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Influence of culture media and environmental factors on mycelial growth, sporulation and spore germination behaviour of *Curvularia eragrostidis* (P. Hennings) Mayer

A. Saha[1], S. Dasgupta[1], P. Mandal[1] and D. Saha[2]
[1]Molecular Plant Pathology and Fungal Biotechnology Laboratory, Department of Botany, University of North Bengal, Siliguri-734013, India.
[2]Plant Biotechnology Laboratory, Department of Biotechnology, University of North Bengal, Siliguri-734013, India.

Abstract

Curvularia eragrostidis, is a foliar fungal pathogen of young tea plants. It causes leaf spot disease of tea. Mycelial growth, sporulation and spore germination behavior of the pathogen were studied. Six different media were tested for mycelial growth. Among these, potato carrot agar (PCA) was found best for the mycelial growth and sporulation. Maximum mycelial growth was attained after 15 days of incubation. Mycelial growth was also studied in different temperatures and pH. Optimum temperature of growth was 25 °C and best growth was obtained at pH 6.0. Glucose and peptone were best carbon and nitrogen sources respectively for growth and sporulation of the fungus. The optimum conditions of spore germination were found to be at pH 7.25 and at incubation temperature of 25 °C.

Introduction

Tea is grown as a major plantation crop in the sub-Himalayan agro climatic zone of north-east India and forms the basis of economy of this region. *Curvularia eragrostidis* (P. Hennings) Mayer was discovered as a foliar fungal pathogen of young clonal cuttings of tea (Saha *et al.*, 2001). Mycelial growth, sporulation and spore germination behavior of a pathogen are essentially required for routine handling of a pathogen in the laboratory or in field studies. Hence, the present investigation was undertaken to observe the growth of *C. eragrostidis* in different media supplemented with different carbon and nitrogen sources. Different environmental factors were evaluated for optimization of spore germination conditions of *C. eragrostidis*.

Materials and methods

Fungal culture

Curvularia eragrostidis (P. Hennings) Mayer was originally isolated from naturally infected tea leaves of young clonal cuttings raised in the nursery of Muhurgong and Gulma Tea Estate of Darjeeling district. It was identified (Identification no. 4150.2 k) using resources of Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi. The fungal culture was maintained on freshly prepared sterile slants of potato dextrose agar (PDA) medium throughout the duration of the work.

Assessment of mycelia growth for determination of optimum growth period

To determine the optimum growth period *C. eragrostidis* was grown in potato dextrose broth (PDB) medium. Mycelial discs of 4mm diameter were inoculated in 250 ml Ehrlenmeyer

flask containing 40 ml PDB. Flasks were harvested at 5 days intervals and the dry weights of the mycelia were recorded until 25 days.

Assessment of mycelial growth in different media

To assess the mycelial growth in different media, *C. eragrostidis* was grown in petriplates containing 20 ml sterile test medium. Six different media viz. potato dextrose agar (PDA), malt extract agar (MEA), czapek dox agar (CDA), oat meal agar (OMA), potato carrot agar (PCA) and Richards' agar (RA) were used for the study. Each plate was inoculated by mycelial disc of 4 mm, cut from the advancing zone of 7 day old culture of *C. eragrostidis*. Diameter of the mycelia grown on the plates was recorded at 2 day intervals until 8 days.

Effect of different carbon sources on growth and sporulation

To study the effect of different carbon sources on mycelia growth and sporulation of *C. eragrostidis*, a basal medium (glucose 1%; asparagines 0.2%; KH_2PO_4 0.1%; $MgSO_4$, $7H_2O$ 0.005%; Zn^{++}, Mn^{++} and Fe^{++} 2 ml/ml) was used in which the fungus was cultured and glucose was replaced individually by the different carbon sources tested in equivalent quantities of carbon as present in 1% glucose. Each flask (250 ml) containing 40 ml basal media substituted with different carbon sources was inoculated in triplicate with 4 mm mycelia discs and incubated at 25 ±1°C. Control flasks did not contain any carbon compound. Sporulation and mycelial dry weight were recorded at 5 days intervals until 25 days.

