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Effects of carbon and nitrogen sources, carbon-to-nitrogen ratio, and initial pH on the growth of nematophagous fungus *Pochonia chlamydosporia* in liquid culture

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Abstract

The effects of carbon and nitrogen sources, carbon-to-nitrogen ratio (C:N) and initial pH value on the growth and sporulation of the nematophagous fungus *Pochonia chlamydosporia* in liquid culture were examined. Among the 21 carbon sources and 15 nitrogen compounds tested, the optimal carbon and nitrogen sources for mycelial growth were sweet potato and L-tyrosine, and for sporulation were sweet potato and casein peptone. A C:N ratio of 10:1 at pH 3.7 gave the maximum yield of conidia and a C:N ratio of 40:1 at pH 6.8 gave the maximum biomass. The initial pH value had a significant effect on mycelial growth and conidial production, with the optimal ranges being 3.5–4.5 for sporulation and 5–6 for growth. Maximum conidial production was obtained at an initial pH of 4.0 and the maximum biomass at pH 6.0. The results also showed that the final pH after 7 days cultivation was always higher than the initial value. The variability in growth and sporulation of seven strains of *P. chlamydosporia* in liquid culture was also compared and discussed.

Key words: carbon, carbon-to-nitrogen ratio, growth, nematophagous fungus, nitrogen, pH, *pochonia chlamydosporia*

Introduction

Pochonia chlamydosporia (Goddard) Zare et al. (= *Verticillium chlamydosporium* Goddard [1]), a nematophagous fungus, is a widespread facultative parasite of the sedentary stages of plant parasitic nematodes. It was first recorded as a parasite of the plant parasitic nematode *Heterodera schachtii* Schmidt by Willcox and Tribe in 1974 [2]. Further studies have shown that it can attack the females and eggs of other cyst and root-knot nematodes [3, 4]. The fungus has since been extensively studied for its potential for development into a biological nematicide [5].

In field trials, suitable inoculum is fundamental for evaluation of fungi as biocontrol agents. Chlamydospores of *P. chlamydosporia* have been used as an inoculum that gives effective and persistent colonization in the soil and rhizosphere [6–8]. However *P. chlamydosporia* does not produce chlamydospore in liquid culture [9] and therefore, an alternative inoculum is required. Stirling et al. [10] reported that a granular formulation of *P. chlamydosporia* made from mycelia and conidia could remain viable for 12 months at 25 °C and was biologically active when introduced into soil. The commercial agent, Xianchongbike, made from mycelia and conidia of *P.chlamydosporia* in liquid culture, also significantly reduced the numbers of root-knot nematodes in tobacco in field trials. These results indicated that mycelia and conidia could be used as a suitable inoculum for *P. chlamydosporia*.

The mycelial growth and sporulation of *P. chlamydosporia* are influenced by components of the medium and culture conditions [11, 12]. The effects of various nutrients on growth and sporulation of *P. chlamydosporia* have also been studied

recently [13]. Most of the growth conditions in submerged culture however, have not been comprehensively investigated. In this study, we optimized carbon and nitrogen sources, C:N, and the initial pH of the media for growth and sporulation of *P. chlamydosporia* in liquid culture. The growth and sporulation variability of seven strains of this fungus in liquid culture were also compared and are discussed.

Materials and methods

Fungal isolates

P. chlamydosporia strains YMF366, YMF325, YMF356, YMF323, YMF164, YMF213 and YMF377 were originally isolated from soil or the eggs of root-knot nematode *Meloidogne* sp. and deposited in MSC (Microorganism Store Center) of our laboratory. These strains were selected from 105 isolates of *P. chlamydosporia* based on the previous evaluation of their biological control potential, as judged by their abilities to produce chlamydospores, parasitize eggs and colonize in the rhizosphere [15]. Isolations were maintained on corn meal agar (CMA) slopes at 4 °C. Isolate YMF366 was used to evaluate the effects of carbon sources, nitrogen sources, pH value and carbon to nitrogen ratio on mycelium growth and sporulation.

Preparation of conidia inoculum

Fungi were transferred to potato dextrose agar (PDA) plates from CMA slopes and incubated at 28 °C for 2 weeks. A plug (2 mm diam.) from the edge of the culture was transferred into 15 ml of autoclaved basal medium in a 50 ml-conical flask and cultivated on a rotary shaker at 170 rpm and 28 °C for 5 days. Conidia were obtained by filtering the cultures through a sieve (150 μm pore size). The conidial suspension was washed three times with distilled, deionized water (ddH_2O) by centrifugation, and finally resuspended in 10 ml of ddH_2O and the conidial concentration determined.

