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Enzymatic activity of *Cyathus olla* during solid state fermentation of canola roots

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Cyathus olla, a bird's nest fungus, is being studied as a biological control agent of stubble-borne diseases of canola. Our objectives in this study were to detect and identify plant cell wall degrading enzymes produced by *C. olla* during solid state fermentation of canola roots. We identified laccase and manganese peroxidase in both 1- and 4-week incubations, and aryl-alcohol oxidase was detected following 4 weeks of incubation. Crude buffer extracts were assayed for cellulases and polygalacturonase, but only the latter was detected. We conclude that *C. olla* has enzymes to degrade lignin and that it may have use as an inoculant to accelerate stubble decomposition.

[Activité enzymatique du *Cyathus olla* lors de la fermentation de racines de canola en milieu solide]

Le *Cyathus olla*, une nidulaire, est étudié comme agent de lutte biologique contre les maladies du canola véhiculées par le chaume. Dans cette étude, notre but est de détecter et d'identifier des enzymes produites par le *C. olla* lors de la fermentation, en milieu solide, de racines de canola et capables de dégrader les parois cellulaires de plantes. Nous avons trouvé des activités laccase et manganèse peroxydase après 1 et 4 semaines d'incubation, et une activité aryl alcool oxydase après 4 semaines d'incubation. Des extraits bruts dans du tampon ont été testés pour la présence de cellulases et de polygalacturonase, mais seulement la polygalacturonase a été détectée. Nous en concluons que le *C. olla* possède des enzymes pour dégrader la lignine et qu'il pourrait être utilisé comme inoculant pour accélérer la décomposition du chaume.

INTRODUCTION

We are studying the white wood-rotting, bird's nest fungus, *Cyathus olla* (Batch) ex. Pers. (Nidulariaceae) as a potential biological control agent for stubble-borne plant diseases. In western Canada, blackleg and blackspot of canola (*Brassica napus* L., *B. rapa* L.), incited by *Leptosphaeria maculans* (Desm.) Ces. et de Not and *Alternaria brassicae* (Berk.) Sacc., respectively, are common and serious diseases of this crop. Both pathogens are stubble-borne, and can overwinter and sporulate on infested debris as long as it remains in the field. The basal stem and root of canola are lignified and may take up to 5 yr to degrade under field conditions (Petrie

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1995). For these reasons, it is recommended that canola be grown in a 4-yr crop rotation.

Accelerated stubble decomposition could reduce the amount of infested stubble in a field, and thereby reduce the primary source of inoculum of stubble-borne pathogens. White wood-rotting fungi, *Cyathus* spp. can degrade lignocellulosic material (Abbott and Wicklow 1984; Shinners-Carnelley and Tewari 2000; Wicklow *et al.* 1984). For example, *C. stercoreus* (Schw.) de Toni can delignify bermuda grass stems (Akin *et al.* 1995), and improve the digestibility of corn and rice residue (Karunanandaa *et al.* 1992), maize stover (Chen *et al.* 1995), and wheat straw (Wicklow *et al.* 1984). Other *Cyathus* spp. can reduce the lignin content of maple hardwood (Wicklow *et al.* 1984). In preliminary studies with *C. olla*, this fungus grew on barley, wheat, and canola residues, but the hyphal growth was more profuse on woody lower parts of the stem and root of canola (Tewari and Briggs 1995). Also, there was a significant degradation of lignin and hemicellulose by *C. olla* upon solid-state fermentation of canola stubble (Shinners-Carnelley and Tewari 2000). The enzymes responsible for lignocellulosic metabolism by these fungi have not been studied extensively. Manganese peroxidase (MnP) and laccase have been identified from *C. stercoreus* (Orth *et al.* 1993), and cellulases have been detected from *C. helenae* Brodie (Kuhad and Johari 1987; Kuhad and Johri 1991), *C. striatus* (Huds.) Willd. ex Pers., and *Cyathus* sp. (Kuhad and Johari 1987). However little information exists on the plant cell wall degrading enzymes of *C. olla.* In a screening experiment, Pelaez *et al.* (1995) detected aryl-alcohol oxidase (AAO) from *C. olla,* but did not detect any other ligninolytic enzymes.