Effect of different nitrogen sources on growth and sporulation

Modified Asthana and Hawker's basal medium 'A' (glucose 1 g; KNO_3 3.5 g; KH_2PO_4 1.75 g; $MgSO_4$, $7H_2O$ 0.75 g; distilled water 1 L) was used for studying the effect of different nitrogen sources on the mycelia growth and sporulation of the fungus. The quantity of various nitrogen sources was adjusted by replacing KNO_3 so as to give the same amount of nitrogen as furnished by 3.5 g KNO_3 in the basal medium. The basal medium only served as control. Sterilized media were inoculated with 4 mm mycelia discs and incubated at 25 ±1°C. Extent of sporulation and mycelia dry weight were recorded at 5 days intervals until 25 days.

Influence of different pH on mycelial growth

Potato dextrose broth (PDB) medium (40 ml taken in 250 ml Ehrlenmeyer flask) was adjusted to different pH (pH 5, 5.5, 6 and 6.5) by adding 1N HCl or 1N NaOH. Following inoculation with 4 mm mycelia discs, the flasks were incubated at 25 ±1°C. Each treatment was replicated thrice. Mycelia dry weight was recorded until 25 days at 5 days interval.

Influence of temperature on mycelial growth

Potato dextrose broth (PDB) medium (40 ml taken in 250 ml Ehrlenmeyer flask) were inoculated with 4mm mycelia disc and incubated at different temperatures (0°C – 45°C at intervals of 5°C) taking three flasks for each temperature conditions. Mycelial dry weight was recorded after 15 days of incubation.

Assessment of spore germination

Spore suspension was prepared from 7 day old sporulated culture of *C. eragrostidis* following the method as described by Saha and Chakraborty (1990). The concentration of the spores in the suspension was adjusted to 10^5 spores ml^{-1} following hemocytometer count. Thirty microlitre of spore suspension drops were then placed on clean, grease free glass

slides. Slides were incubated for 24 hours at 25 ± 1 °C in a sterile humid chamber, stained with cotton blue-lactophenol and observed under microscope. Percent germination and germ tube elongation were recorded at 2 hour intervals. In another set, slides containing spore suspension drops were incubated at different temperatures (0 °C - 45 °C) at intervals of 5 °C in a sterile humid chamber. After 24 hours, the slides were stained with cotton blue-lactophenol and were observed under microscope. Percent germination and germ tube elongation were also recorded.

Assessment of germination of spores in different pH

Six different phosphate buffer of 0.1 M were prepared by mixing sodium dihydrogen phosphate and disodium hydrogen phosphate solutions and the final solutions were adjusted to different pH (pH 4, 6, 6.5, 6.75, 7.25 and 9). Thirty microlitre buffer of each pH were mixed with 30 ml of spore suspension separately and finally mounted on clean, grease free glass slides. After 24 hours of incubation percent germination and germ tube elongation were determined.

Results and Discussions

From the results (Fig.1) it was evident that the maximum growth (mycelial dry weight of 193 mg) of the fungi was after 15 days of incubation. After that period lysis started and mycelial dry weight was found to be declined due to autolysis and depletion of media.

