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Effect of Organic Acids on Production of Auxin-Like Substances by Ectomycorrhizal Fungi

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Abstract

The present study shows that organic acids affect the production of auxin-like substances (ALS) by mycorrhizal fungi growing in L-tryptophan-supplemented media. Fumaric acid decreased the production of these substances in *Rhizopogon luteolus*. Pyruvic acid and α-ketoglutaric acid inhibited their production completely. These organic acids also inhibited the production of ALS in *Amanita muscaria*. On the other hand, pyruvic acid markedly enhanced the production of ALS in *Suillus bovinus*. It is shown that the fungi studied produced IAA.

Keywords: Ectomycorrhizal fungi, organic acids, auxin-like substances

Abbreviations: ALS = auxin-like substances; IAA = 3-indole-acetic acid

1. Introduction

There is general agreement that forest trees gain many advantages from mycorrhizal associations. Detailed data concerning the effects of mycorrhizal fungi on trees have been published by numerous authors (Harley, 1948; Marx, 1972; Bowen, 1973; Slankis, 1974).

In the root zone both mycorrhizal fungi and other microorganisms are first of all affected by root exudates (Katznelson, 1965; Rovira, 1965; Rambelli, 1973). The principal constituents of root exudates are amino- and organic acids, sugars, vitamins, plant growth substances and enzymes. Similar substances are also excreted by non-mycorrhizal microorganisms associated with the roots of forest trees (Kampert and Strzelczyk, 1984, Strzelczyk and Pokojska-Burdziej, 1984; Ró·zycki and Strzelczyk, 1985, 1986, Strzelczyk and Ró·zycki, 1985). The effects of these on growth of mycorrhizal fungi and mycorrhiza formation also seem to be of ecological importance (Davey, 1971; Rambelli, 1973; Slankis, 1973).

Organic substances in root exudates are undoubtedly one of the main causes for the greater development of microorganisms in the vicinity of roots. Organic acids form a substantial part of root exudates (Slankis, Runeckles and Krotkov, 1964; Rovira, 1969; Smith, 1969, 1976) and are also released by microorganisms into the medium (Ró·zycki, 1985).

According to Barea (1986) carbon compounds released from plant roots seem to affect the establishment of mycorrhiza associations by acting on some phases of the pre-infection stage. It cannot be excluded that organic substances, and among them organic acides, available at the root-soil interface affect not only growth but also the physiology of organisms occurring in this habitat. The possible effect of these substances on production of plant growth regulators by mycorrhizal fungi could be of importance in the establishment and functioning of mycorrhizae. This paper reports the results of examining ectomycorrhizal fungi of pine for their production of ALS in the presence of some organic acids.

2. Materials and Methods

Organisms, media and culture conditions

Four mycorrhizal fungi of pine (*Amanita muscaria*, *Suillus bovinus* No. 7, *Suillus bovinus* No. 12 and *Rhizopogon luteolus*) were used.

The fungi were grown in triplicate in the Melin and Rama Das (1954) medium supplemented with fumaric, pyruvic or α-ketoglutaric acid (400 mg C/l). Auxin production was studied in tryptophan-free and L-tryptophan supplemented media. The L-tryptophan (0.2 g/l) was filter sterilized (Millipore, 0.2 μm pore size) and the pH of media adjusted to 5.4.

The media (200 ml aliquots in 1000 ml Erlenmeyer flasks) were inoculated with 2 discs (1 cm in diameter) of the fungi grown for 14 days at 26°C on Potato Dextrose Agar (Difco).

After 21 days of incubation at 26°C the mycelium was separated from the medium by filtration on filter paper and dried to constant weight at 90°C.

Extraction of auxin-like substances (ALS)

The post culture liquids were acidified to pH 2–3 with 1 M HCl and extracted twice with 75 ml peroxide-free ethyl ether. The ether fractions were evaporated to dryness at 40–45°C. The residues were eluted with 5 ml of 0.5 M phosphate buffer (pH 8.0) and shaken during 2–3 hr with 1–2 g of Polyclar AT (water-insoluble polyvinylpyrrolidone for binding phenols, Serva) in 50 ml of 0.1 M phosphate buffer (pH 8.0). Subsequently the suspensions were filtered, acidified to pH 2–3 and subjected to a further double extraction with ether. After evaporation of the ether fractions, the residues were eluted with 2 ml of methanol.

Chromatograpy and bioassay of auxins

Aliquots of 0.2 ml of the methanolic solution obtained from the cultures grown without tryptophan (equivalent to 20 ml of the medium) and 0.1 ml aliquots of the solution obtained from cultures grown with tryptophan were applied to Whatman's No. 3 filter paper for partition chromatography. The descending method was used with the solvent system: isopropanol, ammonia, water (10:1:1 v/v). The chromatograms were developed in darkness up to a length of 25 cm. They were then dried overnight in a stream of air and each part was eluted with 4 ml of 2% saccharose solution in 0.001 M phosphate buffer (pH 6.3). The auxins in the eluates were detected by the Avena coleoptile test (Nitsch and Nitsch, 1956).