Table 1. Growth and sporulation of *P. chlamydosporia* on different carbon sources

Carbon source	Sporulation ($\times 10^6$ conidia/ml)		Biomass yield (mg/l00 ml)
	3 d	7 d	
Sweet potato	40.02 ± 0.21	58.05 ± 0.35	510.02 ± 1.31
D-Fructose	0.12 ± 0.01	4.80 ± 0.11	434.43 ± 0.78
D(+) Raffinose	0.24 ± 0.02	6.05 ± 0.05	400.23 ± 0.55
D-Glucose	0.28 ± 0.03	4.70 ± 0.08	398.47 ± 1.24
Maltose	0.72 ± 0.02	5.53 ± 0.12	387.93 ± 1.32
Sucrose	0.16 ± 0.03	4.35 ± 0.04	378.43 ± 0.69
D-Xylose	0.52 ± 0.05	6.70 ± 0.20	366.52 ± 0.34
α, α–Trehalose	1.01 ± 0.10	8.84 ± 0.17	349.41 ± 1.51
D-Galactose	0.52 ± 0.04	15.52 ± 0.06	343.24 ± 0.30
Wheat bran	7.50 ± 0.12	9.14 ± 0.02	270.03 ± 0.47
D-(+)-Cellobiose	0.51 ± 0.03	0.60 ± 0.07	251.40 ± 1.22
Soluble starch	0.24 ± 0.02	0.44 ± 0.03	242.11 ± 0.27
L(+) Rhamnose	0.24 ± 0.04	3.24 ± 0.22	214.22 ± 0.34
L(+) Arabinose	0.56 ± 0.07	5.68 ± 0.09	194.73 ± 0.69
Mannitol	0.04 ± 0.01	1.60 ± 0.14	193.10 ± 0.19
L-Sorbose	0.08 ± 0.02	2.12 ± 0.08	170.72 ± 0.25
Xycitol	0.16 ± 0.03	0.92 ± 0.03	137.50 ± 0.62
Lactose	0.52 ± 0.05	1.24 ± 0.09	34.93 ± 0.17
Potato	0.35 ± 0.02	1.14 ± 0.15	140.01 ± 0.33
Cornmeal	0	0.27 ± 0.02	120.05 ± 0.77
β-Chloralose	0	0	6.23 ± 0.59
Control (no carbon)	0	0.16 ± 0.01	8.47 ± 0.41
$F(16,34)$	358.5**	1885.7**	17639**
$LSD_{0.01}$	0.21	0.41	15.64

Table 2. Growth and sporulation of *P. chlamydosporia* on different nitrogen sources

Nitrogen source	Conidiation ($\times 10^6$ conidia/ml)		Biomass yield (mg/100 ml)
	3 d	7 d	
L-Glulamic acid	9.31 ± 0.12	10.75 ± 0.08	460.33 ± 0.11
Peptone	15.05 ± 0.14	73.75 ± 0.27	528.33 ± 0.15
DL-Aspartic acid	9.81 ± 0.17	11.95 ± 0.09	457.67 ± 0.08
$(NH_4)SO_4$	4.86 ± 0.09	2.32 ± 0.07	246.13 ± 0.25
Urea	2.02 ± 0.15	3.70 ± 0.14	362.47 ± 0.13
Casein peptone	1.58 ± 0.07	171.75 ± 0.21	467.00 ± 0.09
$NaNO_3$	0.65 ± 0.03	2.75 ± 0.04	443.20 ± 0.34
L-Lysine	0.37 ± 0.04	1.23 ± 0.05	291.87 ± 0.18
Casein	0.34 ± 0.01	51.04 ± 0.02	493.33 ± 0.27
L-Tyrosine	0.15 ± 0.02	1.38 ± 0.09	640.67 ± 0.36
L-Valine	0.04 ± 0.01	10.13 ± 0.12	539.30 ± 0.14
L-Phenyl alanine	0	4.20 ± 0.07	530.02 ± 0.08
L-Threonine	0	0.98 ± 0.04	505.83 ± 0.23
L-Tryptophan	0	0.78 ± 0.02	343.60 ± 0.15
L-Cystine	0	0.51 ± 0.05	262.90 ± 0.31
Control (no nitrogen)	0	0	2.10 ± 0.12
$F(15,32)$	1184.5**	1543.4**	4609.7**
$LSD_{0.01}$	0.58	0.64	14.23

