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Some Factors Affecting Growth of *Mycogone perniciosa* Magn. Causing Wet Bubble in Cultivated Mushroom, *Agaricus bisporus* (Lange) Sing.

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양송이 마이코곤 病菌(*Mycogone perniciosa* Magn.)의 生長에 影響을 미치는 要因

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Abstract : 1. The mycelial growth and sporulation of *Mycogone perniciosa* was compared on modified Czapek's media deficient in carbon source, nitrogen source, K,Mg, P or the heavy metal elements. The mycelial growth was significantly reduced in solution cultures lacking Mg, K or P and only a trace of growth occurred in solutions lacking carbon source or nitrogen source. Most sparse sporulation and smaller chlamydospores than on any of deficient agar media occurred on agar media dificient in carbon source or nitrogen source.

2. In both potato dextrose agar and malt extract solution, growth of the fungus was optimum at 25°C, and undetectable at 10°C and 35°C.

3. Optimum pH for growth of this fungus was 7.0.

4. This fungus was killed in soil when exposed to 50°C or higher for 20 minutes or more.

Introduction

Wet bubble disease on cultivated mushroom, *Agaricus bisporus* (Lange) Sing. caused by *Mycogone perniciosa* Magn. which was reported in 1971 for the first time from commercial farms in Suck Sung Myun area, South Choong Chung province in Korea spread into Ick San Gun, Chun Boock province and became the most important limiting factor for the mushroom growing in these areas.

Although various fungicides were applied for the control of the disease to the infected area, the results were not satisfactory.

Thus, it is considered that utilization of some factors suppressing the development of *M. perniciosa* together with using fungicides can be expected to prevent or minimise effectively the epidemics of the disease.

Some factors affecting the growth of this fungus have been discussed previously by a few workers.

Lambert (1930) reported that the most vigorous growth was made between 21°C and 28°C. Treschow (1941) indicated that the optimum temperature for this fungus was 22°C, also Dough and Hung (1971) reported that optimum temperature for growth of this fungus was 25°C. According to Trechow (1941), the optimum pH for the fungus was 6.7, whereas Dough and Hung (1971) stated that optimum pH was 4.4. Lambert(1930) suggested that vigorous cultures of *M. perniciosa* fail to survive exposure to temperatures higher than 42°C for periods of more than 6 hours. Wuest, Eaker and Conway (1970) stated that *Mycogone* sp. was killed in soil treated at 54.4°C of aerated steam for 30 minutes.

Present experiments were undertaken to check the results of other workers and to obtain further information on the some environmental factors affecting growth of *M. perniciosa* with reference to control of wet bubble in *Agaricus bisporus*.

Materials and Methods

Effects of nutrition on mycelial growth and sporulation.

The basal medium was modified Czapek's liquid medium to which microelements(Cu, Zn, Mn) were added. The formula for the basic nutritional solution was as follows; Sucrose:30g, $NaNO_3$:2.0g, $MgSO_4 \cdot 7H_2O$:0.5g, K_2HPO_4:1.0g, KCl:0.5g, $FeSO_4.7H_2O$: 0.001g, $CuSO_4.5H_2O$: 0.001g, $ZnSO_4.7H_2O$: 0.001g $MnSO_4.7H_2O$: 0.001g ; and distilled water to make 1 litre of madium.

To prepare culture solutions minus known essential elements, the following omissiens and substitutions were made in the basic solution ; Minus carbohydrate, sucrose omitted; minus nitrogen source, $NaNO_3$ omitted; minus Mg, $MgSO_4$ omitted and K_2SO_4 substituted; minus K, K_2HPO_4 and KCl omitted, and Na_2HPO_4 and NaCl substituted; minus P, K_2HPO_4 omitted and K_2SO_4 substituted; minus heavy metal elements, salts containing Fe, Cu, Zn and Mn omitted.

a) Growth in liquid culture solution—50ml portions of nutrient solution were placed in 250ml Erlenmyer flasks, were autoclaved in 121°C for 20 minutes. Spore inoculum, prepared on potato sucrose agar was collected from slant cultures that had been incubated for 17 days at 25±1°C.

Spores were separated from the mycelia by aseptically filtering them through two layers of cheesecloth.

One ml of spore suspension was added to each flask prior to incubation. After the media were inoculated, the flasks were incubated at 25±1°C in still culture. Each treatment comprised 4 replicates.