Cochromatography with authentic IAA (indoleacetic acid, Fischer Scientific Co., USA) was also performed.

The amount of ALS produced was calculated from a standard dose curve prepared for pure IAA and finally expressed as IAA equivalents per 1 g of dry weight of mycelium. Significant differences were established by estimating the least significant difference (LSD) at $P = 0.05$.

Additional chromatograms were examined in the daylight and under UV light after spraying with the Salkowski reagent.

The auxins in the extracts of *Suillus bovinus* No. 12 and *Rhizopogon luteolus* obtained from isolates grown with tryptophan without organic acids and in the presence of fumaric and pyruvic acids also were detected by means of gas chromatography. The samples were methylated with diazomethane and injected into Chromatron GCHF 18.3–4 gas chromatograph (GDR) using a glass column (200 cm×0.4 cm) packed with 5% SE-30 on Gas Chrom

Q, 100–120 mesh. The column temperature of 300°C and detector temperature (FID) of 300°C were used. The carrier gas was N_2, at a flow rate of 40 cm^3/min. Auxins contained in the samples were also spiked with pure IAA and analysed by gas chromatography.

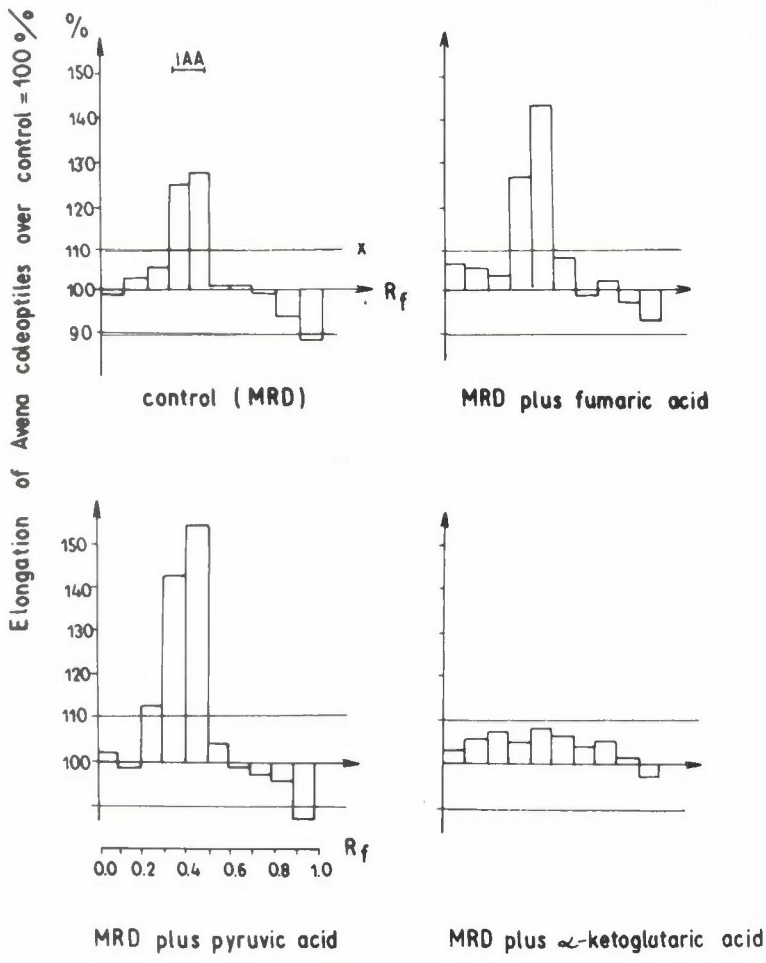

Figure 1. Chromatographic analysis of ALS produced by *Suillus bovinus* No. 12 in media without tryptophan.
Explanations to Figs. 1–3: MRD — Melin-Rama Das medium, the portions above the line × indicate significant differences at P=0.05.

