an incubator at 24°C., the optimum temperature for the spore germination, for about a week. Then under micro-

scope, a single, germinated basidiospore was indicated with a simple object-marker aseptically. Each of these was removed to another fresh agar plate and grown at 24°C. After the germ-tube of the spore had somewhat elongated without contaminations, it was transferred to agar slant. In this way a pure culture was isolated.

2. Effect of culture medium on the mycelial growth.

At first the effects of cultura medium on the mycelial growth were studied. A small circular piece, about 2 mm. in diameter, of the mycelium of *Armillaria Matsutake*, grown on the soil extract agar with 2 per cent. glucose, was transferred to an agar plate. Characteristics of the colonies of *Armillaria Matsutake* on various culture media after 30 days culture at 24°C. are summarized in Table VI.

Table VI.

Summarized Characteristics of *Armillaria Matsutake* Ito et Imai on Various Culture Media after 30 days Incubation at 23°C.

No.	Agar media of 1)	Mycelial growth 2)	Diameter of colonies (mm.)	Aerial myceluim 3)	Characteristics of colonies
1.	Knop's solution	++	6.0	−	Regular, thin
2.	Knop's solution plus 2% glucose	+++	9.5	−	Regular, somewhat thin
3.	Sachs' solution	+	+		
4.	Sachs' solution plus 2% glucose	++++	12.0	−	„ „
5.	Potato extract	−			
6.	Soil decoction with glucose . .	+++	10.0	+	„ compact
7.	Pfeffer's solution	−			
8.	Hopkin's solution	++	6.5	+	„ „
9.	Malt extract solution	−			
10.	Richard's solution	−			
11.	Currie's solution	−			
12.	Soil extract plus glucose, the soil being secured from a pine forest, where *A. Matsutake* was grown	+++++	13.0	++	„ „
13.	Pine leaf decoction	−			

Remarks: 1) In this experiment, agar media of varions nutrient solutions were used.
2) In the column of mycelial growth + sign shows the formation of colonies, visible to the naked eyes, and − sign, no formation. The more the plus signs the better the growth.
3) In the column of aerial mycelium + sign shows the formation of aerial mycelium and − sign, no formation.

According to Table VI, the colonies, visible to the naked eyes, hardly developed on Sachs' solution agar, potato agar, Pfeffer's solution agar, malt extract agar, Currie's solution agar, and pine leaf dection agar even after 30 days culture at 24°C. If 2 per cent. glucose were added to these media, mycelial growth was observed with the naked eyes in many cases. The results show that glucose may be one of the essential components for the mycelial growth.

3. Effect of temperature on the mycelial growth.

Effects of temperatuse on the mycelial growth of *Armillaria Matsutake* were studied by the writers with the soil decoction agar with addition of 2 per cent. glucose. A circular piece, about 2 mm. of pure culture on an agar medium was transferred to an agar slant. The agar cultures, thus tranferred were kept at various temperatures. The diameter of colonies after 15, 30, 45 and 60 days were measured respectively. The results are shown in Table VII and Graph III.

Table VII.

Effect of Temperature on the Mycelial Growth of *Armillaria Matsutake* Ito et Imai.

Temperature C.	Diameter of colonies after			
	15 days	30 days	45 days	60 days
	mm.	mm.	mm.	mm.
0°	—	—	—	—
5°	—	+	5.7	6.0
10°	+	5.0	8.6	11.1
15°	5.0	8.8	15.6	20.6
20°	6.5	11.8	20.5	25.3
24°	6.4	14.2	22.8	26.4
27°	6.4	11.0	14.7	16.5
30°	4.0	4.2	5.4	6.1
32°	—	—	—	—
35°	—	—	—	—
38°	—	—	—	—