Scanning electron microscopy of field stubble infested with *C. olla* revealed the presence of calcium oxalate crystals and structural deterioration of the substrate, indicative of enzymatic activity and subsequent decomposition (Tewari *et al.* 1997). These results suggested that *C. olla* is actively involved in the degradation process. The objectives of this study were to detect and identify plant cell wall degrading enzymes produced by *C. olla* during solid state fermentation of canola root material.

MATERIALS AND METHODS

Detection of ligninolytic isolates

As a screening procedure, 42 *C. olla* isolates including three forms of the species (*C. olla* f. *olla*, *C. olla* f. *anglicus*, and *C. olla* f. *brodiensis* (Shinners and Tewari 1998)) were tested for the ability to degrade a model lignin substrate, the polymeric dye Poly R-478 (Sigma Chemical Co., St. Louis) (Glenn and Gold 1983). The degradation of the aromatic Poly R-478 dye was used as an indicator of ligninolytic activity, whereby the degradation of the aromatic ring resulted in a decolorization of the dye. Isolates were collected from northern and central Alberta, Canada, and obtained from the University of Alberta Microfungus Collection, Edmonton, Alberta, Canada, and the National Mycological Herbarium of Canada, Ottawa, Ontario, Canada (Table 1). *Phanerochaete chrysosporium* Burdsall was used as a positive lignin-degrading control. All isolates were sub-cultured as one plug from potato dextrose agar (Difco) onto a medium containing KH_2PO_4, 0.6 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1}; K_2HPO_4, 0.4 g L^{-1}; $(NH_4)_2$ tartrate, 0.22 g L^{-1}; sorbose, 40 g L^{-1}; Poly R-478 dye, 0.2 g L^{-1}; agar, 15 g L^{-1}; mineral solution 10 mL L^{-1} ($CaCl_2 \cdot 2H_2O$, 7.4 g L^{-1}; ferric citrate, 1.2 g L^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.7 g L^{-1}; $MnSO_4 \cdot 4H_2O$, 0.5 g L^{-1}; $CoCl_2 \cdot 6H_2O$, 0.1 g L^{-1}; thiamine HCl, 10 mg L^{-1}), and adjusted with HCl to pH 4.5 (Paterson and Bridge 1994). All cultures were maintained at 25°C in the dark. Plates were observed and measured weekly, and diam of the zones of decolorization after 4-wk are presented in Table 1. This plate method was used as a screening technique and therefore was not replicated.

Solid state fermentation of *C. olla* on canola root material

Based on the screening for ligninolytic activity using the dye Poly R-478, the isolate *C. olla* f. *anglicus* PR1 was se-

Table 1. *Cyathus olla* accession-information and results of the Poly R-478 plate assay

