NITROGEN NUTRITION OF *AGARICUS BISPORUS*

I. FORM OF INORGANIC NITROGEN UTILIZED BY *AGARICUS BISPORUS*

H. T. Hsu and K. J. Hu

Respectively, Research Assistant and Senior Specialist, Department of Plant Pathology, Taiwan Agricultural Research Institute, Taipai, Taiwan.

Research supported in part by Grant 65-MRF-A-1723, JCRR.
Accepted for publication February 20, 1967.

ABSTRACT

The effect of three inorganic forms of nitrogen, namely nitrates, nitrites and ammonium salts, upon the growth of *Agaricus bisporus* was studied. No growth was observed when nitrates or nitrites were supplied as the sole source of nitrogen in a synthetic solution. The fungus utilized ammonium nitrogen within a limited concentration. Dry weight of mycelial mats of *Agaricus bisporus* decreased when the concentration of ammonium nitrogen increased beyond 1000 mg/l.

INTRODUCTION

One of the most important factors affecting the cellular metabolism of a heterotrophic organism is the kind of available sources of nitrogen in the medium. Nitrogen compounds serve two essential roles in the metabolism of fungi, functional and structural. Present investigation tried to find out the forms of inorganic nitrogen which can support the growth of cultivated mushroom, *Agaricus bisporus, in vitro*. Inorganic nitrogen compounds other than ammonium salts, nitrates, and nitrites are not included under this investigation, since these compounds appear, in general, to be utilized only if easily or spontaneously converted to one of the three mentioned forms.

MATERIALS AND METHODS

Cultivated mushroom, strains 543, 202, and 14, were used throughout the experiment. The fungus was maintained on rice straw compost decoction agar medium. Fifty grams prepared compost and 50 g corn meal were boiled in 500 ml deionized water for 60 min. Eighteen grams agar was disolved into another 500 ml deionized water. The filtrates of the two solutions were added together. The final volume was made up to 1000 ml. The pH was adjusted to 6.8 either by 0.1 N NaOH or 0.1 N HCl.

The inoculum for cultural experiments consisted an agar bit, sized 2 mm in diameter, on which the fungus has grown. Surface cultures were employed. The synthetic solution contained 10 g dextrose, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 10 mg $FeSO_4 \cdot 5H_2O$, 3 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 3 mg $MnCl_2$, 3 mg $ZnCl_2$,

1 mg $CuSO_4 \cdot 5H_2O$, and 3 mg thiamine-HCl, and was brought up to 1000 ml with deionized water.

All glassware used in the experiment was soaked in 4 N HCl for 48 hours, rinsed with tap water and then with deionized water. Fifty ml of the solution was placed in each of the 250-ml Erlenmeyer flasks. Dextrose was sterilized by passing through bacteriological fritted filters. The other chemicals were sterilized by autoclaving at 15 lbs pressure for 20 min. Dextrose was added to the solution aseptically. They were adjusted to pH 6.8±0.1 either by sterilized 0.1 N NaOH or 0.1 N HCl. The prepared medium was let to stand at room temperature for 3 days. The flasks with contamination were removed.

After 3-week culture at 23°±0.5°C, the mycelial mats from 4 replicate flasks were collected. Dry weight of mycelial mats was taken after ovendry at 80°C for 24 hours in terms of fungus growth.

RESULTS

The growth of *Agaricus bisporus* on different forms of inorganic nitrogen is shown in Table 1. Nitrogen which is in form of nitrates or nitrites did not support growth. The fungus could utilize ammonium salts as sources of nitrogen. The ammonium salts in sulfate or tartarate form served equal well for the growth of *Agaricus bisporus*. The effect of concentration of ammonium nitrogen in cultural solution is also shown in Table 1. The most suitable concentration of ammonium nitrogen for the fungus growth was between 800–1000 mg/l (Fig. 1). The growth of the fungus increased proportionally from 0 to 800 mg/l ammonium

Table 1. The effect of inorganic nitrogeneous compounds in a synthetic solution on the mycelial growth of cultivated mushroom *A. bisporus* strain 543 at 23°±0.5°C after 3-week culture (mg/200 ml dry weight of mycelium).

Conc. of N mg/l	Nitrate N		Nitrite N		Ammonium N	
	Sodium nitrate	Potassium nitrate	Sodium nitrite	Potassium nitrite	Ammonium sulfate	Ammonium tartarate
0	0	0	0	0	0	0
200	0	0	0	0	7.02	9.37
400	0	0	0	0	19.81	23.67
600	0	0	0	0	78.82	64.33
800	0	0	0	0	93.27	87.53
1000	0	0	0	0	80.22	86.74
1200	0	0	0	0	51.78	72.92
1400	0	0	0	0	46.27	53.25
1600	0	0	0	0	41.74	46.07
1800	0	0	0	0	30.54	47.39
2000	0	0	0	0	14.65	20.75

nitrogen. When the ammonium nitrogen increased beyond 1000 mg/l, the yield of the mycelial mats decreased.

