EFFECT OF PECTINASE ON THE PRODUCTION OF SESQUITERPENE LACTONES IN THE HAIRY ROOT CULTURE OF *LACTUCA VIROSA* L.

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ABSTRACT

Direct addition of pectinase as an elicitor to the hairy root culture of *Lactuca virosa* L. transformed with *Agrobacterium rhizogenes* stimulates the accumulation of constitutive sesquiterpene lactones – lactuside A and crepidiaside B. The highest amounts of lactuside A (80% above the control level) and crepidiaside B (39% above the control level) have been found 24 and 48 hours, respectively, after pectinase treatment.

KEY WORDS: *Lactuca virosa* L., Asteraceae, hairy root culture, *Agrobacterium rhizogenes*, pectinase, elicitor, lactuside A, crepidiaside B, sesquiterpene lactones.

INTRODUCTION

In a previous study (Malarz and Kisiel, 1999) we demonstrated that exogenous methyl jasmonate increased the constitutive concentrations of sesquiterpene lactones – lactuside A and crepidiaside B in a hairy root culture of *Lactuca virosa* L. (Asteraceae), suggesting that the compounds are involved in defense response.

In this study we report that production of the two sesquiterpene lactones can be stimulated by pectinase. This microbial pectinolytic enzyme has been mainly used to induce phytoalexin formation in suspended callus tissues and cell cultures of plants (Kurosaki et al. 1985; Brooks et al. 1986; Van der Heijden et al. 1988). Pectinase, directly added to the nutrient media of the cultures, mimics initial steps of plant-pathogen interaction. The enzyme causes damages of plant cell wall pectic polymers releasing oligosaccharides which serve as endogenous elicitors inducing defence response of the plant (Kurosaki et al. 1985). This response involves not only de novo production of phytoalexins, but also and increased accumulation of constitutive defensive metabolites present at lower levels in the unchallenged plant tissues (Eilert, 1987). In several recent studies, transformed root cultures have been used as a plant culture system to investigate changes in the secondary metabolite accumulation in response to different elicitors, including pectinolytic enzymes (Mukundan and Hjörtso, 1990; Rijhwani and Shanks, 1998; Sauerwein et al. 1991; Sevon et al. 1992).

MATERIALS AND METHODS

Plant material

Seeds of *Lactuca virosa* L., obtained from the Botanical Garden in Oberholz (Germany), were sterilized for 30s in 70% ethanol and then in 15% solution of Dimanin C (Bayer). The sterilized seeds were rinsed four times with sterile water and afterwards inoculated on a modified MS agar medium (Murashige and Skoog, 1962) without hormones, containing one-fourth of micro- and macroelements. Germination was conducted at 25°C under continuous illumination (40 $\mu\text{mol m}^{-1}\text{s}^{-1}$). Hairy roots of *L. virosa*, transformed with *Agrobacterium rhizogenes* LBA 9402, were induced on leaf explants from sterile grown seedlings and cultivated in a hormone-free MS liquid medium containing macroelements reduced to half concentration and 3% sucrose, on a gyrotory shaker (110 r.p.m.) in the dark.

Addition of pectinase

In the course of the first experiment, commercial pectinase (from *Aspergillus niger*, 8.1 units/mg, Sigma) was dissolved in water and added to the hormone-free MS liquid medium mentioned above, in quantities of 100, 300 and 600 units per liter of the medium.

In the course of the second experiment the enzyme (600 units/l) was added on day 6 after the last inoculation. The transformed roots were harvested 21 days (first experiment), and 24 and 48 hours (second experiment) after pectinase addition. In both experiments the medium without pectinase was

used as control. Enzyme solutions at various concentrations were sterilized by filtering through a Sartorius filter (0.22 μm , MinisartN).

Phytochemical analysis

Lyophilized hairy roots (0.25 g) were extracted twice with 12.5 ml of ethanol at room temperature. The extracts were filtered and concentrated in vacuo. The residue was dissolved in 1 ml of methanol and centrifuged (20000 r.p.m., 4°C, 5 min). The supernatant (5 μl) was injected into the Waters HPLC system. Analysis was performed on a μ -Bondapak C₁₈, 21 mm $\times$ 300 mm, column (Waters, No 86609), coupled with a UV photodiode array detector, using methanol-water (35:65) mixture as mobile phase at 0.5 ml/min flow rate. Each extraction was repeated three times, and each sample was injected three times.