Media preparation, inoculation and cultivation

The medium of Blackburn and Hayes [14] was used as the basal medium for the optimization tests. The medium was composed of 10 g maltose, 2.0 g $NaNO_3$, 0.5 g $MgSO4{\cdot}7H_2O$, 0.5 g KH_2PO_4, 0.65 g Na_2HPO_4 and 0.5 g KCl, per litre of water. A total of 21 carbon sources including 4 natural complex substrates (Table 1) and 15 nitrogen sources (Table 2), were examined. For the tests of carbon or nitrogen sources, maltose or $NaNO_3$ in the basal medium was replaced by 4 g of C l^{-1} of each carbon source or by 0.4 g of N l^{-1} of nitrogen source respectively, and the pH of the media were adjusted to 6.8 by using 1 M NaOH or 1 M HCl. The media with natural complex substrates were prepared as follows: 20 g powder of the substrate was added to 500 ml ddH_2O in a 1000 ml container, after being boiled for 30 min, the mixture was clarified by centrifugation at 8000 rpm for 10 min, and the aqueous supernatant was used as carbon source in the corresponding test medium. The test on C:N ratio used D-fructose as carbon source and L-glutamic acid as nitrogen source, and the C:N ratios were calculated as the amount of carbon to nitrogen while maintaining a constant level of nitrogen at 0.4 g N l^{-1}. The effect of each ratio was evaluated at pH 3.7 and pH 6.8, respectively.

In tests of the effects of different initial pH, the basal medium was composed of 10 g D-fructose, 4.2 g L-glutamic acid, 0.5 g $MgSO4 \cdot 7H_2O$, 0.5 g KH_2PO_4, 0.65 g Na_2HPO_4 and 0.5 g KC1, per litre of water. The pH was adjusted to 2.5, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 by using 1 M NaOH or 1 M HCl before sterilization.

All media were prepared with ddH_2O. Each 250 ml-conical flask contained 100 ml of medium, steam-sterilized at 121 °C for 30 min and this was inoculated with 200 μl of conidial inoculum. The number of conidia used for testing the carbon and nitrogen sources, the C:N ratio and the initial pH value test were 2.40×10^6, 2.34×10^6, 1.75×10^6 and 1.17×10^6, respectively. Each experiment was carried out in triplicate. Incubation was at 170 rpm, 28 °C for 7 days, unless otherwise indicated.

Comparison of growth and sporulation among seven isolates

The medium used was the same as that used to test the effects of initial pH, but the pH was set at 6.8. Flasks were inoculated with a plug (2 mm diam.) from a PDA colony in 100 ml medium. After cultivation, the conidial concentration was counted per milliliter. For the biomass determination, the culture was collected by centrifugation at

Table 3. Effects of medium carbon to nitrogen ratio on the growth and sporulation of *P. chlamydosporia* in liquid culture

C:N ratio	Conidiation ($\times 10^6$ conidia/ml)					Biomass yield (mg/100 ml)	Final pH value
	3 d	4 d	5 d	6 d	7 d		
80:1[a]	0	0	0	0	1.93 ± 0.12	106.41 ± 0.32	3.82
80:l[b]	0	0	0	0	2.18 ± 0.03	213.12 ± 0.50	6.91
40:l[a]	0.32 ± 0.02	1.52 ± 0.05	7.52 ± 0.03	37.54 ± 0.08	40.31 ± 0.05	891.90 ± 0.41	6.34
40:l[b]	0.26 ± 0.01	1.02 ± 0.04	3.45 ± 0.08	6.24 ± 0.07	39.43 ± 0.06	925.71 ± 0.38	7.23
20:1[a]	1.58 ± 0.08	18.23 ± 0.02	23.45 ± 0.06	21.56 ± 0.15	24.17 ± 0.11	762.14 ± 0.52	6.1
20:1[b]	0.31 ± 0.02	0.55 ± 0.07	7.24 ± 0.14	13.92 ± 0.21	14.31 ± 0.09	673.52 ± 0.32	7.45
10:l [a]	6.65 ± 0.05	38.74 ± 0.13	40.25 ± 0.21	51.14 ± 0.09	47.12 ± 0.16	545.13 ± 0.44	6.6
10:1 [b]	0.63 ± 0.03	12.41 ± 0.06	28.58 ± 0.07	14.95 ± 0.14	12.57 ± 0.05	426.72 ± 0.16	8.0
5:1[a]	12.53 ± 0.04	18.51 ± 0.08	26.05 ± 0.10	27.99 ± 0.07	21.62 ± 0.12	341.74 ± 0.35	7.59
5:1[b]	0.33 ± 0.01	0.51 ± 0.03	1.09 ± 0.02	0.71 ± 0.02	0.52 ± 0.05	336.50 ± 0.63	8.8
1:1[a]	1.11 ± 0.04	3.97 ± 0.16	3.32 ± 0.08	2.93 ± 0.12	2.45 ± 0.02	212.22 ± 0.27	7.4
1:1[b]	0.33 ± 0.06	1.l7 ± 0.11	2.13 ± 0.13	2.02 ± 0.05	2.01 ± 0.03	91.93 ± 0.45	7.8
F (11,24)	697**	1170.8**	2103.3**	1900.9**	1477.6**	32175.5**	
$LSD_{0.01}$	0.43	0.62	0.31	0.15	0.32	13.65	

