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Metabolism of Jasmonic Acid in *Pisolithus tinctorius* Cultures

By

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Summary

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The metabolism of the plant growth regulator jasmonic acid was studied in liquid cultures of *Pisolithus tinctorius* and *Laccaria laccata*. After 1 week of fermentation metabolites of jasmonic acid could be detected only in minute amounts in *Laccaria laccata*, whereas *P. tinctorius* converted 20 % of the jasmonic acid into 7-iso-cucurbic acid (14 %), 6-epi-7-iso-cucurbic acid (4 %) and cucurbic acid (2 %) by reducing the keto group of the jasmonic acid. The preferred configuration of the formed hydroxyl group is (6S). A possible role of this metabolic activity in mycorrhization steps was discussed. In the chloroform extract 24-methyllanosta-8,24(24')-diene-3,22-diol could be found as a characteristic compound of the fungus.

Introduction

Jasmonic acid (JA) is an ubiquitously occurring plant growth regulator (CREELMAN & MULLET 1997). In the last decade, JA or its methyl ester have been recognized to be a signal in plant defense mechanisms occurring upon wounding, herbivore attack or pathogen infection (WASTERNACK & PARTHIER 1997). JA and derived compounds named jasmonates could be found also in fungi (MIERSCH & al. 1993). In studying physiological effects of jasmonates, plants are fed usually with JA. However, in most cases possible metabolism of JA was neglected. In studying the effect of exogenously applied JA in mycorrhizal symbiosis, an inhibition of the mycelial growth of *Laccaria laccata* was observed. In *Pisolithus tinctorius*

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mycelium that inhibition was less pronounced (REGVAR & GOGALA, unpublished). Anomalies of spruce (*Picea abies*) roots after JA treatment were seen using scanning electron microscopy, only when the plants were not mycorrhized with *P. tinctorius*. This observation led to the speculation that JA is formed by mycorrhizal fungi or metabolized by them (REGVAR & GOGALA 1995, 1996). First inspection of *L. laccata* and *P. tinctorius* revealed, that both are able to biosynthesize JA. The plant growth regulator could be identified in both the mycelium and the culture medium. In studying mycorrhization the JA content was found to alter within the plant (*Picea abies*) (REGVAR & al. 1998). Furthermore, preliminary experiments revealed that the activity to metabolize JA were different for *P. tinctorius* and *L. laccata*. Therefore, it was of interest to study the metabolic potencies of both fungi in more detail. In the present study we have shown that *P. tinctorius* is able to metabolize JA into cucurbic acid isomers which were isolated and structurally elucidated.

Materials and Methods

Mycorrhizal fungi *Pisolithus tinctorius*, DB 49, isolated in France and *Laccaria laccata*, DB 28, isolated in the SSSR, recultured on PDA at 24°C for 2 weeks. Three disks (diameter 0.7 cm) were used for inoculation of 50 ml medium according to (SHEMAHANOVA 1960) in 150 ml flasks. Media were supplemented with 5 µM JA and adjusted to pH 6.3. The cultures were grown in the dark at 1500 rpm and 24°C for 1 week. 2 l of culture solution were filtered and the filtrate extracted at acidic pH with chloroform (3 x 50 ml) and the combined extracts were evaporated to dryness. TLC on silica gel of 1/100 of the extract was done according to MIERSCH & al. 1987. Subsequently, 1/100 of the extract was acetylated with a mixture of pyridine/acetic acid anhydride (2:1) and 1/100 extract methylated with ethereal diazomethane before TLC. The remaining extract was separated on 6 ml DEAE-Sephadex A25 according to KRAMELL & al. 1997 the acidic fraction further purified on LiChrolut RP-18 (500 ng, Merck) with an increasing gradient of methanol containing 0.2 % acetic acid in water. Fractions containing 20 %, 30 %, 40 %, 50 %, 60 % methanol were collected separately, and were evaporated, methylated and analyzed by GC-MS according to KRAMELL & al. 1997. The steroid-compound was separated from the neutral fraction after separation on DEAE-Sephadex A25 by TLC on silica gel 60 (Merck) with the solvent system n-hexane - ethyl acetate - acetic acid (60:40:1) before GC-MS-analysis. Isolated compounds were compared with authentic substances using TLC and GC-MS.

Results and Discussion

Jasmonic acid (JA) was applied to liquid cultures of *Pisolithus tinctorius* and *Laccaria laccata*. After 1 week the culture filtrate was extracted and inspected on possible metabolites of JA by thin layer chromatography (TLC). In the culture filtrate of *L. laccata* only minute amounts of possible metabolites were found indicating a lack of JA metabolism, on the other hand, in the chloroform extract of *P. tinctorius* 4 main substances were detected by TLC analysis, 3 of them could be found only upon JA treatment. Acetylation procedures, methylation and subsequent TLC analysis revealed that 3 of the compounds contain carboxyl groups and all of them possess hydroxyl groups. Compounds were purified by chromatographic

methods, were methylated and subsequently analyzed by gas chromatography-mass spectrometry (GC-MS). In addition, authentic compounds were used for identification.

