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# A preliminary study on nutritional requirements of nematophagous fungus ARF907 for mycelial growth

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**After studying the effects of carbon sources, nitrogen sources, and mineral elements on the mycelia growth of ARF907 in our lab, this research firstly was concerned with the carbon concentration, carbon to nitrogen ratio on the mycelia growth of this fungus, and the better mycelial yield got with carbon concentration of 6 g $l^{-1}$ and carbon to nitrogen ratio of 160:1. This paper secondly was concerned with the combinations of carbon and nitrogen sources on mycelial growth at certain carbon concentration and carbon to nitrogen ratio. Full experiments of 4 carbon sources and 6 nitrogen sources, were selected and found that fructose with soy peptone got the best mycelia yields.**

**Key words:** Carbon concentration, carbon to nitrogen ratio, carbon source, nitrogen source.

## INTRODUCTION

The nematophagous fungus ARF907 is a promising biocontrol agent (Timper et al., 1998; De et al., 1993) which can infect soybean cyst nematode at any growth stages except second stage juveniles (J2) (Kim and Riggs, 1991; De Leij et al., 1993). High-efficiency production of biomass or vegetative form of the biocontrol fungus is a prerequisite for successful application of relevant fungal biopesticides. Understanding its nutritional requirements is critical for the growth, reproduction, and efficacy of the biocontrol fungus. Whether it can form substantial biomass on the culture medium is largely constrained by medium composition and culture conditions. To date, a number of studies have investigated the influences of carbon (C) source, nitrogen (N) source, C concentration, C/N ratio, vitamins, and minerals on mycelial growth and sporulation of nematode-trapping and endoparasitic fungi (Liu and Chen, 2002, 2003; Mo et al., 2005; Gao et al., 2007). On the basis of a previous work (Sun and Liu, 2006), this study further explored the nutritional requirements of the biocontrol fungus ARF907 for mycelial growth.

## MATERIALS AND METHODS

### Fungal strain

Strain ARF907 was isolated from soybean cyst nematode by Kim (Arkansas, USA).

### Culture methods

Assessing the influence of C concentration and C/N ratio on mycelial growth of ARF907 was done.

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***Preparation of culture media***

After soaking in a 0.05% solution of hydrochloric acid for 24 h, new Petri dishes were rinsed thrice with clear water and once with distilled water, air-dried in the shade, and then sterilized by dry heat at 180°C for 2–3 h before use. PDA medium was prepared according to standard procedure (Fang, 1998). Test media with different C concentrations and C/N ratios were prepared as follows (1 L): Sucrose (C content, 42%) for absolute C contents of 6, 8, and 12 $gl^{-1}$ ; soy peptone (N content, 8%) for the C/N ratios of 10: 1, 20: 1, 40: 1, 80: 1, and 160: 1 at each C concentration; 1.00 g $K_2HPO_4$; 0.50 g KCl; 0.50 g $MgSO_4$; 0.01 g $FeSO_4$; and 13.00 g agar powder. The medium broths were autoclaved at 121°C for 30 min and aseptically dispensed into sterile Petri dishes (10 mL each). After cooling and solidification of the agar medium, the round cellophane membranes (3.5 cm in diameter, autoclaved at 121°C for 30 min) were placed on the medium surface in a triangle pattern. The plates were then kept standing for 2 days to evaporate free water before use.

Inoculation and incubation: Strain ARF907 was grown on PDA medium for 2 weeks. A sterile 5-mm diameter puncher was used to make agar blocks of the same size. The blocks covered with ARF907 mycelium of consistent growth condition served as the inoculum and were placed at the center of cellophane membranes on the test media with different C concentrations and C/N ratios. The inoculated plates were incubated at 25°C for 2 weeks.

**Growth measurement**

Cellophane membranes carrying ARF907 colonies were carefully taken from the test media using sterile tweezers and directly weighed using a precision electronic balance (0.001 g). Net weight of fungal colonies was calculated by subtracting the weight of sterile cellophane membranes from the total weight of cellophane membranes and fungal colonies. Each treatment was repeatedly measured thrice.

**Data analysis**

Data are presented as arithmetic means of triplicate experiments. Significant differences between all means were examined using Fisher's least significant difference test ($P \leq 0.05$). Statistical analysis was performed in Office Excel 2010 (Microsoft Corp., Redmond, WA, USA) and SAS 8.0 (SAS Institute Inc., Cary, NC, USA).

**Assessing the influence of C and N sources on mycelial growth of ARF907**

***Preparation of liquid test media***

Liquid test media with different combinations of C and N sources were prepared using substrates that supported good growth of ARF907 in single-factor experiments, including four C sources (xylose, fructose, sucrose, and galactose), six N sources (yeast extract, soy peptone, urea, peptone, casein, and proline), and two minerals ($MnSO_4 \cdot 5H_2O$, 50 $mgl^{-1}$; and $ZnSO_4 \cdot 7H_2O$, 250 $mgl^{-1}$) (Sun and Liu, 2006; unpublished data). The media were formulated by taking into account the optimal C concentration (6 $gL^{-1}$) and C/N ratio (160:1). After dispensing into 50 ml centrifuge tubes (10 ml each), the liquid test media with different combinations of C and N sources were autoclaved at 121°C for 30 min before use.