ID	Form	Location	Diameter of decolorized zone (mm)
C94	*C. olla* f. *olla*	CANADA, Alberta, Edmonton	39
C95	*C. olla* f. *olla*	CANADA, Alberta, Edmonton	32
C96	*C. olla* f. *olla*	CANADA, Alberta, Edmonton	37
Rav.	*C. olla* f. *anglicus*	CANADA, Alberta, Edmonton	28
Beau	*C. olla* f. *olla*	CANADA, Alberta, Beaumont	46
W240	*C. olla* f. *olla*	CANADA, Alberta, Edmonton	20
PR1	*C. olla* f. *anglicus*	CANADA, Alberta, SE 12-79-21-5[a]	51
PR2	*C. olla* f. *olla*	CANADA, Alberta, NE 12-82-21-5[a]	20
PR3	*C. olla* f. *brodiensis*	CANADA, Alberta, SW 24-78-20-5[a]	32
PR4	*C. olla* f. *olla*	CANADA, Alberta, SW 24-78-20-5[a]	34
PR5	*C. olla* f. *olla*	CANADA, Alberta, NW 9-78-21-5[a]	47
PR6	*C. olla* f. *olla*	CANADA, Alberta, SW 35-79-22-5[a]	59
PR7	*C. olla* f. *brodiensis*	CANADA, Alberta, SW 6-80-20-5[a]	34
PR8	*C. olla* f. *olla*	CANADA, Alberta, SW 6-80-20-5[a]	38
PR9	*C. olla* f. *olla*	CANADA, Alberta, SW 14-78-22-5[a]	40
PR10	*C. olla* f. *olla*	CANADA, Alberta, SE 5-78-21-5[a]	50
PR11	*C. olla* f. *brodiensis*	CANADA, Alberta, SE 13-79-21-5[a]	25
PR12	*C. olla* f. *olla*	CANADA, Alberta, SE 13-79-21-5[a]	21
PR13	*C. olla* f. *olla*	CANADA, Alberta, SE 11-78-21-5[a]	40
PR14	*C. olla* f. *olla*	CANADA, Alberta, SW 2-79 22-5[a]	41
PR15	*C. olla* f. *olla*	CANADA, Alberta, SW 2-79-22-5[a]	49
PR16	*C. olla* f. *olla*	CANADA, Alberta, NE 13-81-21-5[a]	39
PR17	*C. olla* f. *olla*	CANADA, Alberta, Legal	45
PR18	*C. olla* f. *anglicus*	CANADA, Alberta, NW 16-77-21-5[a]	37
PR19	*C. olla* f. *brodiensis*	CANADA, Alberta, NW 10-77-21-5[a]	25
PR20	*C. olla* f. *olla*	CANADA, Alberta, NW 10-77-21-5[a]	24
PR21	*C. olla* f. *brodiensis*	CANADA, Alberta, SE 6-78-20-5[a]	20
PR22	*C. olla* f. *anglicus*	CANADA, Alberta, SE 6-78-20-5[a]	47
PR23	*C. olla* f. *brodiensis*	CANADA, Alberta, Falher	30
PR24	*C. olla* f. *olla*	CANADA, Alberta, Falher	20
PR25	*C. olla* f. *anglicus*	CANADA, Alberta, NE 33-77-20-5[a]	28
PR26	*C. olla* f. *olla*	CANADA, Alberta, NE 33-77-20-5[a]	40
PR27	*C. olla* f. *brodiensis*	CANADA, Alberta, SE 26-78-21-5[a]	30
PR28	*C. olla* f. *olla*	CANADA, Alberta, SE 26-78-21-5[a]	30
PR29	*C. olla* f. *olla*	CANADA, Alberta, NE 20-77-21-5[a]	45
PR30	*C. olla* f. *olla*	CANADA, Alberta, NE 20-77-19-5[a]	37
Fair	*C. olla* f. *olla*	CANADA, Alberta, Fairview	45
UAMH 8276	basidiocarp not available	UAMH collection[b]	49
DAOM 196679	basidiocarp not available	DAOM collection[c]	20
DAOM 197563	basidiocarp not available	DAOM collection[c]	0
DAOM 197577	basidiocarp not available	DAOM collection[c]	0
DAOM 184718	basidiocarp not available	DAOM collection[c]	25

[a] Legal description within the Municipal District of Smoky River, Alberta, Canada.
[b] Obtained from the University of Alberta Microfungus Collection, Edmonton, Alberta, Canada.
[c] Obtained from the National Mycological Herbarium of Canada, Ottawa, Ontario, Canada.

lected for solid state fermentation of canola root material. Incubations were carried out in 1 L Erlenmeyer flasks containing 10 g of canola root material (ground in a Wiley mill, 1 mm screen) and 40 mL of distilled H_2O. The flasks were sterilized three times at 121°C for 35 min to ensure thorough sterilization of the canola root. Following the first sterilization, an additional 10 mL of distilled H_2O was added to replace moisture lost to evaporation during sterilization. Sterile flasks were inoculated with 10 mL of the mycelial suspension, and incubated at about 25°C in the dark for 1- or 4-wk.

The contents of two flasks were harvested for each enzyme preparation. Colonized root material was suspended in 300 mL of cold 25 mM sodium acetate buffer, pH 5.5, and sonicated for two 30 s periods, followed by gentle shaking for 30 min at room temperature (about 22°C). This procedure was repeated twice, for a total of six 30 s sonication cycles and 1.5 h of shaking. The suspension was centrifuged at 26,000 x *g* for 30 min at 4°C. The supernatant was removed, and the pellet washed with 50 mL of 25 mM sodium acetate buffer, pH 5.5 and re-centrifuged. Following the second centrifugation, the supernatant (approximately 400 mL) was filtered through Whatman #1 filter paper to clarify the extract. Aliquots were removed to initially assay the enzyme activities including lignin peroxidase (LiP), MnP, laccase, and AAO. Two 20 mL aliquots were also removed and lyopholized from each preparation for total cellulase and polygalacturonase assays.