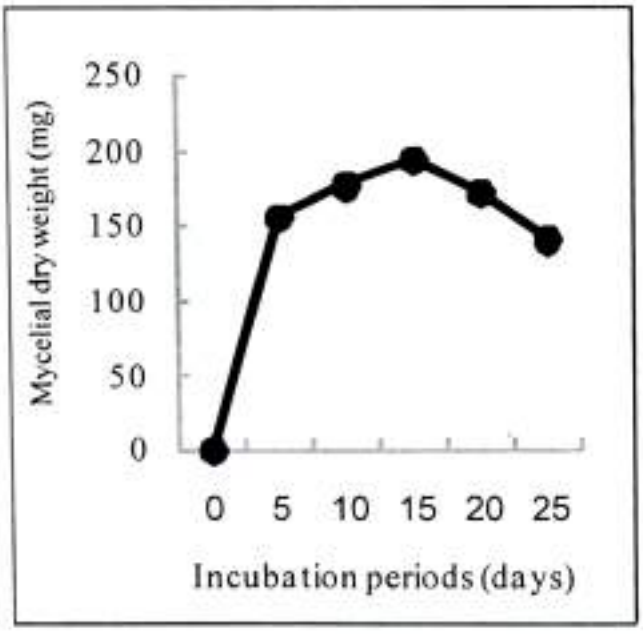

Fig 1. Mycelial growth of *C.eragrostidis* in diferent days of incubation

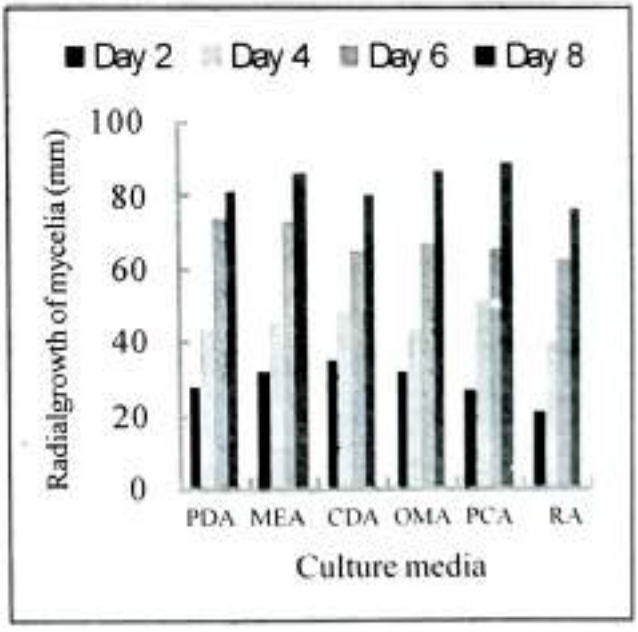

Fig 2. Mycelial growth of *C. eragrostidis* in different solid media

[Abbreviations : PDA = Potato Dextrose Agar, MEA = Malt Extract Agar, CDA = Czapek Dox Agar, PCA = Potato Carrot Agar, OMA = Oat Meal Agar, RA = Richards' Agar]

Among the six solid media tested, PCA showed maximum growth (89 mm in diameter after eight days of incubation) and was found to be the most suitable solid media for mycelia growth. Growth was observed in all the other media tested (Fig.2).Richard's agar and potato dextrose agar supported good growth and sporulation of *C. gloeosporioides* isolated from cashew anthracnose as shown by Sandhyarani and Murthy (2004). Several other workers also stated that PDA was best media for growth and sporulation of some other fungi (Xu *et al* 1984; Maheswari *et al* 1999). Saha *et al* (2008) stated that tea root extract supplemented potato dextrose broth was best for the growth of *Lasiodiplodia theobromae*.

Mycelial growth was observed to be much higher in presence of all the carbon sources tested compared to control, which did not contain any carbon compound (Table 1). Among the various carbon sources tested, glucose and sucrose containing media showed highest growth with mycelia dry weight of 310 mg and 289 mg after 15 days of incubation respectively. Media having galactose as carbon source recorded minimum mycelia growth (115 mg) after 5 days of incubation. Sporulation, which was started after 5 days in all the cases, was found excellent in glucose after 15 days of incubation. In control, *C. eragrostidis* showed insignificant growth which increased up to 25 days and no sporulation was observed. Our result was similar to that reported by Saha *et al.* (2008) who showed that glucose and sucrose was the best carbon sources for the mycelia growth of *Lasiodiplodia theobromae*. Our observation was also partly similar to that of Ray (2004) who reported that lactose and glucose had similar effect on growth of *Botryodiplodia theobromae*.