Crepdiaside B and lactuside A (Fig. 1), isolated previously in our laboratory from *Lactuca* species (Kisiel et al. 1995), were used as authentic samples for co-chromatography. UV absorption maxima of crepdiaside B and lactuside A were 254 nm, Rt=9.6 min, and 235 nm, Rt=5.8 min, respectively.

crepdiaside B

lactuside A

Fig. 1. Chemical structures of the analysed sesquiterpene lactones (Glc= β -D-glucopyranosyl).

RESULTS AND DISCUSSION

The hairy root culture of *Lactuca virosa* L., transformed with *Agrobacterium rhizogenes* LBA 9402, was established in our laboratory and was found to possess the ability to produce sesquiterpene lactones, e.g. lactuside A and crepdiaside B (Fig. 1), characteristic for the roots of the intact plant collected in the first year of its vegetative period (Kisiel et al. 1995). We studied the effect of pectinase directly added to the nutrient medium of the hairy root culture of *L. virosa* on the content of the two sesquiterpene lactones in the plant biomass. The enzyme must be applied at the concentration sufficient to induce defence response. The concentration, however,

cannot lead to the loss of viability of the plant culture. Destruction of a large number of cells in the culture decreases growth indices (Rijhwani and Shanks 1998).

Therefore, in the course of the first experiment three different pectinase concentrations (100, 300 and 600 units per liter of medium) were tested and the hairy roots were harvested 21 days after addition of the enzyme to the culture medium. As shown in Fig. 2, growth indices (G.I.) of pectinase treated hairy roots (calculated as a ratio of the final dry weight of the culture to the dry weight of inoculum) were nearly the same as those of control. Moreover, no marked changes in biosynthetic ability of the culture were observed during the prolonged incubation.

The second experiment showed that treatment of the hairy root culture with 600 units/l pectinase caused increased accu-

Fig. 2. Growth indices (G.I.) of the hairy root culture of *Lactuca virosa* L. treated with pectinase (100, 300 and 600 units/l) during 21 days.

Fig. 3. Accumulation of lactuside A (a) and crepdiaside B (b) in the hairy root culture of *Lactuca virosa* L. treated with 600 units/l pectinase.

mulation of lactuside A and crepidiaside B by 24-48 hours. The content of lactuside A (Fig. 3a) increased by 80% above the control level 24 hours after addition of the enzyme to the culture medium and then, during the following 24 hours, decreased below the control value. In the case of crepidiaside B, the culture responded 48 hours after pectinase treatment and the amount of the compound accumulated after that time was 39% higher than that in control (Fig. 3b). The compounds were not detected in the culture medium. This was in contrast to the findings in *Capsicum annuum* root and suspended callus cultures, where the presence of capsidiol (phytoalexin) in the media was observed after treatment of the cultures with lytic enzymes (Chávez-Moctezuma et al. 1996; Brooks et al. 1986).

In our research (Malarz and Kisiel, 1999), exogenous methyl jasmonate markedly stimulated crepidiaside B accumulation (60% above the control level) in the hairy root culture of *Lactuca virosa* L. By contrast, the culture treated with pectinase produced significantly increased amount of lactuside A (80% above the control level). The time courses of accumulation of crepidiaside B and lactuside A in methyl jasmonate- and pectinase-treated hairy root cultures, respectively, reached maxima by 24 hours. The results support our earlier suggestion that the sesquiterpene lactones are involved in defense response.

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WPLYW PEKTYNAZY NA BIOSYNTEZĘ LAKTONÓW SESKWITERPENOWYCH W HODOWLI KORZENI WŁOŚNIKOWATYCH *LACTUCA VIROSA* L.

STRESZCZENIE

Dodatek pektynazy jako elicytora do kultury korzeni włośnikowatych *Lactuca virosa* L., transformowanych *Agrobacterium rhizogenes*, stymuluje akumulację konstytucyjnych laktonów seskwiterpenowych – laktuzydu A i krepidazydu B. Najwyższy wzrost zawartości laktuzydu A (80% w porównaniu z kontrolą) i krepidazydu B (39% w porównaniu z kontrolą) uzyskano odpowiednio po 24 i 48 godzinach elicytacji.

SŁOWA KLUCZOWE: *Lactuca virosa* L., Asteraceae, korzenie włośnikowate, *Agrobacterium rhizogenes*, elicytor, pektynaza, laktuzyd A, krepidazyd B, laktony seskwiterpenowe.