***Inoculation and incubation***

Strain ARF907 was grown on PDA medium for 2 weeks. A sterile 5 mm diameter puncher was used to make agar blocks of the same size and three blocks with fungal mycelium of consistent growth condition were chosen as inoculums. The blocks were placed in 10 ml of liquid test medium with different combinations of C and N source, and then incubated at 25°C for 2 weeks with oscillation (180 rpm).

***Growth measurement***

Filter paper was oven-dried at 80°C to constant weight and weighed as soon as possible using a precision electronic balance (0.001 g). The filter paper was folded into a funnel shape and placed in a clean, dry 50 ml centrifuge tube. After the filtering of culture broth, the filter paper was rinsed once with sterile water and transferred to a sterile 50-mL centrifuge tube and oven-dried at 80°C to constant weight. The total weight of filter paper and the dried mycelium were measured and the dry weight of fungal mycelium was obtained by subtracting the weight of filter paper. The optimal combination of C and N sources for mycelial growth of ARF907 in liquid medium was identified according to dry weight of the mycelium. Each treatment was repeatedly measured thrice.

**Data analysis**

Data are presented as arithmetic means of triplicate experiments. Significant differences between all means were examined using Fisher's least significant difference test ($P \leq 0.05$). Statistical analysis was performed in Office Excel 2010 and SAS 8.0.

## RESULTS

### Influence of C concentration and C/N ratio on mycelial growth of ARF907

Different C concentrations and C/N ratios influenced mycelial growth of strain ARF907 to varying extents (Table 1). At the C concentrations of 6 and 8 $gl^{-1}$, there were no significant differences in mycelial yield of ARF907 between different C/N ratios. Better mycelial growth was observed at the C concentration of 12 $gl^{-1}$ and C/N ratio of 10:1, showing significant differences from the other treatments. At specific C/N ratio, there existed no significant differences in mycelial yield of ARF907 between different C concentrations. When the interaction between C concentration and C/N ratio was taken into account, there existed significant differences in mycelial yield between all treatments. The optimal combination for mycelial growth of strain ARF907 was C concentration of 6
$gl^{-1}$ + C/N ratio of 160:1.

### Influence of C and N sources on mycelial growth of ARF907

Different combinations of C and N sources had influences of varying degrees on mycelial growth of strain ARF907 (Table 2). The highest mycelial yield was achieved with the combination of fructose + soy peptone; sucrose +

**Table 1.** Effects of carbon concentrations and carbon-to-nitrogen ratios on mycelial growth of ARF907 (g).

| Carbon concentrations（$gl^{-1}$) | Carbon to nitrogen ratios | | | | | $LSD_1$ | $LSD_3$ |
|---|---|---|---|---|---|---|---|
| | 10 | 20 | 40 | 80 | 160 | | |
| 6 | 0.315 | 0.316 | 0.338 | 0.304 | 0.351 | 0.086 | |
| 8 | 0.302 | 0.332 | 0.314 | 0.305 | 0.332 | 0.06 | |
| 12 | 0.342 | 0.312 | 0.316 | 0.316 | 0.308 | 0.025 | 0.009 |
| $LSD_2$ | 0.059 | 0.069 | 0.071 | 0.086 | 0.051 | | |

Values are means of three replicates. $LSD_1$ stands for standard bias of different C:N ratios at the same carbon concentration; $LSD_2$ stands for standard bias of different carbon concentrations at the same C:N ratio; $LSD_3$ stands for standard bias of interactions of different carbon concentrations and C:N ratios within the same isolate.

**Table 2.** Effects of the combiantions of carbon and nitrogen sources on the mycelial growth (g).

| Carbon sources | Nitrogen sources | | | | | |
|---|---|---|---|---|---|---|
| | Tryptone | Yeast extract | Soy peptone | Urea | Casein | Proline |
| Xylose | 0.526bcde | 0.527bcde | 0.538abcde | 0.534abcde | 0.538abcd | 0.524bcdef |
| Fructose | 0.533abcde | 0.53abcde | 0.547a | 0.518ef | 0.506f | 0.522cdef |
| Sucrose | 0.521def | 0.541ab | 0.538abcde | 0.53abcde | 0.531abcde | 0.542ab |
| Galactose | 0.536abcde | 0.534abcde | 0.528abcde | 0.539abcd | 0.538abcd | 0.54abc |

Values are means of three replicates. Values in the same column followed by same letter are not significantly different (LSD; P≤0.05).

proline came second, and sucrose + yeast extract came the third. The lowest mycelial yield was obtained with the combination of fructose + casein. Other combinations of C and N sources had moderate influences on mycelial growth of strain ARF907, with no significant differences in the mycelial yield.

## DISCUSSION

The mycelium of biocontrol fungi is the main acting body for prevention and control of host pests. Rapid mycelial growth to a large extent influences the pathogenicity of biocontrol fungi to their host (Gao et al., 2003). Liu and Chen (2003) reported the 20 carbon source, 18 nitrogen source and 9 vitamins for the growth of ARF18, which indicated that carbon source and nitrogen source play an important role on the growth of this fungi, while vitamins seemed to be unnecessary for ARF18 to grow. In the present study, we systematically studied the influences of C concentration, C/N ratio, and combination of C and N sources on mycelial growth of strain ARF907 and preliminarily explored its nutritional requirements for mycelial growth in liquid culture. The results show that mycelial growth of strain ARF907 was strongly affected by C concentration, C/N ratio, and combination of C and N sources. This work has great implications for shortening the incubation period and increasing the mycelia yield of the nematophagous fungus ARF907.

## Conflict of interest

The author(s) have not declared any conflict of interests.

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