Ligninolytic enzyme assays

All enzyme assays were carried out in 1-mL volumes at room temperature and the absorbance was measured using a Hewlett Packard 8451A diode array spectrophotometer. One unit of activity was defined as the amount of enzyme required to produce 1 μmol of product per min (1 U = 1 μmol min^{-1}, 1 mU = 1 nmol min^{-1}). The concentrations of each assay component given below are final concentrations based on a 1-mL total reaction volume. Prior to performing the assays, veratryl alcohol was purified to prevent trace impurities from interfering with the assays (Bourbonnais and Paice 1990).

LiP was measured by the oxidation of veratryl alcohol to veratrylaldehyde (E_{310} = 9300 M^{-1} cm^{-1}) (Tien and Kirk 1988). Each assay contained 50 mM sodium tartrate pH 2.5, 2 mM veratryl alcohol, and 10 to 550 μL enzyme solution. The reaction was initiated with the addition of 0.4 mM H_2O_2. MnP was assayed according to Paszczynski *et al.* (1988) using 0.01% phenol red as the substrate (E_{610} = 4460 M^{-1} cm^{-1}). Each assay contained 100 mM sodium tartrate pH 5.0, 0.01% phenol red, 0.1 mM $MnSO_4$, and 10-690 μL enzyme solution. The reaction was initiated with 0.1 mM H_2O_2. Laccase activity was measured by the oxidation of 2,2'-azinobis-(3-ethyl benzthiazoline-6-sulphonate) (ABTS) (E_{420} = 3.6 x 10^4 M^{-1} cm^{-1}). Each assay contained 0.5 mM ABTS, 0.1 M sodium acetate buffer pH 5.0, and 10-700 μL of enzyme solution. AAO was assayed according to Guillen *et al.* (1992) by measuring the oxidation of veratryl alcohol to veratrylaldehyde (E_{310} = 9300 M^{-1} cm^{-1}). Each assay contained 100 mM sodium phosphate buffer pH 6.0, 10 mM veratryl alcohol, and up to 550 μL of enzyme solution.

Protein purification

Following filtration on Whatman #1 paper, proteins in the extract were fractionated by anion-exchange chromatography on Q-Sepharose Fast Flow medium (Pharmacia). The column (2.6 cm x 18 cm) was equilibrated with 25 mM sodium acetate, pH 5.5 and approximately 360 mL of extract was loaded, followed by an additional wash with 1 L, 25 mM sodium acetate, pH 5.5. Proteins were eluted with a linear NaCl gradient of 0 to 0.3 M, with a total elution volume of 500 mL. The column was washed with 1M NaCl. The flow rate was 1.6 mL min^{-1}, and the eluted proteins were collected in 3.7 mL fractions. Enzyme activities (LiP, MnP, laccase, AAO) and heme (408 nm) were measured as the fractions were collected. Fractions with activity were pooled and concentrated using Slide-a-Lyzer cassettes (Pierce), and then assayed to determine final enzyme activities using

the previously described assays. Protein determinations (Bradford 1976) for the crude extract and purified fractions were made using the Coomassie Plus (Pierce) micro-plate assay. This procedure was adapted from that of Vares *et al.* (1995) for ligninolytic enzymes from wheat straw inoculated with *Phlebia radiata* Fr.

Cellulases

Lyopholized samples were re-dissolved in 2 mL (one-tenth their original volume) of 50 mM citrate buffer, pH 4.8 and dialyzed against the same buffer. Total cellulase activity was determined using the filter paper method (Mandels *et al.* 1976) and the dinitrosalicyclic acid (DNS) method for determination of reducing sugars (Miller *et al.* 1960). Enzyme and substrate blanks were included to measure background levels of reducing sugars. A glucose standard curve (0-4 mg) was used to calculate enzyme activity.

Polygalacturonase

Lyopholized samples were re-dissolved in 2 mL (one-tenth their original volume) of 50 mM sodium acetate buffer, pH 5.2 and dialyzed against the same buffer. Polygalacturonase activity was determined by the release of reducing sugars from 0.1% polygalacturonic acid. Each assay contained 5-20 µL of the concentrated enzyme preparation in 0.8 mL of 50 mM sodium acetate buffer, pH 5.2 with 0.1% polygalacturonic acid, and adjusted to 1 mL with buffer (Annis and Goodwin 1997). The samples were incubated for 1 h at 30°C and reducing sugars were measured using the DNS method. A galacturonic acid standard curve (0-0.2 mg) was used to calculate polygalacturonase activity.