[a] initial pH value of media was 3.7.
[b] initial pH value of media was 6.8.

Table 4. Effects of initial pH value on growth and sporulation of *P. chlamydosporia* in liquid culture

Initial pH value	Conidiation ($\times 10^6$ conidia/ml)					Biomass yield (mg/100 ml)	Final pH value
	3 d	4 d	5 d	6 d	7 d		
8.5	0.14 ± 0.02	0.64 ± 0.13	1.76 ± 0.11	1.63 ± 0.14	0.73 ± 0.08	401.33 ± 0.41	8.4
8	0.15 ± 0.01	0.44 ± 0.06	1.53 ± 0.05	2.75 ± 0.19	1.05 ± 0.06	412.10 ± 0.23	8.4
7.5	0.98 ± 0.08	1.21 ± 0.04	1.34 ± 0.02	4.52 ± 0.11	2.59 ± 0.05	417.02 ± 0.34	8.6
7	1.09 ± 0.03	5.83 ± 0.07	8.75 ± 0.06	11.85 ± 0.04	8.45 ± 0.14	421.01 ± 0.19	8.5
6.5	1.06 ± 0.01	2.76 ± 0.04	8.32 ± 0.13	9.51 ± 0.08	7.89 ± 0.17	416.21 ± 0.52	8.4
6	2.31 ± 0.06	8.63 ± 0.12	10.65 ± 0.17	18.13 ± 0.13	12.81 ± 0.06	522.14 ± 0.30	8.2
5.5	3.85 ± 0.03	12.02 ± 0.07	15.57 ± 0.14	17.83 ± 0.15	12.23 ± 0.09	512.20 ± 0.27	8.0
5	3.14 ± 0.05	11.62 ± 0.03	14.21 ± 0.14	21.42 ± 0.21	11.75 ± 0.16	508.83 ± 0.23	7.9
4.5	3.67 ± 0.08	41.29 ± 0.06	42.56 ± 0.19	45.55 ± 0.12	53.81 ± 0.15	494.92 ± 0.46	7.2
4	3.56 ± 0.10	40.75 ± 0.08	52.75 ± 0.23	56.75 ± 0.16	24.53 ± 0.11	414.40 ± 0.13	6.9
3.5	4.77 ± 0.06	40.63 ± 0.09	42.12 ± 0.04	46.55 ± 0.18	45.02 ± 0.20	398.82 ± 0.41	5.6
2.5	0	0.21 ± 0.02	1.25 ± 0.02	5.73 ± 0.05	8.65 ± 0.09	182.21 ± 0.26	3.0
F(11,24)	795.5**	2060.4**	2028.4 **	5868. C**	3937.9**	2310.1**	
$LSD_{0.01}$	0.21	0.34	0.18	0.25	0.22	13.24	

8000 rpm for 10 min. The pellet was dried at 80 °C for 24 h and the dry biomass was quantified.

Data analysis

Data were statistically analyzed with the SPSS software package (SPSS Inc. Chicago, USA). Conidial concentration was converted to the natural logarithm values (In [Y + 1], where Y = conidial concentration), prior to analysis of variance (ANOVA). Values for biomass yield were used in ANOVA directly. Least significance differences (LSD) at 0.01 were used for comparisons between treatments.