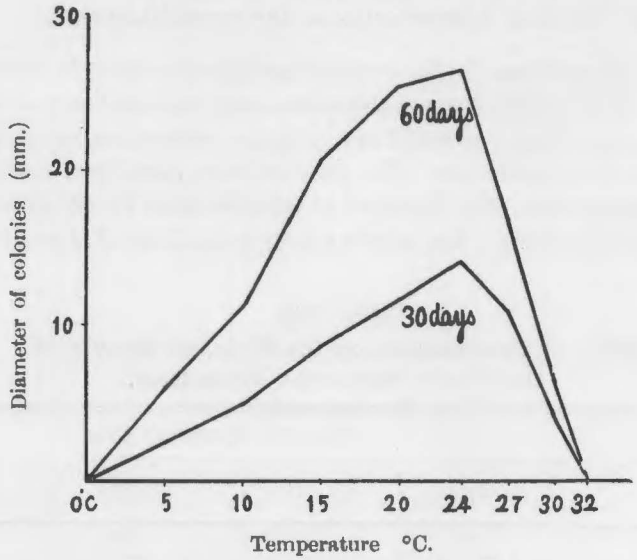
Culture medium: Soil extract agar with addition of glucose, the soil being secured from a pine forest on which *Armillaria Matsutake* was grown. The pH was about 4.0.

As shown in Table VII and Graph III the mycelial development of *Armillaria Matsutake* was slightly observed at from 10° upto 30°C. when it was inspected after 15 days incubation. After 30 days culture a slight mycelial

growth was observed even at 5°C. In short, the optimum temperature for the mycelial growth of this mushroom lies at about 24°C., the minimum at about 5°C. and the maximum at 30°—32°C.

Graph III.

Effect of Temperature on the Mycelial Growth of *Armillaria Matsutake* Ito et Imai.



Culture medium: Soil extract agar with addition of glucose, the soil being secured from a pine forest on which *Armillaria Matsutake* was grown.

Duration of culture: 30 days and 60 days.

V. Summary.

1) The present paper gives the results of the writers' experiments on the germination of the basidiospores and on the pure culture of the mycelium of *Armillaria Matsutake* ITO et IMAI.

2) The basidiospores of this mushroom was able to germinate on the surface of such culture media as soil decoction agar, of which the soil was obtained from a pine forest where the fruit bodies of *Armillaria Matsuioke* were found, the soil decoction agar plus 2 per cent. glucose and malt extract agar. The pH value favorable to the germination seemed to be pH 4.0—6.0.

3) The basidiospores began to germinate at 10°—15°C. The optimum temperature for the spore germination was at about 24°C. and the maximum temperature, 26°—29°C. Even at the optimum temperature 4—10 days were required before the first signs of the spore germination were observed.

4) As the basidiospores showed no signs of germination within the water drops or culture media, they seemed to require a plenty supply of free oxygen for the spore germination.

5) The mycelium of *Armillaria Matsutake* Ito et Imai was able to grow in pure culture on the following culture media so far as studied: the soil extract agar from the forest land of pine trees, Knop's agar, Sachs' agar with addition of glucose, and Hopkin's agar. In general the mycelium grew pretty well on the culture media containing glucose.

6) The minimum temperature for the mycelial growth seemed to be about 5°C., the optimum, 24°C. and the maximum, 30°—32°C.

7) The growth rate of the mycelium was very small. Even at about the optimum temperature, the diameter of the colonies after 30 days culture attained to only 15 mm.

8) In the pure culture of the mycelium, started from more than two basidiospores, the clamp connection of the hyphae were observed.

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Explanation of Plates X—XIII.

Plate X.

Fig. 1. A fairy ring of *Armillaria Matsutake* ITO et IMAI in the forest of *Pinus densiflora* SIEB. et ZUCC. in Mt. Misaoyama near Okayama. The picture was taken in October 1934.

Plate XI.

Fig. 2. Two fruit bodies of *A. Matsutake*, grown in early summer, showing general features of the stems, pilei and gills. They were got in a market in Kurasiki and photographed in June 1934.

Fig. 3. Hymenium of *A. Matsutake*, showing the basidiophores. (×200)

Fig. 4. Basidiospores of *A. Matsutake*. The fruit body was obtained from Saizyô, Pref. Hirosima. (×2,200)

Fig. 5. Germination of basidiospores of *A. Matsutake*, after 8 days incubation on the soil extract agar at 27°C. (×500)

Plate XII.

Fig. 6. Germination of basidiospores of *A. Matsutake*, after 8 days incubation on the soil extract agar at 25°C. (×1,500)

Fig. 7. Germination of basidiospores of *A. Matsutake*, after 20 days incubation on a slide glass kept in a moist chamber at 27°C. (×350)

Plate XIII.

