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LACCASE IN ANACARDIACEAE

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Key Word Index—*Mangifera indica*; *Schinus molle*; Anacardiaceae; laccase; enzyme distribution; chemotaxonomy.

Abstract—The presence of a typical laccase is demonstrated in the cavities of the secretory ducts of a number of species of the Anacardiaceae, including *Mangifera indica* and *Schinus molle*. In addition mango fruit contains catechol oxidase. The presence of laccase may be of chemotaxonomic value.

INTRODUCTION

During the harvest of mango fruits, *Mangifera indica*, a secretion flows from the cut pedicel. This secretion browns and hardens. In bark of the lacquer trees of the genus *Rhus* there is a similar secretion which contains laccase. These secretions although not originating in laticifers, are often termed latex. The *Rhus* laccase has been extensively studied [1, 2], but there are very few reports of laccase in other higher plants [3, 4]. Since *Mangifera* and *Rhus* belong to the Anacardiaceae which are characterised by the presence of secretory ducts in the phloem, we decide to study the distribution of laccase in this family. Catechol oxidase has been reported to be present in the mango fruit [5], but laccase has not been described in it. We now show that laccase is present in the duct content of a number of members of the Anacardiaceae.

RESULTS AND DISCUSSION

The polyphenol oxidase present in the secretion from the cut pedicel of mango fruit behaves as a typical laccase. It oxidases both quinol and 4-methylcatechol, as well as ascorbic acid (Table 1). The amount of enzyme used was small, making it unlikely that ascorbic acid was oxidised by a coupled oxidation. Moreover, addition of ascorbic acid to the reaction mixture in the presence of quinol did not enhance O_2 uptake. As the *Rhus* laccase [6, 7], and in contrast to fungal laccases [8], it does not oxidise *p*-cresol. It has maximal activity around pH 6. It is not inhibited by CO as reported for *Rhus* laccase already by Keilin and Mann [6, 7], nor by 1 mM phenylhydrazine, but as expected it is inhibited 69% by 1 mM diethyldithiocarbamate. Surprisingly the mango laccase did not oxidase syringaldazine [9]. The ability to oxidise this substrate may therefore be characteristic of fungal laccases.

Table 1. Substrate specificity of mango laccase

Substrate	O_2 uptake (μl/min/mg protein)
Quinol	44.5
4-Methylcatechol	50.8
p-Cresol	0
Sodium ascorbate	16.4

Table 2. Laccase activity in secretion of various species of Anacardiaceae

	Total activity	Specific activity
Mangifera indica	730	44.5
Schinus molle	5130	1200
Pistacia palaestina	140	57
Pleiogynium timoriense	238	—

10 mM quinol substrate; total activity: μl O_2/min/ml secretion; specific activity: μl O_2/min/mg protein.

We compared the enzyme from the cut pedicels to that present in the flesh of the fruit. The latter behaved as a typical catechol oxidase, did not oxidize quinol and was inhibited 100% by CO, by 1 mM phenylhydrazine and by 1 mM diethyldithiocarbamate. The mango fruit contains two quite distinct enzymes, a laccase present in the secretory ducts, and a catechol oxidase in the other cells of the fruit tissue. The secretion from the cut fruit stalk originates entirely in the duct system (Joel, unpublished).

Secretory ducts are characteristic of the Anacardiaceae. We therefore tested the secretion from the ducts of other members of this family for laccase activity. All the species tested contained laccase activity. The enzyme was active towards the same substrates as the mango laccase, and did not oxidise *p*-cresol. Their response to inhibitors was also like that of mango laccase. *Schinus molle* had an exceptionally high activity (Table 2). Neither carbon monoxide nor phenylhydrazine reduced the total activity of the secretions towards methylcatechol, indicating the absence of catechol oxidase.

Since laccase is present only in the duct cavities and we found it in three different tribes on the family Anacardiaceae, this is apparently a special characteristic of the family and may be chemotaxonomic value.