Table 1. Mycelial growth and sporulation of *C. eragrostidis* in different carbon sources

Carbon sources	Incubation periods									
	5 days		10 days		15 days		20 days		25 days	
	Mwt (mg)*	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Glucose	254.0 ±0.50	+	292.5 ±0.81	++	310.0 ±0.61	++++	296.0 ±1.15	++++	272.0 ±1.00	++++
Sucrose	215.5 ±0.76	++	266.7 ±0.87	++	289.0 ±1.00	+++	270.0 ±0.81	+++	246.0 ±0.58	+++
Mannitol	124.0 ±0.58	+	176.3 ±0.65	++	206.0 ±0.72	+++	188.0 ±0.58	+++	156.0 ±0.23	+++
Galactose	115.0 ±0.53	+	150.0 ±0.92	+	169.0 ±0.81	++	145.0 ±0.87	++	121.5 ±0.76	++
Control	9.4 ±0.92	-	12.0 ±1.15	-	15.8 ±0.42	-	18.0 ±0.55	-	23.5 ±0.87	-
CD at 5%	5.69		4.37		6.66		5.07		6.76	

*Mwt =Mycelial dry weight; Spn = sporulation

** Mean of three replicates; Data after ± represents standard error.

Among the seven nitrogen sources tested (Table 2), maximum mycelial growth (384.5 mg) of *C. eragrostidis* was found in the basal media supplemented with organic nitrogen source peptone. Other organic nitrogen sources also showed better growth than the inorganic nitrogen sources tested. Sporulation was poor in all the nitrogen sources tested and no sporulation was recorded in the basal media. *Alternaria protenta*, a pathogen of sunflower showed abundant sporulation on glucose peptone agar and leonien agar but not on dextrose nitrate agar (Wu and Wu, 2003). Holb and Chauhan (2005) observed that peptone was the best nitrogen source that produced quickest growth of *Monilia polystoma*. Saha *et al.* (2008) suggested the suitability of peptone and potassium nitrate as organic and inorganic nitrogen sources respectively for the growth of *L. theobromae*.

Table 2. Mycelial growth and sporulation of *C. eragrostidis* in different nitrogen sources

Nitrogen source	Incubation periods									
	5 days		10 days		15 days		20days		25 days	
	Mwt (mg)*	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Inorganic										
Potassium nitrate	144.0 ±1.00	+	172.0 ±0.64	+	215.0 ±0.53	++	199.0 ±0.72	++	187.0 ±0.53	++
Sodium nitrate	120.0 ±0.58	-	155.0 ±0.58	+	189.0 ±0.72	++	199.0 ±0.71	+++	185.0 ±1.08	+++
Ammonium nitrate	105.0 ±0.71	+	133.0 ±0.92	++	175.0 ±0.90	+++	169.0 ±0.53	+++	150.0 ±0.90	+++
Ammonium sulphate	135.0 ±1.02	-	172.0 ±0.58	+	201.0 ±0.50	+++	188.0 ±1.15	+++	175.0 ±0.78	+++
Organic										
Peptone	315.0 ±0.49	+	358.0 ±1.15	++	384.5 ±0.87	+++	336.0 ±0.58	+++	269.0 ±0.60	+++
Yeast extract	155.0 ±0.75	+	224.0 ±0.58	+	265.0 ±0.50	++	207.5 ±0.76	++	144.0 ±0.82	++
Beef extract	210.0 ±0.95	+	290.2 ±0.42	+	355.0 ±0.69	++	329.0 ±0.81	++	272.0 ±0.79	++
Control	4.4 ±0.81	-	8.1 ±0.66	-	11.1 ±1.21	-	14.4 ±0.70	-	20.6 ±0.87	-
CD at 5%	5.99		8.06		5.92		5.78		5.812	