RESULTS

Screening for ligninolytic activity

Of the 42 accessions screened, all but two (DAOM 197563 and DAOM 197577) decolorized the Poly R-478 medium to some degree over the 4-wk period. Zone of decolorization was determined by measuring the diam of the decolorized area (Table 1). *Phanerochaete chrysosporium* decolorized the entire plate (diam 90 mm) after 4-wk, whereas 40 accessions of *C. olla* produced variable decolorized zones ranging in diam from 20-59 mm (Table 1). An uninoculated plate was used as a negative control. The accession (*C. olla* f. *olla* PR 6) that produced the largest decolorized zone (59 mm) was collected from an agricultural field, however, the fibrous substratum could not be positively identified, and for this experiment, it was preferable to have an accession that was isolated from canola. Therefore, the accession *C. olla* f. *anglicus,* PR 1, isolated from field-infested canola stubble was chosen for the solid-state fermentation experiment. This accession produced the second largest zone of decolorization (51 mm diam).

Detection of ligninolytic enzymes

Initial crude buffer extract samples (prior to fractionation) from both the 1- and 4-wk preparations were assayed for LiP, MnP, laccase, and AAO. Laccase and MnP activities were detected from both incubation periods (Table 2), but LiP and AAO were not detected in the initial crude extract.

Table 2. Initial enzyme activities in crude culture extracts from canola root degraded by *C. olla*

	Specific activity (mU mg^{-1} protein)[a]	
Enzyme	1 week	4 weeks
Laccase	923	300
MnP	18	21
AAO	ND[b]	ND
Total cellulases	ND	ND
Polygalacturonase	187	60

[a] 1 mU = 1 nmol of product min^{-1} mL^{-1}.
[b] Not detectable.

Crude buffer extracts from both the 1- and 4-wk incubations were brown in color. When loaded in the column, a brown zone appeared bound to the Q-Sepharose that was not removed with the buffer wash. This zone remained tightly bound through the NaCl gradient, and was not eluted until the column was washed with 1M NaCl. As fractions were eluted, they were assayed for ligninolytic enzyme activities (LiP,

MnP, laccase, AAO), and heme was measured as an indicator for the heme containing enzymes, LiP and MnP. Laccase activity was the first enzyme to be detected in the elution profiles in both 1- and 4-wk incubations (Fig. 1). Increase in heme absorbance was useful in detecting MnP, which was eluted from the column soon after laccase, with some fractions containing both enzymes. After 1 wk, there were two peaks of MnP activity suggesting two forms

