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LIGNIN DEGRADING EFFICACY OF *RHIZOCTONIA SOLANI* A STUDY *IN VITRO*

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Abstract

The lignin degrading capacity of *Rhizoctonia solani*, isolated from *Vanda testacea* Reichb.f. (Orchidaceae) was assessed *in vitro*. Qualitative test showed the positive result while quantitative estimation includes the estimation of xylanase activity, total phenols and total soluble proteins. Xylanase activity revealed that maximum peak was achieved on 14th day, the activity value being 10.21±0.12 IUml^{-1}. It decreased on increasing incubation period. Total phenols were recorded to be high on 8th day of incubation with 12.10±0.02 μg ml^{-1}. The amount of phenols tended to decrease on 10th day having 11.67±0.03 μg ml^{-1}. Extracellular protein was found to be maximum on 12th day of incubation having the value 5.02±0.06 μg ml^{-1}.

Introduction

LIGNIN IS the main component of all vascular plants. It is a recalcitrant heteropolymer of phenyl-propanoid unit present in woody plant tissues and provides the rigidity to them. The term lignin has been derived from Latin 'lignum 'means wood,is the product of the free-radical polymerization of substituted p-hydroxycinnamyl alcohols; the three phenolic precursors coumaryl, coniferyl and sinapyl alcohols which differ in their degree of methoxylation (Kirk and Fenn, 1979). These precursors are synthesized from L-phenylalanine and L-tyrosine generated via the shikimic acid metabolic pathway, where the compounds are initially derived from carbon dioxide fixed by plant photosynthesis (Higuchi *et al*., 1977). Many fungi are known to degrade lignin e.g. *Armillaria, Clavaria, Coprinus, Pleurotus, Poria* etc. As orchids are known to possess the mycorrhizal fungi (Kaushik, 1983), and we had already tested the cellulose degradation efficacy of *Rhizoctonia solani* (Kumar and Kaushik, 2004), the present investigation was carried out to test its lignin degradation efficacy.

Material and Methods

Fungal Strain

The fungus *Rhizoctonia solani* was isolated from *Vanda testacea* Reichb.f. an epiphytic orchid, and maintained on PDA (Potato Dextrose Agar) medium slant at 4°C.

Medium and Culture Conditions

The lignin degrading ability of the fungus was assessed qualitatively and quantitatively. For qualitative estimation, the methyl orange agar medium (Field, 1993; Rodriguez *et al*., 2000) was used.The composition of medium was potato extract: 20.0g / %, glucose: 2.5g / %, methyl orange: 0.5g / %, agar: 2.0g / %, and ampicillin: 0.05g / %.

Quantitatively, the lignolytic capacity of fungus was determined in medium containng KH_2PO_4 (2.0 gl^{-1}), $(NH_4)_2SO_4$ (2.1 gl^{-1}), $MgSO_4.5H_2O$ (0.3 gl^{-1}), $CaCl_2$ (0.3 gl^{-1}), $MnSO_4.5H_2O$ (0.00156 gl^{-1}), $ZnSO_4.7H_2O$ (0.0014 gl^{-1}), $CoCl_2.6H_2O$ (0.00266 gl^{-1}) and 1% lignin sulphonic sodium salt. The medium pH was maintained at 5. Homogenized mycelium from 5-10 day old cultures was used to inoculate 250 ml Erlenmeyer flasks containing 50ml of medium. Three replicate flasks were incubated at 28±2°C in a rotary shaker at 120 rpm. Samples were collected at different time intervals and centrifuged; the filtrate was used to test the xylanase activity, total proteins and total phenols.

The xylanase activity was estimated according to Ghose and Bisaria (1987), the soluble protein was estimated by the method of Lowry *et al*. (1951) and total phenols were estimated according to Bray and Thorpe (1954).

Results and Discussion

The study of lignin degradation has been a neglected area but preliminary efforts have revealed a fascinating and unusual process, even before many of the detailed features have been delineated. Although the only microbes shown to metabolize lignin efficiently are Basidiomycetes, other fungi are known to degrade it slowly (Esyln *et al*., 1975; Haider and Trojanowski, 1980; Lundström, 1973). But little is known about their role in the natural process, and very little is known about the chemical and physiological aspects of their activities. This perhaps is the first attempt using a fungal symbiont isolated from the orchid *Vanda testacea* Reichb.f. growing at Dehradun in outer ranges of the Shivalik Hills. The results are summarized (Table1; Fig.1 a-c) and discussed briefly as follows:

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Table 1. Estimation of xylanase, total soluble production and total phenols.

S.No.	Number. of days	Xylanase (IU ml^{-1})	Total soluble proteins (μg ml^{-1})	Total phenols (IU ml-1)
1.	2	2.48±0.30	2.02±0.06	10.08±0.04
2.	4	4.92±0.62	3.0 ± 0.25	11.02±0.03
3.	6	6.28±0.27	3.78 ± 0.09	11.89±0.06
4.	8	7.62±0.33	4.02 ± 0.09	12.10±0.02
5.	10	8.10±0.30	4.66 ± 0.08	11.67±0.03
6.	12	9.75±0.19	5.02 ± 0.06	11.02±0.03
7.	14	10.21±0.12	5.72 ± 0.05	10.78±0.02
8.	16	9.08±0.17	6.13 ± 0.09	10.32±0.02
9.	18	7.62±0.19	6.78 ± 0.05	9.67±0.03
10.	20	7.30±0.37	7.08 ± 0.07	9.21±0.04
11.	22	6.21±0.52	7.98 ± 0.02	8.80±0.01
12.	24	5.68±0.16	8.10 ± 0.04	8.56±0.03
13.	26	4.92±0.12	8.21 ± 0.05	8.10±0.02
14.	28	3.61±0.32	8.76 ± 0.04	7.90±0.02
15.	30	2.98±0.20	9.14 ± 0.05	7.45±0.02

