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Study on Gloeostereum Inoarnatum S. Itoetimai - Fermentation Cultivation(Liquid Fermentation)

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ABSTRACT

It was reported in our Previous paper that the fermented products from Gloeostereum incarnatum strongly inhibit the growth of six kinds of bacteria in human bodies. In this paper the appropriated conditions of immersing culture for the strain 8 903 of Gloeostereum incarnatum was analysed. And the output of the hypha and fermentative product was determined or compared. The prelimenaryresults showed that the appropriated conditions for the growth of Gloeostereum incarnatum are: (1)culture medium:glucose 3%; protein peoptne 0.2%; soybeancake power 1%; yeast power 0.3%; KH2PO40.05%; MgSO4 0.03%; CaCO3 0.01%; vitamin B1 0.001%; befor sterilization pH Value of six should be maintained; (2) temperature; 27℃ ~28℃; (3) time; about 200 hours; (4) ventilation; (30% ~50%)/min. The sigh of the end culture are: pH coming down about 4: remnant glucoses less 1%; amino nitrogens about 20%; time about eight days. In the aforementioned conditions, the output of fermentative product achieve to 2.5~3g/L.

Key Words : Gloeostereum incarnatum S.Ito et Imai;immersing culturef; fermentative product

The scientific name of Gloeostereum incornatum S. Itoet Imai is Ba-Sidiomycotina, Hymetnomycetes, Aphyllophorales, Corticiaceae and Gloeostereum, the wild Gloeostereum incornatum S. Itoet Imai grows in China, northeastern district and the north sea of Japan. As a famous medicinal and edible mushroom, it includes the wild mushroom cultivated artificially and the liquid fermentation hypha. This mushroom tastes like a sea cucumber with abundant nutrition and various physiological activity(anti-inflammation and anti-bacterial) and the demand in the domestic and national markets is increasing. In China, the wild mushroom cannot meet the demand so it is cultivated artificially. Also as for the fungi used for the production of antiseptic, it is used after the fermentation(liquid).

However, as the study on fermentation condition is still in the initial stages, which prevents the liquid fermentation in a large scale.

In this condition, we(School of Life Science Northeast Nomal University) are studying the liquid fermentation condition. We are introducing the experimental results as follows:

1. Materials and method

1.1. Type of Fungus: School of life science northeast Nomal university in China separated Gloeostereum incornatum S. Itoet Imai from the wild mushroom collected in the forest of Mt. Baekdu area, Kilimsung, China.

Medium(culture medium): PDA
Culture temperature: 26 ℃~28 ℃

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Culture duration: 4-5 days

After 4-5 day cultivation of fungus in the medium, hyphae in the form of white villosity grow around the mushroom. If it is transplanted in another medium, this special fungi is obtained after 4-5 days.

You should make sure to confirm that this fungi is the Gloeostereum incornatum S. Itoet Imai at the reproduction experimentation.

1.2 Medium:

In vitro medium: PDA

Fruit body medium: sawdust

Liquid fermentation medium: glucose medium (corrosive elm tree extract liquid cannot be used)

1.3 Culture method:

Culture temperature: 27 ℃～28 ℃ PH=5.5～6

Speed of revolution: 100～120 r/min

Volume of fermentation liquid:

100 ml/250ml = per bottle

250 ml/500ml = per bottle

Inoculation amount: 5～10%(liquid inoculation)

Inoculated 4 bottle(250ml) per slant hypha.

Fermentation tube: Automatic fermentation facility of Verrt company in America.

1.4 Measurement method:

Wet weight of hyphae is measured in general and normal method. The dry weight of hyphae is also measured in general and normal method. PH is measured with 25 type PH agent. Reducing sugar is measured in I2 measurement method. Amino acid is measured in HCHO titrating method.

Antibiotics(C9H4O) of Gloeostereum incornatum S. Itoet Imai is measured by using 751 type ultraviolet spectrometer.

