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The aggression of Hevea brasiliensis by Rigidoporus lignosus and Phellinus noxius: some biochemical events

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Abstract

The activity and spatial distribution of eight enzymes in adult tap root tissues colonized by *R. lignosus* and *P. noxius* are investigated. Their physiological significance in the pathogenic process is discussed.

1 Introduction

Rigidoporus lignosus and *Phellinus noxius*, Polyporaceae, are widespread white rot parasites in equatorial and humid tropical zones. Even though they attack a large number of shrubby species (CHEVAUGEON 1959), they exist in equilibrium in the natural forest. They are of

practical importance, at least in Africa, since they are the most dangerous root parasites of rubber trees.

The main purpose of the present work is to correlate the parasite attack with changes of host tissues (lignified roots) and with enzyme activities chosen for their possible participation in tissue degradation. After an estimation of the level of the biochemical disturbances, the origin of the enzymes (host or parasite) and their possible role in the pathogenic process are discussed.

2 Materials and methods

The enzyme excretion abilities of *R. lignosus* and *P. noxius* were tested by measuring, in culture filtrates, the activity of carboxymethyl-cellulase, pectinase, phosphatase, β-glucosidase, α- and β-galactosidase, laccase and peroxydase.

In order to estimate the degree of enzyme disturbances in host tissues caused by the parasite attack, extracts of healthy (H) and infected (I) tissues from partially invaded *Hevea* tap roots were examined for quantitative and qualitative enzyme activities.

The spatial distribution of enzymes in partially invaded tap roots was investigated on the following samples:

- Healthy tissues (H), sampled much ahead of parasite progression (Control),
- Healthy front tissues (HF), sampled near the progression front,
- Parasitized front tissues (IF),
- Parasitized tissues (I) removed much behind the front ("old" colonized tissues),
- Parasitized alveolar type tissues (IA) (only in the case of infection by *P. noxius*).

Electrophoresis of enzymes were realized as described by SMITHIES (1955).

Culture medium (Wood dust), culture filtrate, wood extracts, enzyme assays were prepared or realized as earlier described (GEIGER 1975; GEIGER et al. 1976; NICOLE et al. 1982).

3 Results

The main results are as follows:

1. Compared to the enzyme activities which can be extracted from healthy tissues, extracts from parasitized tissues exhibit a number of enzymatic modifications, both at quantitative and qualitative levels.
   - qualitatively: three enzymes are present only in parasitized tissues: CM-cellulase, pectinase and laccase for which their major participation in the pathogenic process may be presumed.
   - quantitatively: with the exception of phosphatase and partially of β-glucosidase, enzyme activities in infected tissues are much higher than those in healthy tissues; these enzymes are: α and β-galactosidases and peroxidase.
2. *R. lignosus* and *P. noxius* both excrete glycosidases, polyoxidases and laccase, but at activity levels which are specific to each fungus; there is a predominance of laccase activity in the case of *R. lignosus* and hydrolases in the case of *P. noxius*. These same quantitative variations exist in parasitized tissues; so that in terms of the relative proportions of the different enzymatic activities, the extracts of these tissues are quite similar to those of the homologous culture filtrates.
3. The spatial distribution of enzyme activities shows that, with exception of the phosphatase and the peroxidase, the activities increase according to the sequence H → HF → IF → I → IA tissues. They are highest in the alveolar tissues which are thus incapable of any type of reaction requiring the integrity of the protein synthesis system. Therefore it can be assumed that all those enzymes present in infected tissues are of fungal origin.
4. Electrophoretical comparisons of isophosphatases and isoperoxidases from healthy and

infected tissues (by *R. lignosus* and *P. noxius*) and from homologous culture filtrates show that the phosphatase in infected tissues is of fungal origin, whereas the peroxidase increase in IF type tissues corresponds to a host reaction.

5. The enzymes secreted by the pathogens are well adapted to the degradation of plant tissues. The action of enzymes contained in culture filtrates causes the release of various saccharides (monomers and oligomers) from insoluble *Hevea* lignocellulose.

Similarly, laccase must play a role in lignin degradation as shown by the fact that one of these enzymes (laccase L1) produced by *R. lignosus* causes both the condensation and the depolymerisation of lignin (GEIGER et al. 1983).

4 Discussion – Conclusion

According to all these data we can propose some hypotheses or conclusions about the disturbances caused by the parasite attack of host roots, their signification and the role of the enzymes that are involved.