Results

Effects of the carbon sources on growth and sporulation

Twenty-one carbon sources had different effects on the mycelial growth and sporulation of YMF366 in liquid culture (Table 1). For mycelial

growth, four carbon sources, sweet potato, D-fructose, D(+) raffinose and D -glucose, supported significantly higher yields than maltose. The optimal carbon source was sweet potato, which gave the greatest biomass of 510 mg of dry biomass per 100 ml medium after seven days' cultivation. Sucrose had a similar effect to maltose. Other carbon sources gave a lower biomass than that of maltose. For sporulation, sweet potato and D-galactose gave the highest yield of conidia (58 or 15.5×10^6 conidia/ml respectively). Five carbon sources, wheat bran, α, α-trehalose, D-xylose, D(+) raffinose and L(+) arabinose, resulted in more conidia than maltose, but the differences were not significant. The other five carbon sources, D-fructose, D-glucose, sucrose, L(+)rhamnose and L-sorbose, supported similar levels of sporulation to that of maltose. The remaining carbon sources, mannitol, lactose, potato, xylitol, D-(+)-cellobiose, soluble starch, cornmeal and β-chloralose, gave significantly lower conidial yields.

Effects of the nitrogen sources on growth and sporulation

The fungus grew well in all nitrogen sources, but the biomass yield varied (Table 2). For mycelial growth, nine nitrogen sources, L-tyrosine, L-valine, L-phenyl alanine, peptone, L-threonine, casein, casein peptone, L-glutamic acid and DL-aspartic acid, gave better yields than $NaNO_3$. The optimal nitrogen source was L-tyrosine which gave a biomass of 640.67 mg per 100 ml culture. Urea, L-tryptophan, L-lysine, L-cystine and $(NH_4)_2SO_4$, gave lower biomass yields than $NaNO_3$, but these differences were not significant. For sporulation, casein peptone, peptone and casein gave better yields than $NaNO_3$ and the optimal nitrogen source for sporulation was casein peptone which gave the highest yield of conidia (171.75×10^6 conidia/ml). Other nitrogen sources, except cystine, which gave a lower conidial yield than maltose, resulted in a similar level of sporulation to maltose. The results also showed that L-glutamic acid, DL-aspartic acid and peptone supported sporulation throughout the period of seven days, while with casein peptone, casein and L-valine sporulation only occurred in the later stages.

Table 5. The variability of growth and sporulation capability among isolates of *P. chlamydosporia* in liquid culture

Isolate	Conidiation ($\times 10^6$ conidia/ml)	Biomass yield (mg/100 ml)	Final pH value
YMF356	32.53 ± 0.12	412.41 ± 0.32	8.3
YMF366	25.85 ± 0.06	438.23 ± 0.27	8.6
YMFZK7	19.25 ± 0.07	383.51 ± 0.20	8.2
YMF323	14.57 ± 0.11	485.40 ± 0.13	8.4
YMF164	13.91 ± 0.03	407.72 ± 0.31	8.0
YMF325	11.36 ± 0.05	342.74 ± 0.12	8.3
YMF213	7.13 ± 0.06	286.41 ± 0.24	8.2
$F(7,16)$	549.8^{**}	2452.7^{**}	
$LSD_{0.01}$	0.17	17.52	

Effects of C:N on growth and sporulation

For sporulation, the C:N ratio of 10:1 at pH 3.7 gave a maximum yield of 51.4×10^6 conidia/ml. At pH 3.7, C:N ratios from 5:1 to 40:1 gave between 24.17 and $51.l4 \times 10^6$ conidia/ml. With C:N ratios from 40:1 to 10:1, each medium gave less conidia at pH 6.8 than at pH 3.7. The C:N ratio also affected the rate of sporulation. When the ratio was ≥20:1, the maximum yield occurred on Day 7; when the ratio was ≤10:1 the maximum yield occurred on Day 6 or earlier.

For biomass, a C:N ratio of 40:1 at pH 6.8 gave a maximum yield of 925.7 mg/100 ml. C:N ratios from 5:1 to 40:1 gave 336.5–925.7 mg/100 ml.