After 13 days of incubation, the contents of the flask were transferred to a 50ml centrifuge tube, centrifuged for 5 minutes at 1,500 r.p.m., then the suspension was rinsed. The mycelial mat placed in preweighed aluminum cup, dried for 24 hours at 80~90°C and weighed.

b) Growth on agar culture—Since agar is a complex polysaccharide sulfate ester and radial growth measurements on solid media ignore tangential growth of fungus, agar cultures were used to observe the sporulation of this fungus. 2% agar media was made with powder agar of the complete nutrient solution and all solutions deficient in elements. The inoculum discs, 6mm in diameter, were cut from the advancing margin of 6 day-old culture on potato sucrose agar and placed in the center of each plate. All plates were maintained at 24±1°C and experiments were repeated twice with 7 plates of each treatment.

Radical growth measurements were made 5 and 8 days after inoculation. In addition, the plates were observed at regular intervals under a microscope for the amount of sporulation of chlamydospores and conidiospores.

Effect of temperature on mycelial growth

a) Growth on agar media—10ml of autoclaved potato sucrose agar medium were added to each Petri dish. Discs 5mm in diameter, cut from the outer margin of a potato sucrose agar culture of the fungus were placed in the center of each plate. The plates were incubated at 5°C intervals through the range of 10°C to 35°C with 5 replicates, and

radical growth measurements were taken 7 days after inoculation.

b) Growth in liquid culture solution—100ml of malt extract solution were added to each 300ml Erlenmeyer flask. Each flask was inoculated with 1 ml of spore suspension and each treatment replicated 4 times.

After they were incubated for 12 days under quiescent condition, the mycelial mats were transferred to pre-weighed filter paper(TOYO ROSHI KAISHA, No.6), washed with distilled water to remove the surplus of nutrient solution, and dried in a drying oven at 80°C for 40 hours to constant weight. The dried material was then weighed to determine yield of the fungus from the culture medium

Effect of pH on mycelial growth

The pH of the media was adjusted between 4 to 7.6 by addition of Citrate-phosphate buffer solution (Colowick and Kaplan, 1955) into Yeast starch solution.

The 0.5ml of spore suspension of *M. perniciosa* was inoculated to 50ml of medium in 250ml Erlenmeyer flask with four replicates in each treatment.

They were grown for 16 days at 25°C in still culture, growth was recorded as the oven-dry weight of the mycelial mats.

Thermal death point

Test-tubes were packed with 40g of soil (sandloam 3 : loam 7) wetted with potato sucrose solution. They were sterilized in an autoclave at 121°C for 2 hours. Conidial suspension in the amount of 1 ml of *M. pernicosa* was inoculated into each sterilized test-tube. After the inoculation, test-tubes were placed in an incubator at 25°C for 20 days.

The temperature in the water bath varied at 10°C intervals ranging from 40°C to 80°C. The exposure time in each water bath temperature were 10, 20, 40 and 60 minutes. The timing commenced when the soil heated to the treatment temperature. Following treatment with four replicates, the inoculated and treated soil was placed on a plate of potato sucrose agar and incubated for 4 days to obtain the thermal death point of the fungus.

Results and Discussions

Effects of nutrition on mycelial growth and sporulation

Mycelial growth of *M. perniciosa* was the best in the complete liguid medium and was satisfactory in the solution minus heavy metal elements. When carbon or nitrogen source were lacking, only a trace of growth occurred and growth was significantly reduced in solutions lacking the essential elements Mg, K, P. On the other hand, the fastest radical growth occurred on agar medium deficient in K, but mycelial density was more sparse than that on complete agar medium. The mycelial growth and density on agar media deficient in Mg or heavy metals were about equal to those on complete agar medium.

The radical growth on agar medium lacking in P was the slowest but was more dense than those on carbon source, nitrogen source or K deficient agar medium. On agar medium lacking in carbon source, the mycelial growth was comparatively slow, most sparse and difficult to see as well as that on the water agar. The amount of sporulation of chlamydospore and conidiospore was abundant on complete agar medium and on Mg or heavy metals deficient agar medium, whereas the most sparse sporulation and smaller chlamydospores than on any of above agar media occurred on agar medium deficient in carbon source or nitrogen source, all of which were of comparable amounts of sporulation and spore sizes.

On agar medium dificient in P, in the early stage, chlamydospore formation retarded to some extent and was very sparse as well as that on carbon deficient agar medium, but amount of sporulation was gradually increased as the colony aged.