2. Results and examination

2.1 Form observation

Hyphae: Hyphae is of white string form and has diaphragm. The diameter of hypha is 1.5～25 mm. Weak strings form build up to be thick villosity on

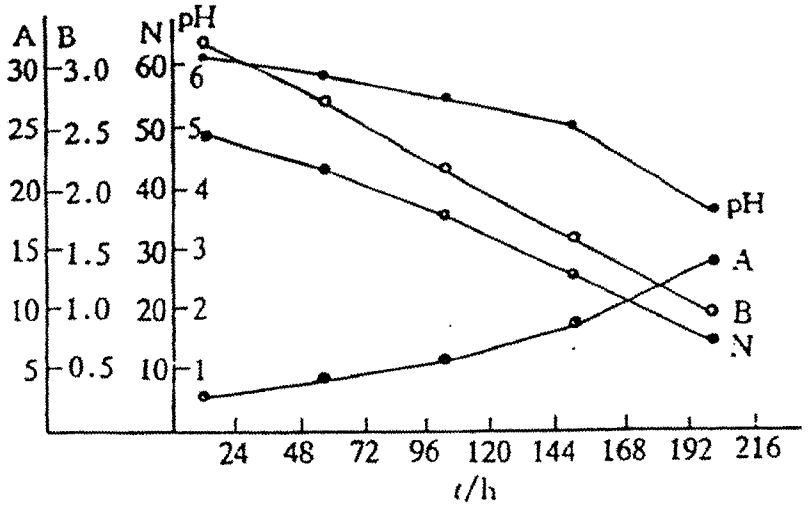


Fig. 1. Decrease of nutrition in the fermentation liquid of Gloeostereum incornatum S. Itoet Imai

A. Wet weight of hyphae/% B. Reducing sugar/%

N. Amino acid/mg (10mL)

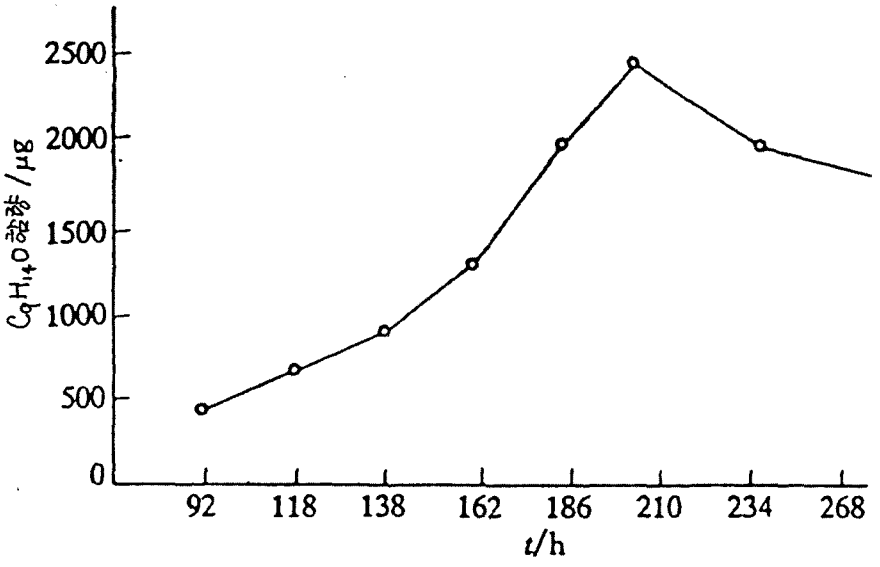
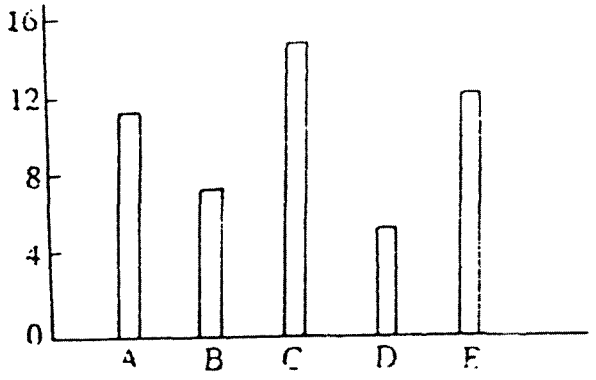


Fig. 2. Relation between fermentation time and C9H14O content



A: Tryptone medium
B: Corn flour medium
C: 두병가루 medium
D: Potato medium
E: Malt skin medium

Fig. 3. Effect of different medium on hyphae production

PDA medium and as the culture time passes on, its color turns into light yellow sticking to substrate, generating many aerial hyphae and branches to be thick hyphae so we can observe the unique shape of hyphae through the microscope.