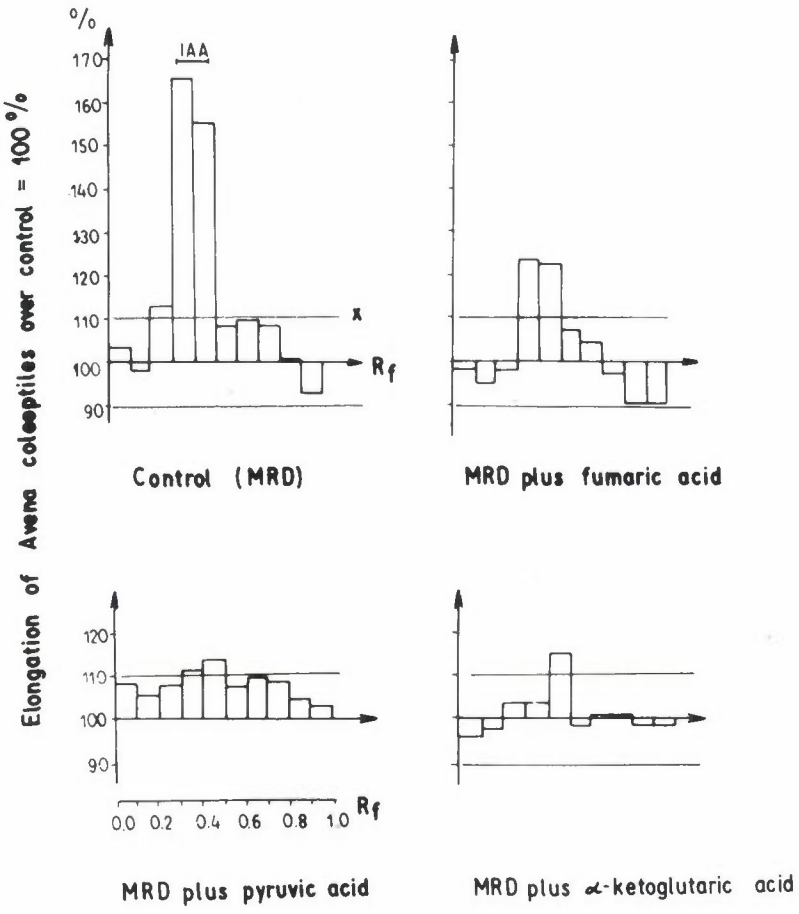

Figure 2. Chromatographic analysis of ALS produced by *Amanita muscaria* in media with tryptophan.

3. Results

The results obtained in this work are presented in Table 1 and Figs. 1–4. It appears from the data that in the control medium (without organic acids) without tryptophan, trace amounts of auxins were produced only by both *Suillus bovinus* isolates. However in the presence of tryptophan all isolates produced ALS. *Rhizopogon luteolus* exhibited the highest activity of ALS.

In tryptophan-free media with organic acids only the isolates of *S. bovinus* produced ALS, located on the chromatograms at R_f 0.3–0.5. In the presence of fumaric and especially pyruvic acid an increased synthesis of these com-

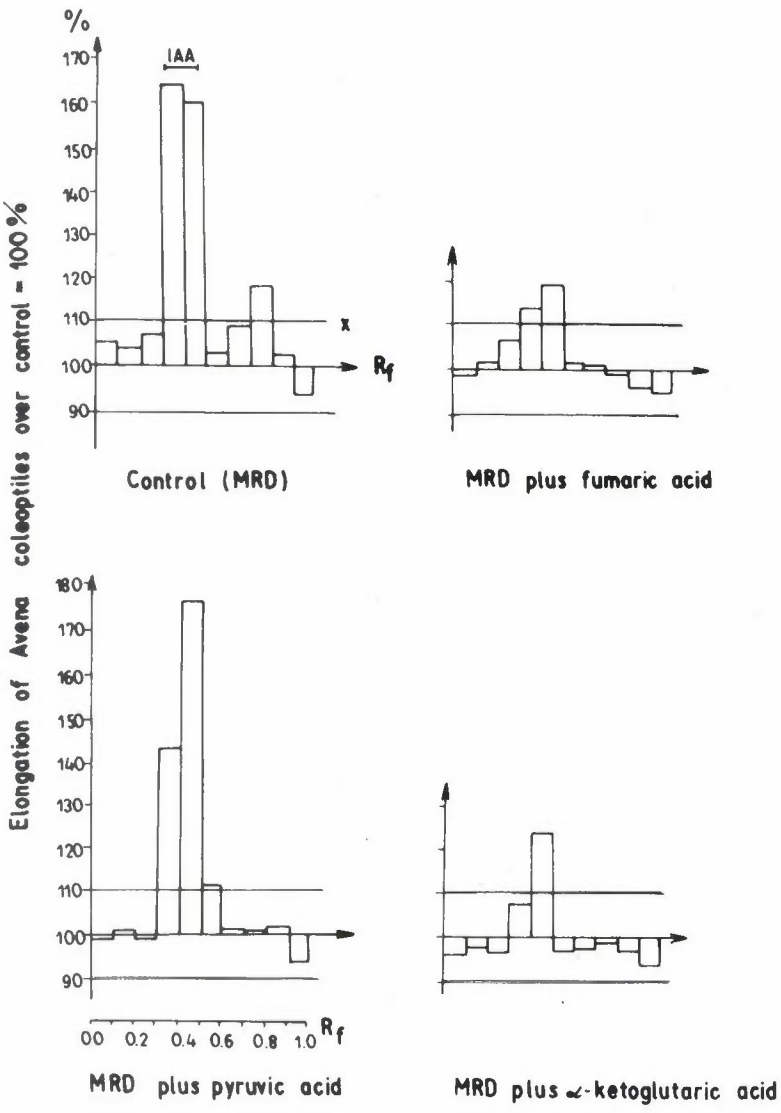

Figure 3. Chromatographic analysis of ALS produced by *Suillus bovinus* No. 7 in media with tryptophan.

pounds was observed in *S. bovinus* No. 12 (Fig. 1). A reverse phenomenon occurred with α-ketoglutaric acid.