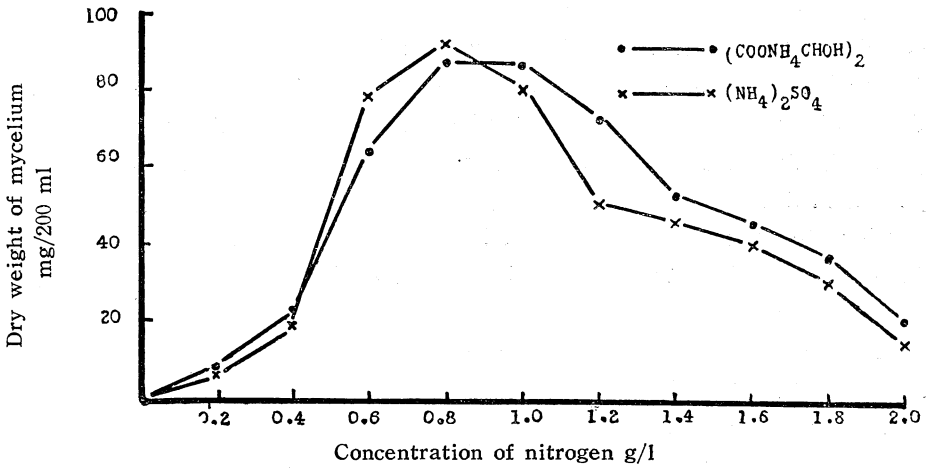

Figure 1. The effect of concentration of ammonium nitrogen in a synthetic solution on the yield of mycelium of *A. bisporus* strain 543 at 23°±0.5°C after 3-week culture.

To determine whether or not the ammonium salts other than ammonium sulfate and ammonium tartarate are available for fungus growth, ammonium chloride, ammonium carbonate, ammonium nitrate, ammonium acetate and ammonium citrate were studied at the concentration of 800 mg/l nitrogen. Table 2 shows that all ammonium salts used in this experiment could support the growth of *Agaricus bisporus*.

Table 2. The effect of ammonium salts in a synthetic solution on the mycelial growth of cultivated mushroom *A. bisporus* at 23°±0.5°C after 3-week culture (mg/200 ml dry weight of mycelium)

Ammonium salts	Mycelial dry weight mg/200 ml		
	Strain 543	Strain 202	Strain 14
Ammonium carbonate	81.2	81.7	76.0
Ammonium chloride	97.3	84.3	85,5
Ammonium nitrate	61.5	60.4	66.9
Ammonium sulfate	89.4	79.6	83.2
Ammonium acetate	80.2	76.8	77.5
Ammonium citrate	86.5	89.8	79.4
Ammonium tartarate	85.4	91.2	70.6

DISCUSSION

Nitrates as excellent sources of nitrogen for many fungi is well known (Lilly and Barnett, 1951; Cochrane. 1958) Apparently, *Agaricus bisporus* cannot utilize

nitrates to support its growth. Failure to utilize nitrates as sources of nitrogen was not overcome by the presence of molybdenum in the medium as suggested by Steinberg (1937) and Mulder (1948). Evidence shows that the fungus grows well when the ammonium nitrate was used as the source of nitrogen (Table 2). This indicated that nitrates did not support fungus growth was not due to the toxicity of nitrates to the fungus. It also suggested that the fungus was unable to form nitrate reductase, an inducible enzyme, even at the presence of the substrate in the medium, since no nitrate reductase activity could be detected (unpublished data from senior author).

Failure to utilize nitrites, which is the more common situation among fungi, could be explained on the basis of its known toxicity.

Ammonium nitrogen was generally utilized by *Agaricus bisporus*. The amount of growth of the fungus was restricted within a certain limits of concentration of nitrogen (Fig. 1). The inhibition of the growth of *Agaricus bisporus* beyond 1000 mg/l nitrogen was presumably, in part at least, by the fungistatic action of ammonium ions, since ammonia in high concentration is toxic.

LITERATURE CITED

1. Cochrane, V.W., 1958. Physiology of Fungi. John Wiley and Sons, Inc., New York, p.524.
2. Lilly, V.G. and H.L. Barnett, 1951. Physiology of the Fungi. McGraw-Hill Book Co., New York, p. 464.
3. Mulder, E.G., 1948. Importance of molybdenum in the nitrogen metabolism of micro-organisms and higher plants. Plant and Soil 1:94-119.
4. Steinberg, R.A., 1937. Role of molybdenum in the utilization of ammonium and nitrate nitrogen by *Aspergillus niger*. Jour. Agr. Research 55:891-902.

洋菇之氮素營養

I. 無機態氮的利用

徐惠廸　胡開仁

1. 本文詳述三種無機態氮素對洋菇生長之情形。
2. 硝酸態及亞硝酸態的氮不能供洋菇生長。
3. 銨鹽類的氮爲洋菇之良好氮素源。
4. 銨鹽類的氮在 800 mg/l 之濃度時最適合洋菇之生長。
5. 銨鹽類的氮超過 1000 mg/l 時能減低洋菇之生長。