1. With exception of peroxidase, the enzymes that are present in infected tissues are of fungal origin. They are involved in root tissue degradation. *R. lignosus* and *P. noxius* degrade both polysaccharidic and lignin fractions and therefore they must be classified among the white rotting fungi.

2. The increase of the peroxidase activity in IF tissues is a response of the host against the parasite aggression. As shown by GEIGER and HUGUENIN (1981) this enzyme polymerizes the p-coumarylic and sinapinylic alcohols and thus it may be involved in reaction lignin synthesis leading to the cell wall thickening that could be observed in infected tissues (NICOLE et al. 1982).

3. Quantitative differences and especially the ratio of laccase and polysaccharidases should have an effect on the relative capacity of the two fungi to degrade the different polymers of the cell-wall, and thus the different root tissues. In practice, it is expected that *P. noxius* is more active than *R. lignosus* on polysaccharide structures and thus in tissues which are richest in cellulose, xylan and pectic materials, whereas the inverse should be true on highly lignified tissues.

The comparisons between lignin contents of wood sticks infected either by *R. lignosus* or *P. noxius* agree with this hypothesis. In fact the former exhibits a tendency to a more rapid degradation of the lignin fraction whereas the latter degrades somewhat more rapidly the polysaccharidic fraction as the lignin one.

In agreement with the biochemical data, the histological observations show that *P. noxius* degrades cortical tissues i. e. weakly lignified tissues, more rapidly and more completely than *R. lignosus* does. On the other hand, *R. lignosus* degrades more rapidly the woody lignin-rich structure of the xylem than the cortical tissues. Nevertheless *P. noxius* degrades quite well the lignified tissues of xylem. This suggests that the lignin degrading equipment of *P. noxius* is not a limiting factor but that, on the contrary, the weak hydrolitic equipment of *R. lignosus* limits the degradation abilities of this parasite on polysaccharidic structures (NICOLE et al. 1984).

Summary

Parasite aggression causes a considerable disturbance in the enzyme equipment of *Hevea* root tissues. The enzymes present in those tissues are of fungal origin and participate in the degradation of polymers in different cell walls of the host. Regarding the ratio between polysaccharidase and laccase activities *P. noxius* may preferentially attack weakly lignified tissues whereas *R. lignosus* would be more active on lignified tissue of the xylem than on cortical tissues. The increase of peroxidase activity corresponds to a host reaction and may participate in the defence mechanisms of *Hevea brasiliensis* against the invading parasites.

Résumé

L'attaque d'Hevea brasiliensis par Rigidoporus lignosus et Phellinus noxius: différentes manifestations biochimiques

L'agression parasitaire provoque une perturbation considérable au niveau de l'équipement enzymatique des tissus racinaires de l'Hévéa. Les enzymes présentes dans les tissus parasités sont d'origine fongique et participent à la dégradation des différents polymères pariétaux des tissus de l'hôte. La proportion entre hydrolases (plus particulièrement polyosidases) et laccase diffère suivant le parasite et suggère une attaque préférentielle des structures polysaccharidiques par *P. noxius* et une activité privilégiée à l'encontre des tissus lignifiés par *R. lignosus*.

L'accroissement de l'activité peroxydasique dans les tissus parasités correspond à une réaction de l'hôte. Cette enzyme est susceptible de participer aux mécanismes de défense de l'Hévéa.

Zusammenfassung

Der Befall von Hevea brasiliensis durch Rigidoporus lignosus und Phellinus noxius: einige biochemische Zusammenhänge

Der Angriff von Pathogenen verursacht erhebliche Störungen im Enzymhaushalt des Wurzelgewebes von *Hevea brasiliensis*. Die Enzyme in den befallenen Geweben stammen von den Pilzen und sind am Abbau der verschiedenen Zellwandbestandteile der Wirtszelle beteiligt. Betrachtet man das Verhältnis zwischen Polysaccharidase- und Laccaseaktivität, so dürfte *P. noxius* bevorzugt schwach lignifiziertes Gewebe befallen, während *R. lignosus* eher im lignifizierten Gewebe des Xylems als in der Rinde aktiv sein dürfte. Der Anstieg der Peroxidaseaktivität geht mit einer Reaktion des Wirtes Hand in Hand und dürfte Teil einer Abwehrreaktion von *H. brasiliensis* gegen die eindringenden Pathogene sein.

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