Fig. 8. Germination of basidiospores of *A. Matsutake*, after 18 days incubation on the soil extract agar at 25°C. (×350)

Fig. 9. Hyphae of *A. Matsutake* grown in pure culture. Clamp connections of the hyphae are shown. (×1,500)

PLATE X.

Fig. 1.

PLATE XI.

Fig. 2.

Fig. 3.

Fig. 4.

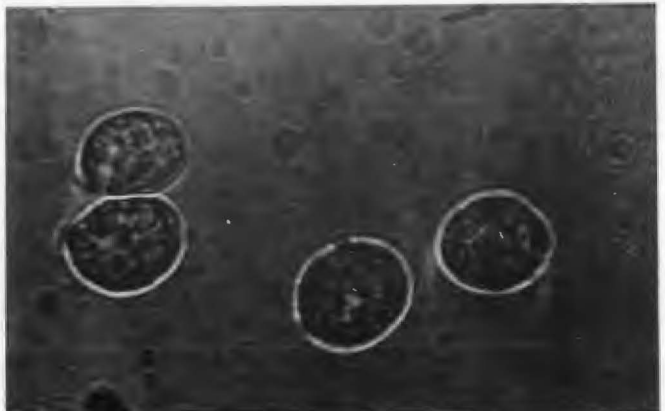

Fig. 5.

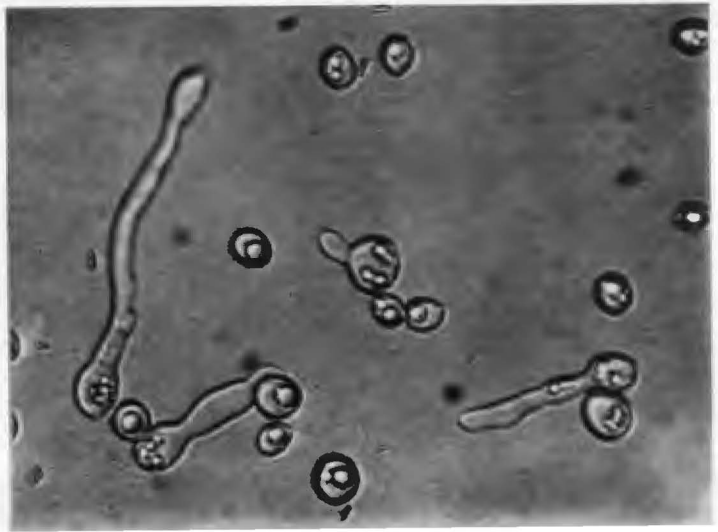

PLATE XII.

Fig. 6.

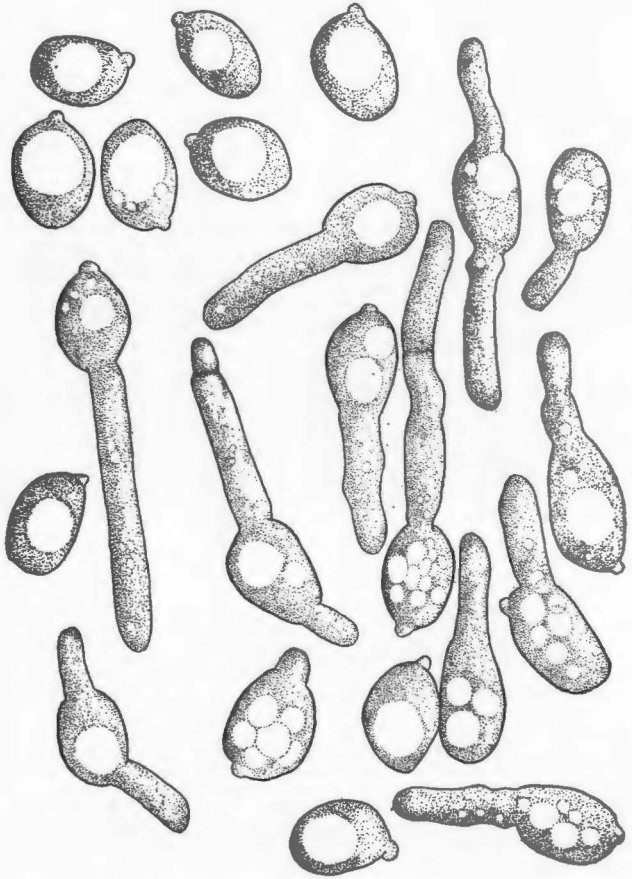

Fig. 7.