*Mwt =Mycelial dry weight; Spn = sporulation

** Mean of three replicates; Data after ± represents standard error.

The growth of *C. eragrostidis* was evaluated in potato dextrose broth adjusted to four different pH. Mycelial dry weight was maximum (235.0 mg) after 15 d when the fungi was grown in the media at pH 6.0 (Table 3). These results correlate with the work of Mendoza *et al.* (2005) where the influence of the composition of the culture media and the pH for the growth, sporulation and morphology of the conidia of *S. schenckii* was emphasized. Kang *et al.* (2003) observed that optimum growth of the phytopathogenic fungus *C. gloeosporioides* was around the pH 6.0. Thakare and Patil (1995) suggested that the optimum pH for growth of *C. gloeosporioides* was 4.1-6.8.

Table 3. Mycelial growth of *C. eragrostidis* in different pH

Medium of growth	pH	Mycelial dry weight (mg)*				
		5 days	10 days	15 days	20 days	25 days
PDB	5.0	156±0.53	160±0.85	195±0.32	172±0.26	140±0.71
	5.5	150±0.64	180±0.80	205±0.60	190±0.58	175±0.72
	6.0	154±0.20	210±0.40	235±0.30	220±0.26	199±0.58
	6.5	160±1.11	183±0.11	213±0.56	198±0.26	185±0.60
CD at 5%		6.44	5.44	4.18	3.20	5.69

*Mean of three replicates; Data after ± represents standard error.

C. eragrostidis was grown in different temperatures to find the optimum temperature of growth. From the results (Table 4) the optimum temperature of growth of the fungi was recorded to be 25°C.

Table 4. Mycelial growth of *C. eragrostidis* in different temperature

Medium of growth	Temperature (°C)	Mycelial dry weight (mg)*
PDB	0°C	40.60±0.83
	5°C	127.50±1.04
	10°C	188.40±0.78
	15°C	210.00±0.72
	20°C	253.80±1.13
	25°C	290.50±0.78
	30°C	225.00±0.53
	35°C	130.50±0.55
	40°C	77.60±0.50
	45°C	22.50±0.29
CD at 5%		5.34

*Mean of three replicates; Data after ± represents standard error.

Since spore germination is a determining factor at the onset of host colonization by a fungal pathogen, several studies were undertaken to evaluate the influence of environmental factors like pH, temperature, incubation periods etc. on the germination of spores *in vitro*. *C. eragrostidis* showed maximum spore germination (88.80%) and germ tube elongation (46.40 mm) after 24 hours of incubation (Fig.3).Germination started within 2 hours in case of *C. eragrostidis*. *In vitro* studies with the spores of *B. carbonum* showed that germination started between 2-4 hours (Saha and Chakraborty 1990). The maximum spore germination (76.23%) and germ tube elongation (53.00 mm) were found at 25 °C. At 45 °C spore germination of *C. eragrostidis* was completely inhibited (Fig. 4). It was observed by Saha and Chakraborty (1990) that spore germination of *B. carbonum* reduced to 27% when pretreated at 50 °C but pretreatment at 0 °C for even 12 hours had no effect on germination and germ tube elongation. The high temperature also reduced the viability of spores (Flett and Wehner, 1989). Achar (2000) reported that conidia from *Stenocarpella maydis* exposed at temperatures below 22 °C germinated only after 17 hours of incubation and rate of germination increased from 22-27 °C, after which the germination rate declined.