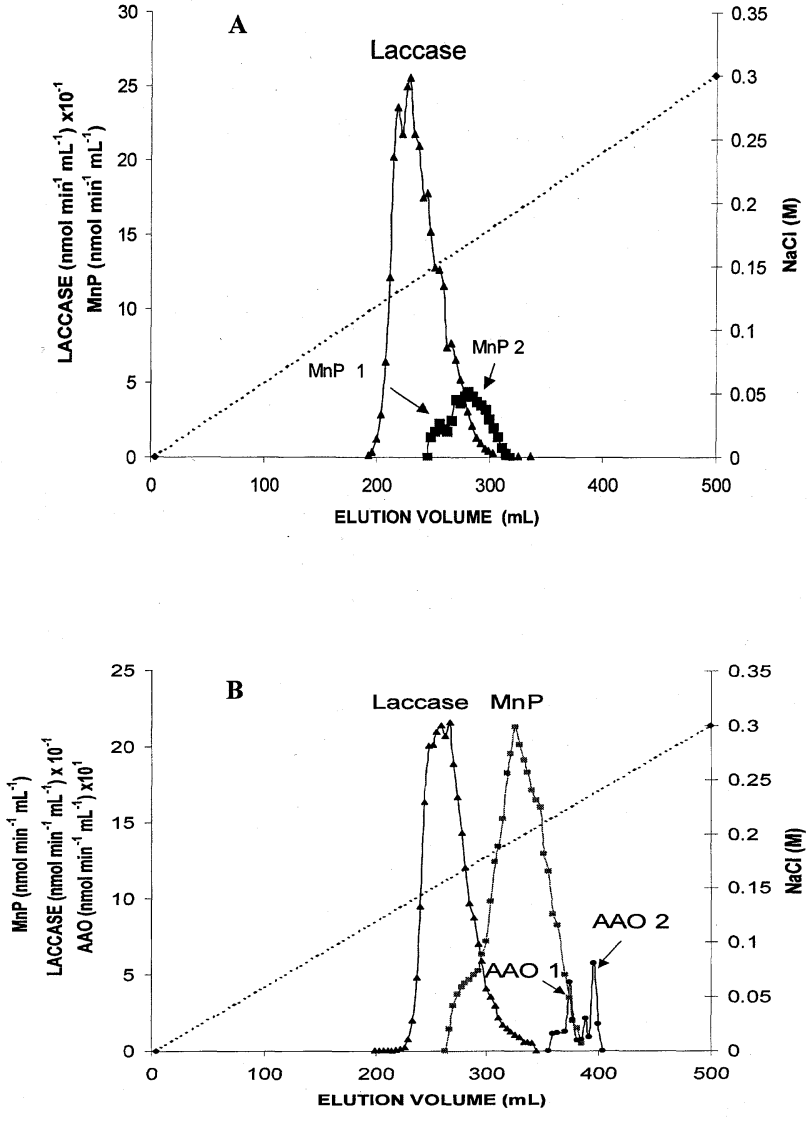

Figure 1. Enzymes separated from 1- (A) and 4-week (B) solid state fermentation of *C. olla* in canola root material.

of this enzyme. On the 4-wk incubation period elution profile, a shoulder on the MnP peak suggested two MnP peaks (Fig. 1), however this was not clearly discernible and would require protein characterization to identify the isozymes of MnP. AAO was not detectable in enzyme assays prior to fractionation, but two peaks of enzyme activity were consistently observed in a narrow range of the elution profile from the 4-wk preparation. LiP was not detected throughout the experiment.

Eluted fractions with activity were pooled and concentrated using the Slide-a-Lyzer cassette technique (Pierce). Enzyme and protein assays of these concentrated fractions were used to determine the specific activity of each enzyme (Table 3). Specific activity values increased as a result of anion-exchange chromatography on Sepharose-Q Fast Flow medium and concentration techniques, demonstrating that these methods were successful at separating, and to some degree, purifying the ligninolytic enzymes present. Replicate experiments of these incubation periods and extractions produced repeatable elution profiles, whereby, the enzymes were eluted at the same place in the gradient, however, the activities of each enzyme were variable between replicate incubations. Laccase was the most abundant enzyme produced. Initial extracts from 1-wk incubations ranged from approximately 12-27 U of total activity, but consistently produced approximately 20 U of activity in the 4-wk incubations. Manganese peroxidase from the initial extract was more variable, ranging from being not detectable to approximately 1.3 U of total activity from both incubation periods. Detection of AAO was confounded by the apparent instability of the enzyme. Aryl alcohol oxidase activity decreased sharply within 24 h following fractionation, but this was not a problem with laccase or MnP.

Table 3. Specific activities of pooled, concentrated fractions

	Specific activity (mU mg^{-1} protein)[a]	
Enzyme	1 week	4 weeks
Laccase	7110	1150
MnP	MnP 1 100 MnP 2 257	438
AAO	ND[b]	AAO 1 3.4 AAO 2 3.2

[a] 1 mU = 1 nmol of product min^{-1} mL^{-1}.
[b] Not detectable.

Cellulase and polygalacturonase

Using the filter paper method, no cellulase activity was detected from either the 1- or 4-wk incubation preparations. Assays of substrate blanks determined that the enzyme solution had a high background of reducing sugars, with the 1- and 4-wk incubation preparations having 2.03 and 2.19 mg of reducing sugar mL^{-1} of enzyme solution, respectively. Polygalacturonase activity was detected from both the 1- and 4-wk incubation preparations (187 and 60 mU mg^{-1} protein respectively) (Table 2).