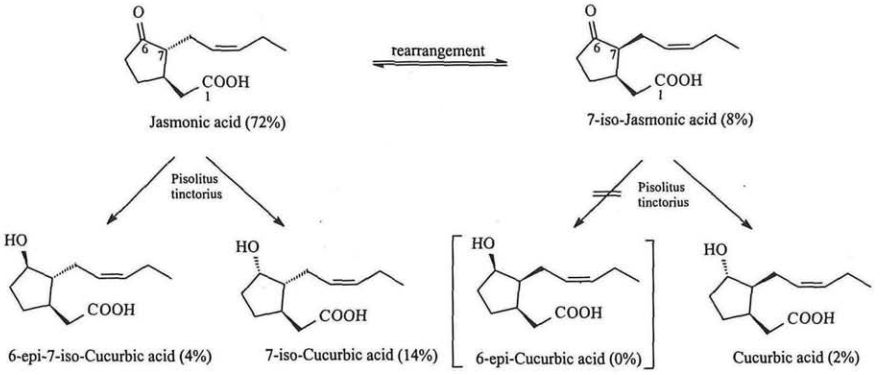


Fig. 1. Metabolism of jasmonic acid by *Pisolithus tinctorius* and jasmonates (quantities in parentheses) isolated from the culture filtrate after 1 week of fermentation at 24° C. 6-epi-Cucurbic acid was not detected.

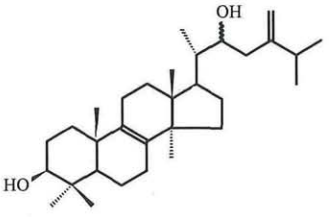
The 3 acidic compounds were identified as 7-iso-cucurbic acid, 6-epi-7-iso-cucurbic acid and cucurbic acid (CA). As shown in Fig. 1, the cucurbic acid isomers are products of JA. Synthetic JA consists of an isomeric mixture of about 90 % JA and 10 % 7-iso-JA. Both isomers were metabolized up to 20 % during cultivation described. 7-iso-cucurbic acid and 6-epi-7-iso-cucurbic acid are metabolites of JA. Cucurbic acid (CA) is formed from 7-iso-JA. The metabolite profile indicates that the fungus was able to reduce the keto group of JA, leading to the (6S)-isomer as the preferred configuration of the hydroxyl group in the main product 7-iso-CA and the minor product CA.

The experiment was not done with radioactive-labeled JA. Therefore, the found metabolites may be products of both endogenous JA and exogenously applied JA. Independent of the origin of JA the ability to metabolize JA was shown.

Cucurbic acid isomers are natural constituents of various higher plants and fungi. 7-iso-CA was found recently in *Fusarium oxysporum* (MIERSCH & al. 1999a) and conjugated in *Pinus* pollen (KNÖFEL & SEMBDNER 1995).

Among the cucurbic acid isomers exhibiting biological activity only 6-epi-CA was found to be active in various assays on plant growth or induction of genes (MIERSCH & al. 1999b). However, this isomer was undetectable in the culture filtrate of *P. tinctorius*. Therefore, it is tempting to speculate that metabolic conversion of JA into cucurbic acid isomers represents a type of inactivation of JA known to be a signal of plant response to biotic or abiotic stress (WASTERNACK & PARTHIER 1997). If *P. tinctorius* is able to metabolize JA in a plant root, CA should be formed predominantly, since the natural occurring JA is the 7-iso-JA (cf.

Fig. 1). It will be interesting to analyze wether inactivation of JA is of advantage for the plant within the process of mycorrhization. In addition, JA can occur in root exudates as shown for wheat seedlings (DATHE & al. 1994). *P. tincotrius* could metabolize JA in the rhizosphere and therefore influence the process of mycorrhization. The weak inhibition of mycelial growth corresponds with a lower susceptibility of *P. tinctorius* to applied JA.



24-Methyllanosta-8,24(24')-diene-3,22-diol

Fig. 2. Fungal metabolite produced by Pisolithus tinctorius without relation tojasmonates.

Under the culture conditions used *P. tinctorius* released a neutral compound into the culture medium. After isolation and purification procedures this compound could be identified by GC-MS and the spectra data base to be 24-methyllanosta-8,24(24')-diene-3,22-diol (cf. Fig. 2). Authentic substance verified the chemical structure found. The „lanostandiol" was identical to a recently described endogenous metabolite of the fungus (BAUMERT & al. 1997).

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