According to preliminary experiments, malt extract solution and Czapek's solution were very satisfactory for growth, but the growth in Petri mineral solution lacking a carbon source was poor. Also, growth of this fungus was most rapid in Czapek's solution supplemented by 3% sucrose, and somewhat inhibitive at higher concentration, while the

Table. 1. Mycelial growth and sporulation of *Mycogone perniciosa* in modified Czapek's solution and on agar culture minus indicated elements.

media	Dry weight mycelial mats(mg) a)	Radius of colony(mm) b)	Relative density of mycelia c)	Relative amount of sporulation d)	
				Chlamydospore	Conidiospore
Complete medium	140	68.0	5	4	4
Minus C	3	64.0	1	2	1
Minus N	16	65.6	2	2	2
Minus Mg	56	68.7	5	4	4
Minus K	32	71.1	3	3	2
Minus P	48	62.8	4	3	3
Minus heavy metals	111	68.3	5	4	4

L.S.D.$_{05}$ 25.5

a) Dry weight of mycelial mats in liquid culture after 13 days.

b) Radius colony on agar culture 5 days.

c) Relative density of mycelia on agar culture after 8 days.
1 : Highly sparse 2 : Sparse 3 : Moderate 4 : Dense 5 : Highly dense

d) Relative amount of sporulation of chlamydospore and conidiospore on agar culture after 9 days
1 : Highly sparse 2 : Sparse 3 : Moderate 4 : Abundant

growth in solution minus sucrose was very meager. Therefore, it is evident that the nutritional requirements of this fungus are definite and the growth is singnificantly inhibitive not only carbon source or nitrogen source deficiency but in the mineral elements. The results of these studies demonstrate that avoiding the use of soil containing excessive organic matters and mineral elements might be helpful in reducing the occurrence of wet bubble.

Effect of temperature on mycelial growth.

In both solid and liquid cultures, growth of the fungus was most rapid 25°C, very slow at 15°C but undetectable at 10°C and 35°C (Fig.1).

Lambert (1930) reported that *M. perniciosa* grew most vigorously when subjected to temperature between 21°C and 28°C, which is above the normal range of temperature for growing mushroom. According to Treschow (1941), the optimum temperature for the growth of *M. perniciosa* was 22°C and no fungus developed at 8°C. Dough and Hung (1971) reported that growth of *M. perniciosa* was best at

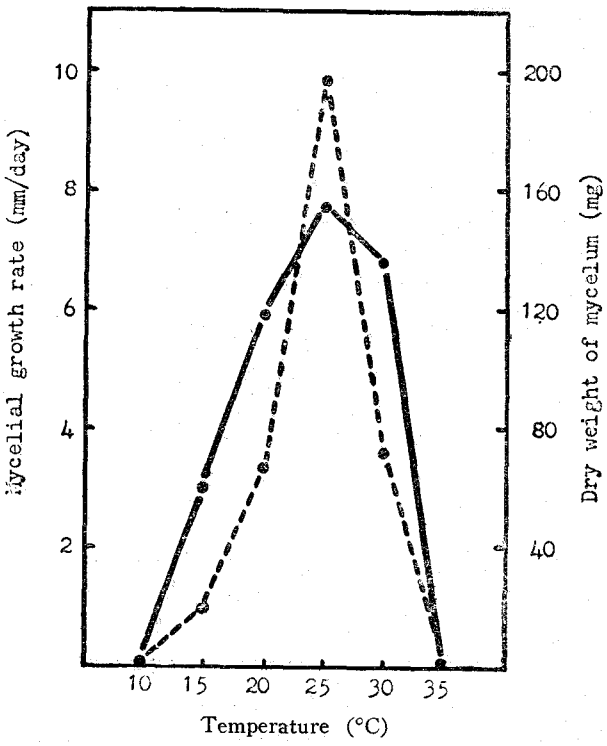

Fig. 1. The effect of temperature on growth of *Mycogone perniciosa* in liquid (●·····●) and on solid medium (●——●)

temperature of 25°C. Last and Gandy (1941) indicated that the cropping temperature should be maintained below 15.5°C. Flegg (1968) reported that there was no difference in the weight of the crop when the cropping temperatures were 13.9 or 16.7° C but 19.5°C resulted in a reduced yield when compared with 16.7°C. The results of this experiment indicate that during the cropping the temperature should be kept at 15°C or lower for high yield of mushroom as well as reducing the incidence of this disease.

Effect of pH of medium on mycelial growth.