EXPERIMENTAL

The source for enzyme were the crude content of ducts of mango fruit, *Mangifera indica*, ducts of the shoots of *Schinus molle* and of the inflorescences of *Pistacia palaestina*, and dry secretory droplets from the surface of shoots of *Pleiogynium timoriense*. Unripe mango fruits were plucked and the secretion from the cut pedicels collected in vials. In the case of *Schinus* the bark of the trunk and main branches was cut, the secretion collected, while in *Pistacia* it was collected from the stalk ducts by cutting fruit stalks. The crude secretions were diluted suitably with 0.1 M Pi buffer, pH 6.2. Enzyme activity was determined using a Clark type O_2 electrode [10], but without a gas phase above the soln. Substrates were used at the following concns: quinol 10 mM, 4-methylcatechol, *p*-cresol and Na ascorbate 5 mM. Inhibitors were added to the enzyme before the assay. CO from a cylinder was bubbled through the enzyme soln for 5 min prior to assay. Enzyme preps were made from the exocarp

of unripe mango fruit, after draining the duct content of the tissue, by blending fruit tissue in 0.1 M Pi buffer, pH 6.2 using 1 ml buffer/g fruit.

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PECTOLYTIC ENZYME ACTIVITY FROM *NICOTIANA TABACUM* POLLEN

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Key Word Index—*Nicotiana tabacum*; Solanaceae; pollen; pectolytic enzymes.

Abstract—Ungerminated pollen of *Nicotiana tabacum* contains a pectolytic enzyme which has its optimal activity between pH 5.5 and 6.5. Pectic lyase was not detected.

INTRODUCTION

Since the first light microscopic observations that pollen tubes grow within the pistil to the egg cells [1], it has been assumed that pollen tubes digest their way through the tissues by means of enzymes [2–5]. The results of electron microscopic studies of pollinated styles supported this view [6, 7]. Depending upon the plant species, pollen tubes grow in general in the intercellular substance of a stylar transmitting tissue or in a secretion product present in a stylar canal. Both materials consist mainly of carbohydrates which are related to complex pectic substances [8–10]. By means of labeling experiments, it has been shown that carbohydrate material of the secretion product of styles as well as of the intercellular material is used by growing pollen tubes for tube wall synthesis [9, 11, 12]. Up to now scant experimental evidence has been given for the presence of an enzyme system in pollen which digests pectin material [3, 13, 14].

The present study was undertaken to determine if pollen of *Nicotiana tabacum* contains enzymes which break down pectin.

RESULTS AND DISCUSSION

A crude, non-particulate preparation of *N. tabacum* pollen has been tested for pectolytic activity. When it was incubated with commercial citrus pectin, a decrease in viscosity of the reaction mixture as well as an increase in reducing groups released into the reaction mixture was observed over a 24 hr period (Fig. 1). The reducing groups released represent 1.2, 1.7 and 4.6% hydrolysis in 6, 12 and 24 hr respectively, based on the simplifying assumption that the units of pectin were anhydrogalacturonic acid. In the same time periods, the viscosity decreased 12, 26 and 42% respectively. There was no evidence of transeliminase activity in these reaction mixtures, since the samples did not show an increase in absorbance at 235 nm. The enzyme showed slightly lower rates of hydrolysis and viscosity decrease when it was incubated with polygalacturonic acid.

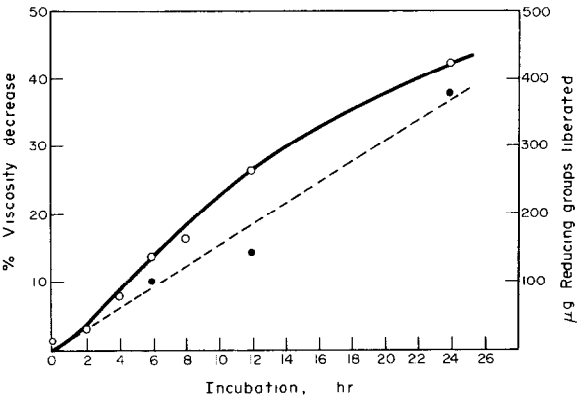

Fig. 1. Viscosity decrease (—○—) and liberation of reducing groups (—●—) of pectin by pollen enzyme. (The best straight line is drawn through the points for reducing groups).

The 6, 12 and 24 hr incubations of pectin and crude enzyme, after removal of protein by heat, were passed through Dowex 1-formate and the acidic components absorbed to the exchanger eluted with 0.1, 1 and 3 M

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