

## Physiological studies on strains of *Pestalotiopsis versicolor* isolated from a mangrove plant

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A series of Physiological experiments were conducted to ascertain the strain variation, if any, in *Pestalotiopsis versicolor* isolated from *Ceriops decandra* (Grifi) Ding Hou, growing in different localities of Sundarbans. The growth characteristics, sporulating abilities of the strains collected from various sources were studied under different cultural conditions. Texture of mycelial mats, rate of growth and intensity of sporulation differed significantly. All three strains showed maximum growth at 30°C. The pH values 5.5, 4.5 and 5.0 were also favourable for the growth of A, B and C strains respectively. Strain C failed to sporulate under continuous darkness for 7 days while other two strains sporulated profusely under similar conditions. Salt (NaCl) tolerance of different strains was tested *in vitro*. Strain A was found to be most tolerant among the three. The strain A showed moderate growth (89.33 mg. dry weight) at 2.04 M NaCl.

**Key words :** *Pestalotiopsis versicolor*, *Ceriops decandra*, Sporulation, Salt tolerance, Phytopathogenic fungus

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A phytopathogenic fungus was isolated for the first time from an infected leaf of *Ceriops decandra* (Grifi) Ding Hou, an economically important mangrove plant of Sundarbans, West Bengal. The organism was identified as *Pestalotiopsis versicolor* (Speg.) Steyaert. This fungus was found to grow abundantly on the living leaves of the aforesaid host in a number of localities in Sundarbans. The differential growth characteristics of isolates of the same species growing on *C. decandra* collected from different localities created an interest and hence, this investigation was undertaken to determine whether any strain variation exists in the aforesaid fungal species.

## MATERIALS AND METHODS

The infected leaves of *Ceriops decandra* was collected from different localities of Sagar Island, Sundarbans ( West Bengal ) during September, 1990. Two strains ( B and C ) were isolated from infected leaves and identified as *Pestalotiopsis versicolor* ( Speg. ) Steyaert. The identity of the organism was also kindly confirmed by Dr. J. E. M. Mordue, International Mycological Institute ( IMI ), Kew, Surrey, England ( IMI No. 343606 ). The strain A was collected from the Departmental stock culture.

For the assessment of mycelial growth, the fungal strains were grown in a medium ( Glucose 30g, asparagine-1g,  $MgSO_4 \cdot 7H_2O$ -0.5g,  $KH_2PO_4$ -1.5g, Distilled water 1000ml ; 50 ml/250 ml flask ) for a desired period. The mycelia were finally collected, dried at 60 C for 96 hr. and weighed after cooling in a desiccator. Usually, three replicates were used for each treatment. To study the effect of any chemical organic or inorganic on the mycelial growth, desired quantity of test chemical was added to the liquid medium before sterilization. Initial pH was noted.

## EXPERIMENTAL RESULTS

### *Comparative growth characteristic of different strains of P. versicolor*

The growth characteristics of strains of *P. versicolor* were compared after growing them in both solid and liquid media. The observations are summarised in Table 1.

It appears from the Table—1 that the growth rate of the strains are more or less similar but the texture of mycelial mat and intensity of sporulation differ significantly among three strains. In liquid medium ( Glucose-30g, Asparagine-1g,  $MgSO_4 \cdot 7H_2O$ -0.5g,  $KH_2PO_4$ -1.5g, distilled water-1000 ml ) the rate of growth was quite different. Strain B exhibited maximum mycelial growth (  $686.33 \pm 4.84$  mg ) while strain A showed minimum growth (  $439.67 \pm 6.06$  mg ) Strain C could be placed in the intermediate category (  $559.33 \pm 4.41$  mg ). Significant difference in rate of growth among the three strains was observed in liquid medium unlike the solid medium.

### *Pairing of isolates*

To confirm the strain variation exists in *P. versicolor* the cultures were paired in all possible combination in Petri dishes containing Potato-dextrose-agar medium and incubated for 21 days at  $25 \pm 1^\circ C$ . The results are summarised in Table-2. The results of pairing experiment also confirm that strain variation exists in *P. versicolor*.

Table 1. Comparative growth characteristics of different strains of *P. versicolor* (on solid medium)

Fungus (Strains)	Average diameter of mycelial mat (mm) after 4 days**	Advancing Zone	7 days	30 days	Remarks (After 30 days)
<i>P. versicolor</i> (Str. A)	67.00	Even, appressed	Sub-felty	Sub-felty	Acervuli scattered throughout, but 1-2 faint concentric rings observed around the inoculum.
<i>P. versicolor</i> (Str. B)	67.66	—do—	Floccose with 2 prominent concentric rings around the inoculum	Felty	Acervuli on the concentric rings around the inoculum
<i>P. versicolor</i> (Str. C)	69.33	Wavy, appressed	Felty growth with several prominent, raised concentric rings around the inoculum	Felty	Acervuli on the raised concentric rings, watery exudation present on the mycelial mat

\*\* Average of 3 replicates ; Temperature  $30 \pm 1^\circ\text{C}$  ; Initial pH of the PDA medium — 6.8

**Table 2.** Pairing of isolates of *P. versicolor*

STRAINS	REACTIONS
A x A	Homogeneous
B x B	-do-
C x C	-do-
A x B	Overgrowth
A x C	-do-
B x C	Line of Contact

*Effect of temperature*

The fungal strains were grown on Potato-dextrose-agar medium and incubated at 15°, 20°, 25°, 30°, 35°, and 40°C for 5 days. The results are given in Table 3.