Effects of pH value on growth and sporulation

Different initial pH values had significant effects on sporulation. The optimal pH range for sporulation was 3.5–4.5. At pH 4.0, conidial production peaked at 56.75×10^6/ml on Day 6. Initial pH values higher than 4.5 or lower than 3.5 reduced sporulation. For mycelial growth, the optimal pH range was 5–6; with pH 6.0 giving the greatest biomass (522.1 mg/100 ml). Lowering the initial pH from 4.5 to 2.5 led to a rapid decrease in biomass, while increasing the pH from 6.5 to 8.5 led to a gradual decrease in the amount of mycelium. The pH values of the media increased significantly after 7 days' cultivation with the exception of pH 2.5 where only poor mycelial growth occurred (Table 4).

Variability of growth and sporulation among different isolates of P. chlamydosporia

The results showed that sporulation was highly variable among the isolates tested (Table 5). Strain YMF356 gave the most conidia, and this was significantly more than obtained from YMF366, YMFZK7, YMF323, YMF164 and YMF323. Four strains, YMF366, YMFZK7, YMF323 and YMF164 produced similar yields. YMF2l3 produced the lowest conidia and biomass yields. The biomass of these isolates indicated a significant difference in their growth rate. YMF323 had the greatest biomass (485.4 mg per 100 ml) after 7 days. The rank order of the other strains' biomass was YMF366, YMF356, YMF164, YMFZK7, YMF325 and YMF2l3 (438.2–286.4 mg/100 ml). All isolates increased the pH value of the medium from 6.8 to above 8.0 after 7 days' cultivation.

Discussion

Hyphomycetes can grow in various forms in liquid culture including as blastospores and submerged conidia. In some cases, the propagules produced by liquid culture can be as virulent as, or more virulent than, aerial conidia [16–18]. In this study *P. chlamydosporia* was found to produce conidia directly on phialides in liquid media and these conidia were not different from those formed in solid cultures in terms of their size and shape. Other kinds of propagules, such as blastopores and chlamydospores were not formed in liquid culture. The pathogenicity of different propagules to nematodes including chlamydospores, aerial conidia, conidia produced in liquid culture, blastospores and mycelial fragments, needs to be compared.

An optimized medium needs to be defined for a given biological control agent before large-scale production can be developed, and the nutritional components of this medium, such as carbon and nitrogen sources, carbon loading, C:N ratio and initial pH value, need to be optimized. In the previous study by Liu and Chen [13], most of the carbon and nitrogen sources tested supported extensive growth of *P. chlamydosporia* in liquid cultures, except that β-chloralose inhibited it. It was also found that L-(−)-sorbose dramatically inhibited the growth of *P. chlamydosporia* in solid culture, as did α-cellulose and citric acid in liquid culture [13]. In this study, L-(−)-sorbose and D-(+)-cellulose supported mycelial growth and sporulation. With the exception of sweet potato, the optimal carbon and nitrogen sources for mycelial growth, e.g. D-fructose, L-tyrosine, were not optimal for sporulation, likewise, the optimal carbon and nitrogen sources for sporulation, e.g. D-galactose and casein peptone, were not optimal for growth. These results indicated that the nutritional requirement for growth and sporulation are different. Once a medium has been optimized, a production medium can be formulated by replacing the nutritional components of the defined medium with low-cost, complex substrates. Based on our results, sweet potato can be used as the main carbon source for submerged fermentation of *P. chlamydosporia*, but its working concentration needs to be optimized. Related research has also shown that barley, milled maize and wheat flour are good substrates for chlamydospore production by the fungus in solid culture [8].

Previous reports have suggested that two nutritional factors, carbon source concentration and C:N ratio, had significant impacts on propagule formation in submerged cultures of fungi [19–21]. Our results suggested that the highest conidial yield was achieved when isolate YMF366 was cultured in the medium at a C:N ratio of 10:1 with an initial pH 3.7. Increasing or decreasing carbon concentration both decreased conidia production. Similar results were obtained by Jackson and Bothast [17] with *Colletotrichum truncatum* and with *Helminthosporium solani* [21]. The biocontrol efficacies of propagules produced under different nutritional conditions need to be determined, and the C:N ratio has an impact not only on biomass yield but also on the biocontrol efficacy [22].

In this work, pH values have been shown to have significant effects on propagule formation of *P. chlamydosporia* though the fungus can grow and sporulate within a wide pH range. The optimal initial pH values for sporulation were found to be 3.5–4.5. It had been shown that radial growth of the fungus was affected significantly at a pH <4, but the effect on sporulation was not determined [11].

The growth and sporulation variation among isolates of *P. chlamydosporia* suggested that the

optimization of the medium for each specific strain should be considered when evaluating the biocontrol potential of isolates.

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