M. perniciosa grew well in a neutral solution of pH 7 (Fig.2).

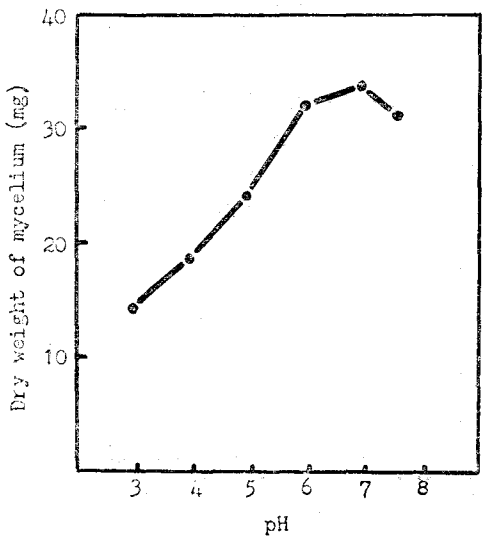

Fig.2. The effect of pH on mycelial growth of *Mycogone perniciosa*

The result of this test is contray to the finding of Dough and Hung (1971) who showed that optimum pH for growth of *M. perniciosa* was 4.4. Treschow (1941) reported that the optimum pH for *M. perniciosa* was determined as 6.7.

Lambert and Humfeld (1939) concluded that an approximately neutral soil (pH 7.0) gave better mushroom yields than excessively acid or alkaline soils, but that any pH value between pH 5.5 and 8.0 seemed suitable, also Allison and Kneebone (1962) reported that a pH of 5.5 to 7.5 is not a limiting factor in the suitability of either the compost or the casing soil for the production of mushrooms. Therefere, the optimum pH level of *M.perniciosa* is similar to that of *A. bisporus* and the results of this study lead to the conblusion that artificially altering the pH of a casing soil for the pervention of this disease is pointless.

Thermal death point

This fungus was killed in soil when exposed to temperatures 50°C or higher for 20 minutes or more (Table 2).

Table 2. The thermal death point of *Mycogone perniciosa* in soil when exposed to different temperature for different lengths of time.

Treated minutes	*Mycogone* recovery from the heat-treated soil a)				
	40°C	50°C	60°C	70°C	80°C
10	+	+	−	−	−
20	+	−	−	−	−
40	+	−	−	−	−
60	+	−	−	−	−

a) 4 replicates/treatment. +: Recovery on Potato-sucrose agar −: Non recovery

Lambert (1930) reported that in agar custure *M. perniciosa* was killed by exposure to temperature of 42°C for 6 hours Whest, Baker and Conway (1970) stated that *Mycogone* sp. and some other mushroom pathogens could not be isolated from soil treated at 54.4°C for 30 minutes. The reason for the difference of thermal death points and time among the reports may be assumed to be the difference of testing methods and materials, In additien, when a soil, naturally contaminated with *Mycogone* sp., was steamed at 80°C or higher for 30 minuts, only 4 infected sporophores or sclerodermoid masses were appeared in the beds cased with steamed soil but 1,222 in beds with untreated ones.

Occurrence of small amount of wet bubble in bed cased with steamed soil might be due to a part of soil not thoroughly exposed with the aerated steam

or accidental contamination. It is considered that for satisfactory disinfection of this disease the casing soil should be thoroughly exposed for 20 minutes or more at a temperature of 50°C or higher.

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摘　要

양송이〔*Agaricus bisporus*(Lange)Sing〕에 마이코곤病을 일으키는 *Macogone perniciosa* Magn.의 生長에 미치는 環境的 要因의 影響을 本病의 防除와 關聯하여 試驗하였던 바 本 病原菌은

1. 차펙스培養基에 數種의 微量元素를 添加하여 製造된 基本培養基에서 炭素源, 窒素源, K,P,Mg 및 重金屬元素를 각각 缺如시켜서 菌絲生長및 胞子形成을比較한 結果, 炭素源혹은 窒素源이 缺如된 培養基上에서는 菌絲生長이 거의 微微하였으며 胞子形成도 顯著히 줄었고 厚膜胞子의 크기도 比較的 작았다. Mg,K,P 등 無機元素가 缺如되었을 때에도 菌絲生長이 低調하였다.

2. 감자寒天培養基와 麥芽抽出液體培養基에서 生育最適溫度는 25°C 이었으며 10°C와 35°C에서는 生育되지 않았다.

3. pH 3~4의 强酸性의 培養基에서는 生育이 不良한 反面에 中性및 弱酸性에서 生育이 良好하였고 生育最適酸度는 pH 7 이었다.

4. 土壤試料中에서 生育되었을 때 50°C의 水槽上에서 20분간 熱處理함으로써 完全히 死滅되었다.

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