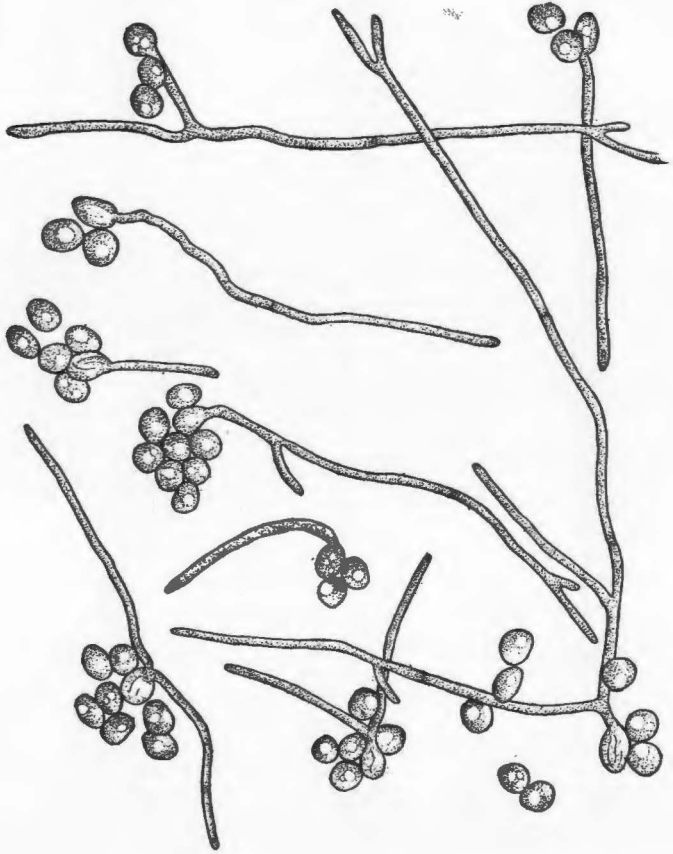

PLATE XIII.

Fig. 8.

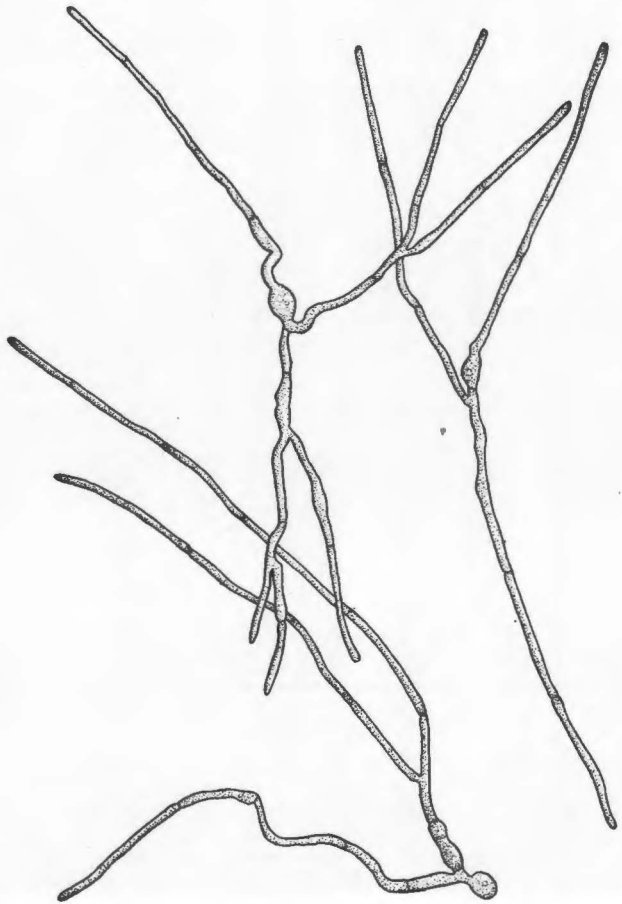

Fig. 9.