DISCUSSION

Our results demonstrate that *C. olla* has a ligninolytic enzyme system that is active during solid state fermentation in a canola substrate. This is the first report of laccase and MnP production by this fungus. AAO was not initially detected, but was present after 4 wk of growth. These results are similar to those of Pelaez *et al.* (1995) who did not detect this enzyme from *C. olla* after 7 d of growth, but did find AAO in the artificial culture medium following 21 d of incubation. As cultures age, the enzyme profiles change. Vares *et al.* (1995) observed such changes at weekly intervals over a 4-wk period of growth of *Phlebia radiata* on wheat straw.

From figures 1 A and B, it appears that laccase activity maintained at almost similar levels, and MnP activity increased by the fourth wk of incubation, relative to the 1-wk preparations. This could be a valid trend, but in this situation, it cannot be assumed that the differences are significant since enzyme activities were variable between repli-

cate incubation periods. It was not the intent of these experiments to quantify the individual enzymes, but rather to identify ligninolytic enzyme production by *C. olla*. The enzymes described in this study were produced in all replicates, and were eluted at the same place in the NaCl gradient, but relative activity was variable from preparation to preparation. The canola root material was used as substrate based on practical considerations relative to the objectives of these experiments, but was not as well defined as an artificial medium. It was therefore, a possible reason for the variability observed in these experiments. The optimum conditions required for ligninolytic activity by *C. olla* have not been defined, but if determined could result in more consistency with respect to enzyme activities. Further research should be conducted to elucidate these conditions, but for the purpose of this experiment, the results contributed to understanding the role of *C. olla* in canola stubble decomposition. Previous studies revealed that this fungus produces calcium oxalate crystals (Tewari *et al.* 1997), suggesting oxalic acid secretion and calcium sequestration. This study provides further information regarding these activities by confirming the production of plant cell wall degrading enzymes that are thought to be active following calcium sequestration, and subsequent weakening of the substrate (Bateman and Beer 1965).

No cellulases were detected under these cultivation conditions. Cellulases are induced by low glucose or sugar concentrations, and are repressed when these sugars are in excess of fungal requirements. The canola substrate used had soluble sugars that may have fulfilled the carbohydrate requirement of the fungus throughout the time course of this experiment. Previous studies have determined that the soluble fraction of canola root accounts for 28% of the total weight (Shinners-Carnelley and Tewari 2000). This fraction includes soluble carbohydrate, starch, and organic acids. Polygalacturonase activity was detected from both incubation periods. There are very few reports in the literature of pectinase enzymes associated with white rot fungi. The fungi in this group are specialized to degrade cellulose and lignin, and the assumption is often made that it is not ecologically significant for white-rot fungi to degrade pectin, since it makes up only a small fraction of wood. However, after detecting polygalacturonase from brown-rot fungi, Green *et al.* (1995) suggested that the importance of pectinase enzymes in wood decay may be underestimated. The ability of a wood-rotting fungus to hydrolyse pectic substances may be advantageous, resulting in solubilization of bordered pit membranes, allowing access to adjoining tracheids. Therefore, the location of pectic substances in woody tissues may be more significant than the quantity.

The ability of *C. olla* to produce polygalacturonase is a significant finding, although it is not known whether this ability would be induced in a field situation within the time used in this experiment. Stubble decomposition is a complex process, wherein many ecologically specialized microorganisms and soil fauna are involved in natural succession on the substrate. From field observations, *C. olla* is normally found growing on decorticated stubble. In the natural succession of organisms, it is assumed that the first to colonize and decorticate stubble would be microorganisms most successful at competing for, and utilizing the soluble sugars, pectic substances, and other easily degraded components. *Cyathus olla* would likely colonize after these other saprophytes could no longer obtain nutrients from the remaining lignocellulose. At this point, polygalacturonase production by *C. olla* may be significant, and aid in colonization of the substrate, as suggested by Green *et al.* (1995).

The results presented here have identified that *C. olla* is capable of producing laccase, MnP, AAO, and polygalacturonase during solid state fermentation of canola root material. These findings contribute to the growing body of knowledge on biochemical and potential ecological attributes of this fungus that is required to assess its potential of being developed into a microbial inoc-

ulant to accelerate stubble decomposition, and ultimately reduce the incidence of stubble-borne diseases of canola. This innovative application would be beneficial to the agricultural industry. Moreover, *C. olla* may also prove to be suited to other applications requiring degradation of aromatic rings such as soil remediation, pesticide degradation, or delignification of other lignocellulosic substrates.

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