

SIMPLE METHODS FOR DETECTING LIGNOLYTIC ENZYMES IN SOLID MEDIUM

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ABSTRACT

White rot fungi are the most capable micro-organisms to degrade lignin. Lignin degradation is not catalysed by any particular enzyme but by concerted action of a group of enzymes termed as lignolytic enzymes. A simple petriplate screening procedure in solid medium for testing extracellular production of these enzymes based on change in colour of the medium and visual detection has been proposed and evaluated.

INTRODUCTION

Lignocelluloses are the most abundant natural materials present on the earth, comprising 50 per cent of all biomass (Goldstein, 1981). Biological processes for the economic conversion of lignocelluloses to products useful for man or his activities involve the short circuiting of part of the global carbon cycle, which returns carbon fixed into lignocelluloses to the atmosphere as CO₂ via the activities of micro-organisms capable of efficient utilization of the lignocellulosic biopolymers. Several groups of micro-organisms can utilize all or part of the lignocellulosic complex found in intact plant tissue (Bisaria and Ghose, 1981; Kirk, 1983; Tsao and Chiang, 1983). The white rot fungi in particular have been shown to be among the most efficient degraders of the whole polymer complex (Kirk, 1983). *Cyathus* sp., a white rot fungus, degrades lignocellulose in cereal crop residues (Kuhad and Johri, 1983; 1987; 1991). Our previous studies have shown *Cyathus* spp. to be a producer of cellulolytic and lignolytic enzymes in liquid medium (Gupta, 1991). In this paper, we describe simple and rapid qualitative assay for detecting laccase, catechol oxidase and lignin peroxidase enzymes on solid medium.

MATERIAL AND METHODS

Four species of *Cyathus*, *C. bulleri*

(Brodie 195062, *C. africanus* (Brodie 196686), *C. striatus* (Huds.) wild ex. pers. 196675 and *C. stercoreus* (Schw) de Toni 196676 were screened for their potential to produce extracellular laccase, catechol oxidase and lignin peroxidase enzymes in solid medium. All the four fungal cultures were grown and maintained on Brodie's basal agar medium (Brodie, 1975). Petriplates of Brodie's basal agar medium were prepared and inoculated with fungus bearing disc of 8 mm diameter each, taken from the growing edges of the cultures of an appropriate isolate. The plates were then incubated at 26 ± 1°C for 12 days so that the fungal mycelium covers the whole plate. Four 8 mm diameter wells were cut in each plate in the peripheral region of the colony at 4 radii 90° apart.

Test for detection of laccase : One ml of freshly prepared solution of 1% (v/v) guaiacol in 95% ethanol was added to two wells. Ethanol was added to the remaining two wells as a control. The appearance of a red to purple colour indicated the presence of laccase.

Test for detection of catechol oxidase: One ml of freshly prepared solution of 0.1 M catechol dissolved in 0.1 M phosphate buffer, pH 6.0 was added to two wells whereas to control wells, 1ml of 0.1 M phosphate buffer (pH. 6.0) was added. The appearance of a

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golden yellow to brown colour indicated the presence of catechol oxidase.

Test for lignin peroxidase detection: 0.5 ml each of freshly prepared 1% (w/v) aqueous solution of pyrogallol and 0.4% hydrogen peroxide was added to two wells, whereas distilled water was added to the remaining two wells as a control for the test. Development of a golden yellow to brown colour indicated the peroxidase activity.

The plates in all the tests were wrapped in black cloth and incubated at 28 ± 2 °C. Periodic observations were made at 1, 16 and 24 h to detect change in colour of the medium. All the tests were performed twice and each time in triplicates.