Fungus ball: In liquid culture, the hypha forms fungus ball in a arch-shaped combination by the table revolution and in the standstill culture no fungus ball is produced. The diameter of the fungus ball varies from 1mm~10mm.

2.2 Reduction of C and N in the fermentation liquid and determination at the end of fermentation

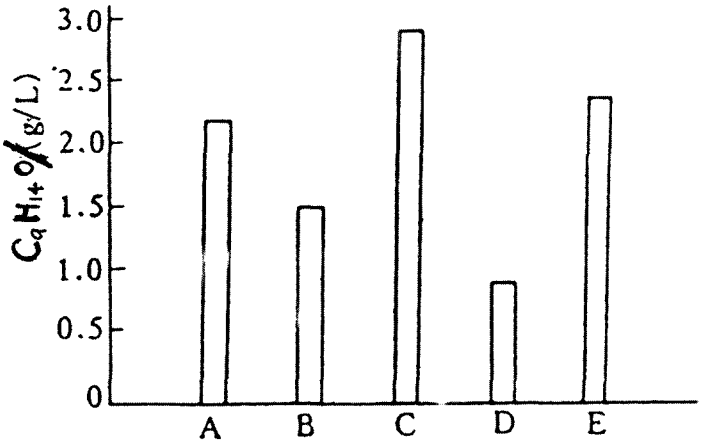


Fig. 4. Effect of different medium to C9H14O production

As the fermentation time passes, the amount of hyphae increases, its color becomes gradually lighter, the liquid becomes clear, the viscosity of the fermentation liquid increase and the liquid produces the smell of fruit. At the observation with the microscope 150 hours after the fermentation, the color of the hyphae is not uniform nor pure, vacuoles are generated, PH decreases, the percentage of reducing sugar is about 1%, amino acid turns to be 20% or lower and the fermentation stops when the wet weight of the hyphae is 11%～14%(figure 1). The fermentation time of the Gloeostereum incornatum S. Itoet Imai and content change in the activated product of the fermentation(C9H14O) are indicated in the diagram(figure) 2.

In figure 1 and 2, when the fermentation time is 8, ph, reducing sugar and amino acid show the lowest values, with the highest wet weight and C9H14O content of hyphae.

2.3 Effect of diverse medium on hyphae and fermentation product

At the result of the fermentation in 5 different medium for 8903 strain of Gloeostereum incornatum S. Itoet Imai-glucose medium(C) has the best result, followed by malt skin and then Tkyptone medium.(as indicated in figure 3 and 4)

2.4 Effect of medium content on hyphae growth

2.4.1 Effect of different carbon source on the hyphae growth

We observe the effect on the hyphae growth by changing the carbon source after selecting the basic medium. Its results are indicated in the table 1.

Table 1. Effect of different carbon source on the hyphae growth

Carbon source	Glucose	Suger	Malthouse	Corn starch
Hyphae weight/g(100ml)	0.86	0.9	0.65	0.7

Based on the table 1, there is no big different between glucose and sugar and glucose is more suitable in the industrial production.

2.4.2 Effect of glucose amount on the hyphae growth

We indicate the hyphae grown according to the

change of glucose amount and based on the table 2, when the glucose amount is less than 3%, it has an influence on the hyphae growth and even if it is higher than 3%, there is no big increase in the hyphae growth so in the economic sense, 3% is suitable.

Table 2. Effect of different glucose amount on hyphae growth

Used amount/%	1	2	3	4	5
Remaining after using/%	0.5	0.8	1	1.4	2.5
Hyphae weight/(100ml)	0.5	0.7	0.9	0.95	0.95

2.4.3 Effect of different nitrogen(N) source on hyphae growth

We observe the hyphae growth by changing the nitrogen source into minerals and organic carbon(same proportion) with the same basic medium. The results indicated in the table 3.