**Table 3.** Effect of temperature on growth of different strains of *P. versicolor*

Temperature (°C)	Diameter of the mycelial mat (mm)* with S. E.			Overall effect due to temperature
	Str. A	Str. B	Str. C	
15	29.33 ±1.45	23.00 ±1.53	19.67 ±1.20	24.00 ±1.58
20	63.00 ±4.04	64.00 ±3.06	65.67 ±3.76	64.22 ±1.86
25	72.00 ±1.00	78.67 ±0.67	73.33 ±0.88	74.67 ±1.11
30	79.00 ±1.15	75.67 ±1.86	77.00 ±1.00	77.22 ±0.85
35	75.00 ±1.15	44.00 ±1.15	70.33 ±0.88	63.11 ±4.85
40	32.67 ±0.67	35.66 ±1.20	33.33 ±0.33	33.89 ±0.61
Overall average growth due to strain	58.50 ±4.91	53.50 ±5.06	56.56 ±5.34	—

\* Average of 3 replicates/treatment

Initial pH-6.5

Incubation period - 5 days

Critical difference (C.D.) values at :

Main effect due to	5%	1%
Temperature	3.00	4.02
Strain	2.12	2.85
Interaction	5.20	6.90

In all cases optimum temperature was 30°C although strain B showed slightly higher growth at 25°C. Growth of strains A and C declined abruptly beyond 35°C. Considerable difference in mycelial growth was also observed among the strains at 15°C.

#### *Effect of pH*

The effect of pH on growth was also studied after growing the strains in glucose asparagine medium as stated earlier. Buffer solution with corresponding pH values ranging from 4.5 to 8.0 were prepared by mixing appropriate volumes of  $\text{KH PO}_4$  and  $\text{K}_2\text{HPO}_4$  at a concentration of M,30. Both buffer solution and medium (double strength and adjusted pH) autoclaved separately and after cooling, equal volumes of both (similar pH grade) were mixed, inoculated and incubated for 9 days at  $19 \pm 1^\circ\text{C}$ .

All the strains of *P. versicolor* grew in a wide range of pH (4.5 to 8.0). Strains A, B and C exhibited maximum growth at pH 5.5, 4.5 and 5.0 respectively (Table-4). In all cases mycelial growth was minimum at pH8.

**Table 4.** Effect of pH on growth of 3 strains of *P. versicolor*

pH	Dry weight of mycelia (mg)* with S. E.			Overall effect due to pH only
	Str. A	Str. B	Str. C	
4.5	473.33 ±2.03	595.00 ±6.11	610.67 ±7.54	559.67
5.0	486.33 ±5.36	563.00 ±6.81	653.33 ±8.88	568.22
5.5	544.00 ±5.13	589.00 ±4.58	636.67 ±1.33	589.89
6.0	509.67 ±6.06	507.33 ±6.33	607.33 ±11.41	541.44
6.5	436.00 ±4.53	420.33 ±5.04	508.33 ±11.05	454.89
7.0	221.33 ±6.57	308.00 ±9.29	331.67 ±8.41	287.00
8.0	198.67 ±9.53	310.33 ±9.02	210.00 ±6.93	239.67
Overall effect due to strain	409.90	470.43	508.52	—

\* Average of 3 replicates/treatment  
Temperature  $19 \pm 1^\circ\text{C}$   
Incubation period - 9 days

*Effect of light and dark on growth and sporulation*

Petri dishes (95 mm. diameter) each containing Potato-dextrose-agar medium were inoculated with agar discs (5 mm. diameter) with mycelia and incubated under 3 different light conditions for 7 days. One set of Petri dishes was incubated under continuous light (500 lux) and second set was placed under continuous darkness (0 lux) while the remaining one was exposed to diffused light (300 lux). The results are presented in Table-5.

**Table 5.** Effect of light and dark on growth and sporulation of *P. versicolor*

Fungus	Continuous darkness (0 lux)			Continuous light (500 lux)			Diffused light (300 lux)		
	Diam. of mycelial mat (mm)	Texture	Sporulation	Diam. of mycelia mat (mm)	Texture	Sporulation	Diam. of mycelial mat (mm)	Texture	Sporulation
Str. A	95.00	Felty	+++	95.00	Felty	—	95.00	Felty	++
Str. B	95.00	Subfelty	+	30.66	Farina- ceous	++	95.00	Sub- felty	+++
Str. C	95.00	Felty with prominent ridges	—	28.66	Farina- ceous	++	95.00	Felty with Prominent ridges	—

— = No sporulation ; + = few sporulation ; ++ = moderate sporulation ; +++ = profuse sporulation ; All data are average of 3 replicates ; Temperature  $24 \pm 1^\circ\text{C}$  ; Initial pH-6.8 ; PDA medium. Incubation period-7 days.

