Symbiosis, 2 (1986)
Balaban Publishers, Philadelphia/Rehovot

Polysaccharide Degrading Enzymes Excreted by Two Root Rot Fungi

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Abstract

The culture filtrate of *Rigidoporus lignosus* and *Phellinus noxius* exhibit endo- and exo-glucanase as well as cellobiase activities. A very weak pectinolytic activity was detected in the *R. lignosus* culture filtrate whereas *P. noxius* showed both a pectinase (endo-PG) and a pectate-lyase activity. Besides these enzymes, xylanase, laminarinase and glycosidase activities were also observed. Partial purification of the *P. noxius* extracellular enzymes was carried out. Molecular weight and optimal pH of these enzymes were determined.

Keywords: extracellular polysaccharide degrading enzymes, glycosidases, root rot fungi, *R. lignosus, P. noxius, Fomes*, rubber tree

1. Introduction

Rigidoporus lignosus and *Phellinus noxius* are two rubber root parasites causing a white rot of wood (Geiger et al., 1986a, b and c). They were previously shown to secret lignin as well as polysaccharide degrading enzymes, both *in vitro* and *in vivo* (Geiger et al., 1986a). The objectives of the present study were: to estimate the ability of the enzymes released into the culture filtrates to degrade the polysaccharide fraction of the natural lignocellulose isolated form the rubber tree wood, to characterize the nature of the cellulolytic and pectinolytic complex, and to achieve a partial separation of the enzymes that are very actively secreted by *P. noxius* in order to determine some of their physicochemical properties.

0334-5114/86/$03.00

2. Materials and Methods

The fungi were cultured on either a wood saw-meal or on a filter paper medium. Subsequently on the 13th day, the culture filtrates containing the soluble extracellular enzyme activities were recovered.

The total lignocellulose degrading abilities of the crude culture filtrates were determined by incubating them, at pH 4.6, with a 1% extractive-free lignocellulose substrate. The reaction media were analyzed (using dinitro salicylic acid (DNS) method) for total reducing sugar released and their mono- and oligo-saccharide composition identified by thin layer chromatography (TLC) on silica gel.

The α- and β-galactosidase, β-glucosidase, CM-cellulase and pectinolytic activities were measured as previously reported (Geiger, 1975). The cellobiase, exoglucanase, xylanase and laminarinase activities were characterized at pH 4.6 using reaction media containing 0.02 M cellobiose, 1% Avicel (insoluble microcristalline cellulose), 0.2% xylan or 0.2% laminarin, respectively.

The qualitative characterization of the cellobiase and the cellulolytic and pectinolytic complexes was achieved by TLC analysis of the reaction products. The discrimination between the two types of pectinolytic activities (hydrolytic and lyase splitting of the pectic polymer) was achieved using the Sherwood's (1966) thiobarbituric acid (TBU) test.

Partial purification of *P. noxius* extracellular enzymes was achieved by successive column chromatography on DEAE-cellulose and on Sephadex G75, G100 or G150 gel. The latter technic was used for the molecular weight (MW) determination of the different enzymes.

3. Results

Qualitatively the same oligo- and polysaccharide degrading enzyme activities could be detected both in *R. lignosus* and in *P. noxius* culture filtrates. The main differences between these two culture filtrates are at the quantitative level of the activities. The culture filtrates of *P. noxius* were always much higher (3 to 100 fold, depending on the nature of the enzymes) in glycosidase and polyosidase activities than that of *R. lignosus*. The same quantitative difference was observed when lignocellulose was used as substrate: the total reducing sugar released during the enzymatic hydrolysis was about 10 fold higher in the presence of *P. noxius* culture filtrate than that in the presence of the *R. lignosus*. As previously reported (Geiger et al., 1986a), the two fungi exhibit *in vivo* (host infected tissues) the same kind of enzyme activities as they do *in vitro*. Therefore their ability to excrete an enzymatic complex

that is effective, *in vitro*, on lignocellulose, indicates that similar complex excreted *in vivo*, should be effective in the host cell wall degradation during pathogenesis (host penetration and tissue colonization).

The qualitative TLC analysis of the lignocellulose hydrolysis products shows an accumulation of glucose, cellobiose and some galacturonic acid. Several additional "spots" were also revealed on the chromatogram. Most probably they refer to oligosaccharides of varying composition and size.

TLC analysis of the degradation products of Avicel, when incubated with crude culture filtrates, allowed the identification of both cellobiose and glucose. It indicates that these filtrates contain an exoglucanase (cellobiose hydrolase) activity, i.e. hydrolysing the Avicel into cellobiose. This disaccharide is subsequently hydrolysed into glucose by the cellobiase activity of the crude filtrate. The latter activity was characterized in the same way: glucose was shown to accumulate in the reaction medium containing the cellobiose substrate.

The CM-cellulase is actually an endoglucanase as shown by: (a) the high viscosimetric activity versus the very weak one when measured by the DNS (reducing sugar) method; (b) the oligosaccharidic fractions, having variable size, resulting from the CM-cellulose hydrolysis.

The pectinolytic activity of *R. lignosus* culture filtrate was low, while that of *P. noxius* was very high, consisting of a high pectinase (hydrolytic) and a weak pectate lyase activity. The pectinase was purified and characterized as an endo polygalacturonase (endo-PG) according to Bateman and Millar's (1966) classification of the pectic enzymes.

Finally a partial separation of some of the enzymes belonging to the oligo and polysaccharide degrading complex secreted by *P. noxius* was carried out by DEAE-cellulose chromatography and Sephadex gel filtration. It allowed the identification of: one α-galactosidase (MW: 112–115,000; opt. pH: 5.0), two β-galactosidases (MW: 74,000 and 43,000; opt. pH: 4.3), two β-glucosidases (cellobiases; MW: 69,000 and 88,000; opt. pH: 5.0), two CM-cellulases (MW: 33,000 and 31,000: opt. pH: 4.8), one pectinase (endo-PG; MW: 39,000; opt. pH: 4.5) and one xylanase (MW: 38,000; opt. pH: 3.8).

4. Conclusion

No qualitative differences were observed in the ability to degrade polysaccharide fraction of the lignocellulose by *P. noxius* and *R. lignosus*. The degrading complex excreted by the two fungi appears to be exceptional in the diversity of the enzymes of which it is composed. In addition, *P. noxius*

secretes those activities at an exceptionally high level, explaining its high efficiency in degrading the cellulosic tissues (phloem) of the rubber tree (Geiger et al., 1986a; Nicole et al., 1982; 1986). From a "biotechnological" point of view, *P. noxius* should be a valuable source of glycosidase and polyosidase enzymes or isoenzymes.

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