Each value is expressed as mean ± S.D. (n=3)

Qualitative tests for lignin degradation yielded positive results. The decolourization of methyl orange agar plates from dark brown chocolate to light brown was seen with passage of time. The lignolytic activity of isolates was assessed by xylanase activity, total phenols and total soluble proteins. Studies on xylanase activity revealed that maximum peak was achieved on 14 days the activity value being 10.21±0.12 lUml^{-1}. It decreased with increase in incubation period. Total phenols were highest on day 8 after incubation with 12.10±0.02 μg ml^{-1}. Their amount tended to decrease on 10th day having the value of 11.67±0.03 μg ml^{-1}.

Interestingly, the amount of extracellular proteins invariably increased with increase in incubation period.

Studies on lignin degradation showed that temperature was an important factor for the degradation. Temperature of 28°C and shaking condition in rotary shaker at 120 rpm was maintained through out the process, results were verified according to Kirk *et al.* (1978) and da Silva and Carmona (2008). Earlier, Seyis and Aksoz (2005) using xylan as the only carbon source for xylanase production from *Trichoderma harzianum* obtained the maximum level of activity at the end of 13 days as is also true in our studies.

Wahleithner *et al.* (1996) studied four different laccases (monophenol oxidase) in *Rhizoctonia solani*, and at least three laccases in *Botrytis cinerea* (Marbach *et al.*, 1984; Pezet,1998; Viterbo *et al.*, 1994;). Laccase was also reported in *Coniochaeta* (Barbosa *et al.*, 1996), *Hortaea acidophila* (Palonen *et al.*, 2003), *Penicillium chrysogenum* (Rodriguez *et al.*, 1996) and *Xylaria* (Liers *et al.*, 2006). *Fusarium proliferatum*, which is able to mineralize synthetic lignin, also secretes superoxide radicals during lignin mineralization (Regalado *et al.*, 1999). Such peroxide radicals may generate highly reactive hydroxyl radicals, which are known to be involved in lignin degradation (Guillen *et al.*, 2000).

Presently, the phenolic content was also recorded high, in accord with similar finding by Krishna and Bagyaraj (1984). The higher amount of phenol might be one of the factor responsible for increased disease resistance found in mycorrhizal plants. The phenolic substances like alexins, hircinol, loroglossal and orchinol have been isolated and identified from orchids during the process of infection. Thus the mycorrhizal fungus in orchids can be exploited for degradation of lignin.

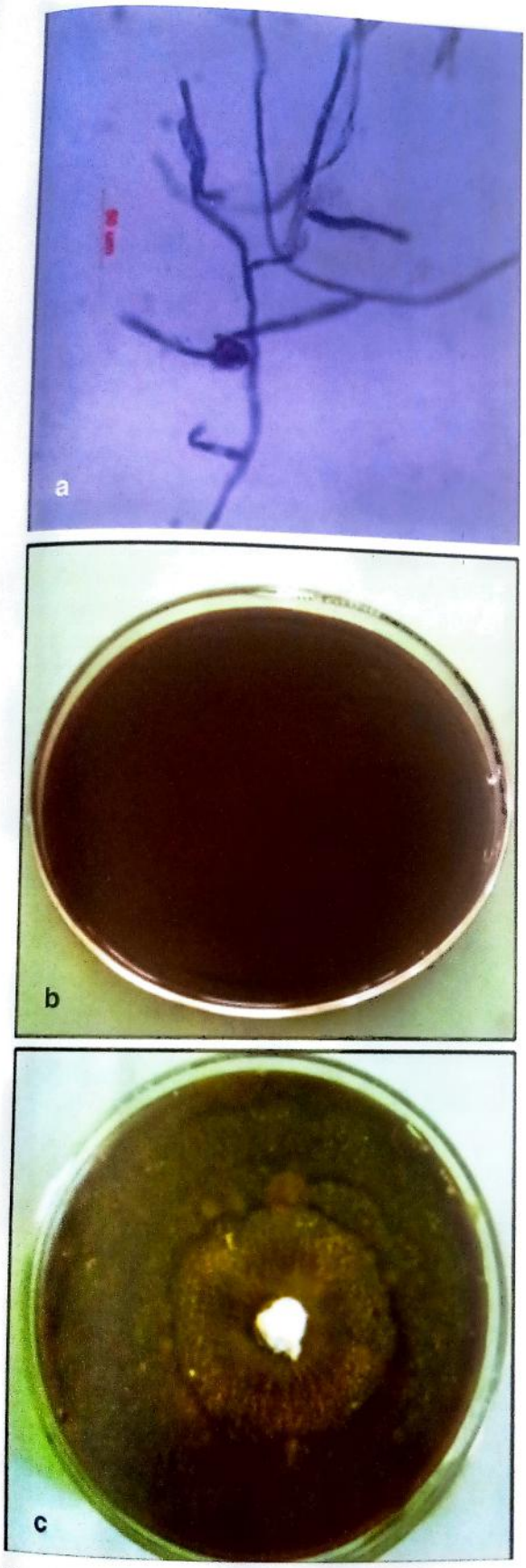

Fig. 1 a-c. *Rhizoctonia solani* cultures : a, Isolates from *Vanda testacea*; b, On Methyl orange agar medium (control); c, Positively testing for lignin degradation.

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