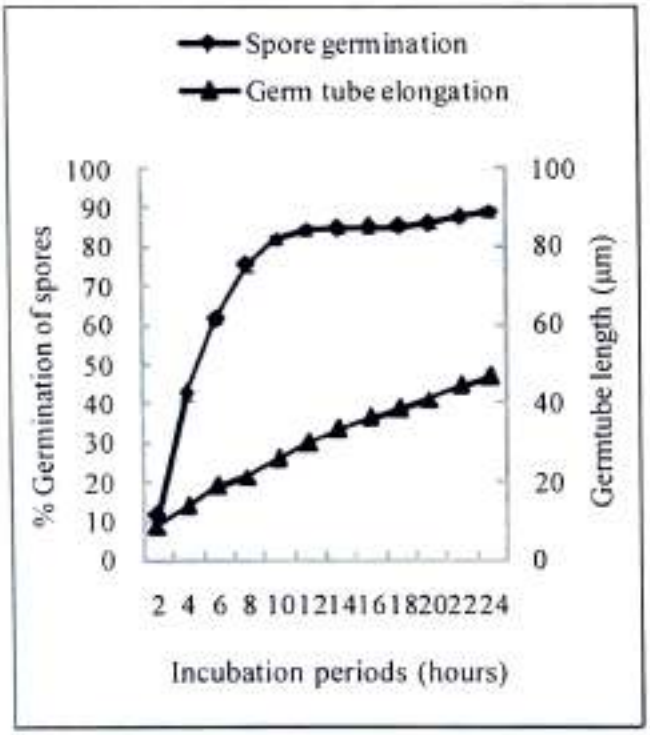

Fig 3. Percent germination of spores and germtube elongation of *C. eragrostidis* in different incubation periods (hours)

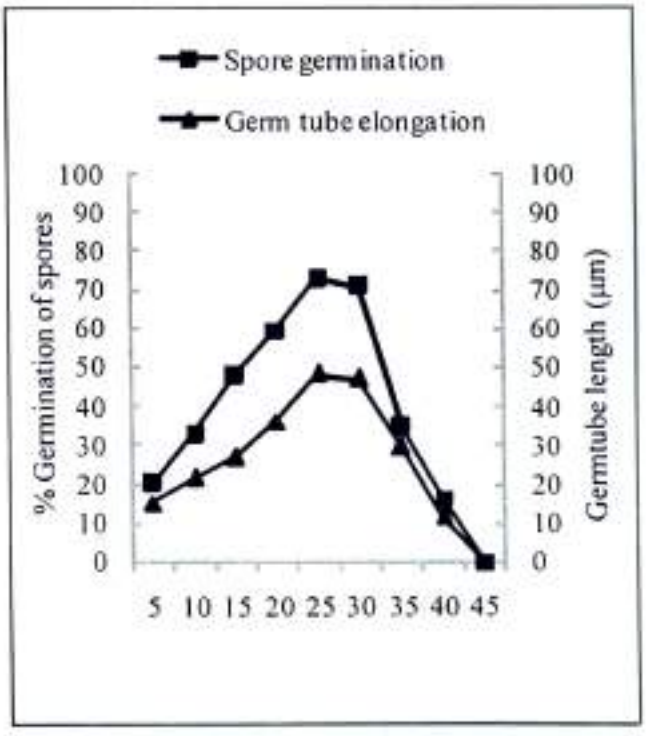

Fig 4. Percent germination of spores and germtube elongation of *C. eragrostidis* in different temperatures

Table 5. Spore germination and germ tube elongation of *C. eragrostidis* at different pH after 24 hours

pH	Percent germination when control raised to 100	Germ tube length (μm)
4.00	40.90±0.97	30.00±0.50
6.00	44.23±0.62	46.40±0.70
6.50	47.16±0.58	47.50±0.36
6.75	55.55±0.29	49.20±0.40
7.25	83.15±0.44	50.40±0.30
9.0	26.60±0.83	29.20±0.42
CD at 5%	4.83	2.23

*Mean of three replicates; Data after ± represents standard error.

It has been established that pH has some role on spore germination behavior of *C. eragrostidis*. Highest Spore germination (83.15 %) and germ tube elongation (50.40 mm) were recorded at pH 7.25 (Table 5). Saha and Chakraborty (1990) reported that pH 6.75 was best for germination of spores of *B. carbonum* while pH 7.2 was best for germ tube elongation. More than 97% of conidia of *Basidiobolus ranarum* germinated at pH range of 7-9 (Callaghan, 1974).

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