Table 3. Effect of different nitrogen source on hyphae growth

Nitrogen source	Nitric acid	Lactic acid	Trypton	두병가루
Growth density	-	+	++	+++
hyphae weight/g(100ml)	-	3.5	14	12

Note) We classified into three classes such as +, ++ and +++, changing from low density to high density.

According to the experimentation result, 28 ℃ is the optimum temperature.

As shown in Table 3, organic nitrogen is more advantageous to growth than inorganic nitrogen and the fungi do not use the nitrogen in the form of nitric acid.

2.4.4 Effect of different content on hyphae growth

We observe the hyphae growth in the condition to change content. The result is indicated in Table 4. According to the result, 1% of content is most advantageous to hyphae growth. Its increase or decrease has adverse effect on hyphae growth. The combined use of and Tryptone is advantageous to hyphae growth.

Table 4. Effect of different content on hyphae growth

Used amount/%	1	2	3	4	5
Growth density	+++	+++	+++	++	+
Hyphae weight/g(100ml)	14	14	13.5	11	10

2.5 Effect of various culture conditions on hyphae growth

2.5.1 Temperature and growth

Although it is believed that the optimum temperature for hyphae growth does not exist since before 60' s, we still use the concept of "optimum temperature". The growth temperature of Gloeostereum incornatum S. Itoet Imai range from 20℃ to 30℃ in the plane medium and the optimum temperature is 27℃ to 28℃ and in the liquid fermentation culture, the hyphae weight is heaviest at 28 ℃. Therefore it is believed that 27℃ to 28℃ is the most suitable temperature to hyphae growth. Refer to Table 5.

According to the experimentation result, 28 ℃ is the optimum temperature.

2.5.2 PH and growth

The relation between PH and growth is effected by

Table 5. Effect of temperature on hyphae growth

Temperature	20	22	24	26	28	30	32	34
plane선형대 extension length(cm)	4	5	5.5	9	13	2	4	2
Hyphae wet weight in revolution liquid fermentation	2.5	3.8	5	10.6	12	9.5	3	

several factors. For example, it is effected by temperature, density of ions such as Ca, Mg, etc and nitrogen source and we perform the experimentation making only the change of PH in the medium effect on the growth through all these factors. We indicate the result in table 6 and PH=6 is most advantageous for the growth before the liquid fermentation.

Table 6. Effect of PH on hyphae growth

PH	3	4	5	6	7	8
Hyphae weight g/100ml	-	3.5	7.9	11.5	3.3	-

2.5.3 Air amount and growth

We measere the hyphae amount by injecting 80ml, 100ml, 150ml and 200ml of medium in 750ml triangle wall for fermentation culture. The result is indicated in Table 7. As indicated in Table 7, 80ml medium has the highest percentage of wet weight of hyphae. The result shows that sufficient dissolvable oxigen is consumed during fermentation.

Table 7. Effect of air amount on hyphae growth

Fermenyayion liquid volume(ml)	80	100	150	200
Hyphae wet weight	12.5	12	11	9.5

2.6 Measurement of fermentation component (C9H14O)

We measure the maximum absorption rate in RF-540 type spectrum photometer by dissolving the activated material, the only component(C9H14O).(= 355mm)

Next, taking =355 as standard wavelength, we make the diagram showing the relation between optical density and concentration by measuring the optical density with different concentration with 751 type violate spectrum photometer.(Standard curve). Using this diagram, we measure the density of the effective material produced by hyphae during the fermentation duration. We can observe the fermentation situation.

We determine the liquid fermentation condition of Gloeostereum incornatum S. Itoet Imai in an elementary stage with various experimentations on fermentation duration. We believe that it might be helpful for the mass fermentation production in the future. However, we believe that the fungi cultivated by us are short of generality due to the limited facility in Northeast Nomal University so we need expanded experimentation for the industrial production based on this result.

Note: (1) We omit the references.

(2) The chemical formula for C9H14O is as follows:

(antibiotics)