RESULTS AND DISCUSSION

Brodie's basal agar medium plates of *Cyathus africanus*, *C. striatus* and *C. stercoreus* showed change in colour of the medium to red by 1 h, which remained for upto 24h. *C. bulleri* did not show any colour change till 24 h and thus does not produce laccase enzyme into the medium (Table 1). In contrast to this, the enzymes catechol oxidase and peroxidase were produced by all the four fungal strains which was detected in terms of change in colour of Brodie's basal agar

medium upon reaction with their specific substrates (Table 2 and 3). *C. bulleri* and *C. stercoreus* showed development of brown colour after only 1 h of initiating the reaction. *C. africanus* showed positive test after 16 h and *C. striatus* only after 24 h. The colour change among various fungal species ranged from light brown to chocolate brown. In case of lignin peroxidase enzyme, all four *Cyathus* species gave positive result after 16 h of reaction. The change in colour of the medium from creamish yellow to dark brown lasted till 24 h of incubation.

Since the discovery of lignolytic enzymes in 1983, there has been exceptional interest in these enzymes. This is because of many possibilities of using the enzyme commercially in various fields, including pulp and paper industry, chemical industry, detoxification of pollutants, waste water treatment and for feed (straw) modification to improve digestibility (Mehta and Gupta, 1989). White rot fungi are the most potent lignin degraders and have been surveyed for their capacity to produce these enzymes. Many groups of workers have dealt with detection and quantification of extracellular lignolytic enzymes in liquid media (Kuhad and Johri, 1991; Gupta, 1991).

Table 1. Laccase Detection on Brodie's basal medium plates

S.No.	Incubation Period (hr)	Test organism	Test for Laccase	Colour of the medium
1.	1	<i>C. bulleri</i>	-	creamish yellow
		<i>C. africanus</i>	+	red
		<i>C. striatus</i>	+	red
		<i>C. stercoreus</i>	+	red
2.	16	<i>C. bulleri</i>	-	creamish yellow
		<i>C. africanus</i>	+	red
		<i>C. striatus</i>	+	red colour
		<i>C. stercoreus</i>	+	dark red
3.	24	<i>C. bulleri</i>	-	creamish yellow
		<i>C. africanus</i>	+	red colour
		<i>C. striatus</i>	+	red colour
		<i>C. stercoreus</i>	+	dark red

+ = Positive ; - = Negative

Table 2. Catechol oxidase Detection on Brodie's basal medium plates

S.No.	Incubation Period (hr)	Test organism	Test for catechol oxidase	Colour of the medium
1.	1	<i>C. bulleri</i>	+	golden brown
		<i>C. africanus</i>	-	creamish yellow
		<i>C. striatus</i>	-	creamish yellow
		<i>C. stercoreus</i>	+	dark brown
2.	16	<i>C. bulleri</i>	+	brown colour
		<i>C. africanus</i>	+	brown
		<i>C. striatus</i>	-	creamish yellow
		<i>C. stercoreus</i>	+	dark brown
3.	24	<i>C. bulleri</i>	+	chocolate brown
		<i>C. africanus</i>	+	brown
		<i>C. striatus</i>	+	light brown
		<i>C. stercoreus</i>	+	chocolate brown

+ = Positive ; - = Negative

Table 3. Peroxidase Detection on Brodie's basal medium plates

S.No.	Incubation period (hr)	Test organism	Test for peroxidase	Colour of the medium
1.	1	<i>C. bulleri</i>	-	creamish yellow
		<i>C. africanus</i>	-	creamish yellow
		<i>C. striatus</i>	-	creamish yellow
		<i>C. stercoreus</i>	-	creamish yellow
2.	16	<i>C. bulleri</i>	+	blackish brown
		<i>C. africanus</i>	+	golden brown
		<i>C. striatus</i>	+	dark brown
		<i>C. stercoreus</i>	+	brown
3.	24	<i>C. bulleri</i>	+	blackish brown
		<i>C. africanus</i>	+	golden brown
		<i>C. striatus</i>	+	dark brown
		<i>C. stercoreus</i>	+	brown

+ = Positive ; - = Negative

The method described by us for qualitative detection of lignin-degrading enzymes is simple, can be easily done without the aid of any equipment/instrument and can be detected visually. Moreover, the use of petridishes for culturing the isolates makes the method easier to handle than the methods in which Erlenmeyer flasks were used. The method developed may find wide application for rapid screening of the white rot fungi for their ability to elaborate lignin-degrading enzymes into the medium.

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