It is interesting to note that strain C showed no sporulation in either diffused light or in continuous darkness but continuous light induced sporulation. On the other hand continuous light inhibited sporulation of strain A. Profuse sporulation occurred when cultures of strains A and B were incubated in continuous darkness and diffused light respectively. Although there was no significant difference in the rate of growth of 3 strains on PDA medium under continuous darkness or diffused light, exposure to continuous light, however, increased the growth of strain A markedly.

*Effect of Sodium Chloride*

Salt tolerance of the three strains of *P. versicolor* of which two were isolated from halophytic plants was also tested. Glucose-asparagine medium was prepared as described earlier for pH experiment and different amount of Sodium

Chloride were supplemented to the medium to obtain 0.17, 0.34, 0.51, 0.68, 1.36, 2.04 (M) concentration. The results are incorporated in the Table-6.

Of the 3 strains, strain A and C showed maximum growth at 0.17, and 0.34 M respectively while B strain exhibited highest growth in control medium. About 95% reduction in growth was recorded for strains B and C and about 80% for strain A at 2.04 M level.

**Table 6.** Effect of NaCl on growth of *P. versicolor* strains

Conc. of NaCl (M)	Dry weight of mycelia ( mg ) * with S. E.			Overall effect due to conc. of NaCl.
	Str. A	Str. B	Str. C	
Control (without NaCl)	439.67 ±4.84	686.33 ±6.06	559.33 ±4.41	561.78
0.17	533.33 +9.84	561.33 ±8.29	569.33 ±6.17	554.67
0.34	449.67 ±8.37	547.67 ±4.98	584.33 ±9.02	527.22
0.51	432.33 ±6.84	516.33 ±7.69	539.33 ±10.40	426.00
0.68	415.33 ±7.69	388.67 ±5.93	409.00 ±8.50	404.33
1.36	118.67 ±6.33	223.67 ±8.15	286.00 ±13.05	209.44
2.04	89.33 ±5.84	36.33 ±1.86	36.67 ±4.98	54.11
Overall effect due to strain	349.05	422.90	426.29	

\* Average of 3 replicates/treatment : Temperature— $23 \pm 1^\circ\text{C}$  : Initial pH of the medium—5.6  
Incubation period—9 days.

*Effect of different concentrations of Sodium Chloride on germination and germ tube growth of P. versicolor*

About 61% spores of strain A and 20% of strains B and C germinated in control (sterile distilled water). The average germ tube length of strain C was, however, three times longer than that of strain B, Results suggest that strains A and B were most and least tolerant to NaCl.

## DISCUSSION

The results of present investigation reveal the existence of strain variation in *P. versicolor* obtained from various sources. Significant variations in texture

of the mycelial mats, growth rate and intensity of sporulation were noted. Variation among strains of a fungal species is not unnatural since it has been reported earlier that strains of *Alternaria alternata* were isolated from *Oxalis* sp., *Phaseolus* sp. and *Vigna* sp.; strains of *Corynespora cassicola* were isolated from *Elephantopus* sp. and *Hemigraphis* sp. while the strains of *Fusarium solani* were isolated from *Acalypha* sp., *Aegle* sp. and *Ruellia* sp. (Mallik, 1977). The result of pairing experiment (pairing of cultures of *P. versicolor* in all possible combinations) also confirms the validity of the strains. Significant difference in growth of strains at lower temperature was also noted. Utikar *et al* (1980) reported that the optimum temperature for the growth of *P. versicolor* was 27-30°C. This report substantiates the findings of the present study. The said author noted 5.8 as the optimum pH value for the growth but the present strains of *P. versicolor* showed different pH values (4.5-5.5) for their optimum growth. This pH range is also not unusual since Thind and Madan (1979) demonstrated that *Pestalotia psidii* could grow well even at pH<sub>3</sub> although *P. funerea* grew at pH 7.5 (Upadhyay and Dwivedi 1973). It is significant to note that both strains A and B sporulated profusely under continuous darkness for 7 days but sporulation of strain C was significantly inhibited under similar conditions. Swamy and Mani (1978) reported that light was essential for the sporulation of *P. palmarum* isolated from coconut. But the results of present study suggest that light is not always essential for sporulation of all species of *Pestalotiopsis*. However, further investigation is necessary to determine why strains of *P. versicolor* show differential behaviour with regard to sporulation. Considerable variation among the strains was also recorded with regard to their salt tolerance. There was no indication that the strains isolated from mangrove plants was more salt tolerant. It is also necessary to mention that spores of *P. versicolor* are more sensitive to NaCl than mycelia. The strains B and C showed total inhibition of growth and germination at 0.08 and 0.34 M concentrations of NaCl respectively. Mani and Swamy (1983) studied the sporulation and growth of *P. palmarum* on Czapek's medium containing 0.85 to 2.72 (M) NaCl. They observed that the growth of the fungus was increasingly inhibited at concentrations above 0.34 M. The results of this study also reveal that one of the strains of *P. versicolor* is significantly more tolerant (2.04 M) to NaCl than that of other two strains.

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