The organic acids affected ALS production by the mycorrhizal fungi grown with L-tryptophan. In *R. luteolus* fumeric acid decreased the production of these substances several fold (Table 1) and pyruvic and α-ketoglutaric

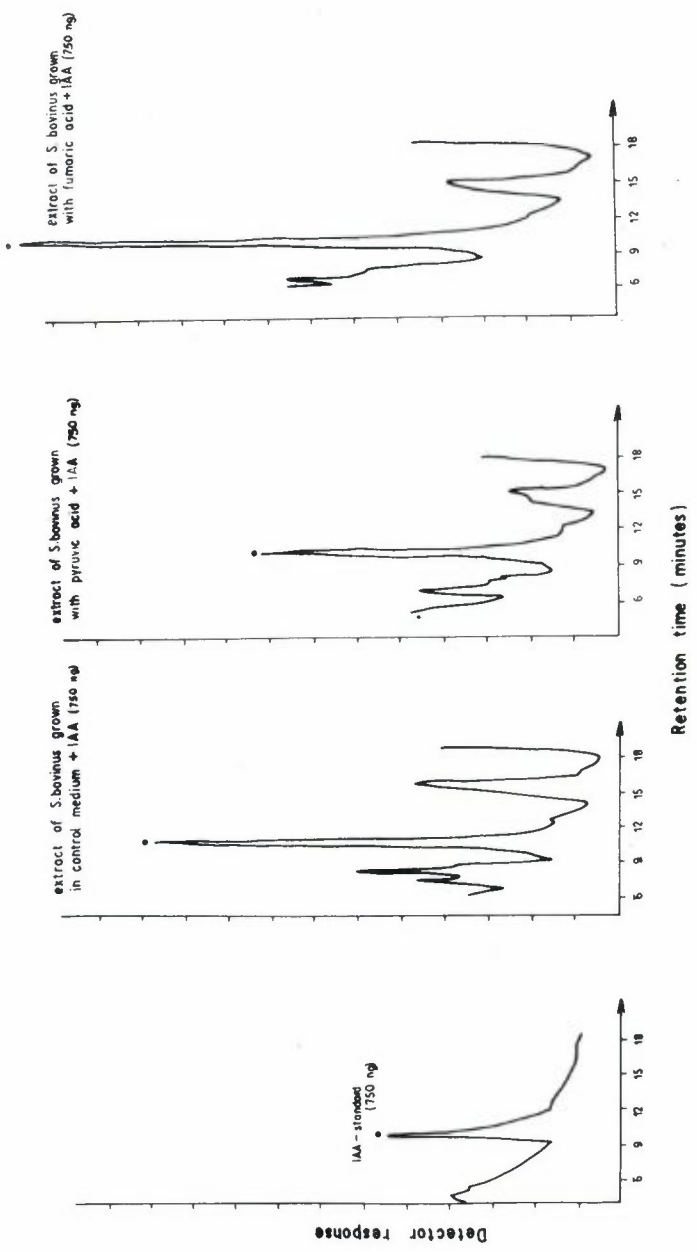

Figure 4. Gas chromatographic analysis of ALS in *Suillus bovinus* No. 12 grown in different media with tryptophan.

Table 1. ALS production by mycorrhizal fungi in Melin-Rama Das medium with organic acids

Fungi	Medium without tryptophan		Medium with tryptophan	
	Amount of ALS expressed as IAA equivalents			
	μg/g of dry weight	R_f	μg/g of dry weight	R_f
	Control			
Amanita muscaria	0	–	56.2	0.2–0.5
Suillus bovinus No. 7	trace	0.3–0.4	33.1	0.3–0.5 0.7–0.8
Suillus bovinus No. 12	5.1	0.3–0.5	37.2	0.2–0.5
Rhizopogon luteolus	0	–	78.5	0.2–0.4 0.5–0.7
	Fumaric acid			
Amanita muscaria	0	–	29.5	0.3–0.5
Suillus bovinus No. 7	trace	0.4–0.5	17.7	0.3–0.5
Suillus bovinus No. 12	17.4	0.3–0.5	100.4	0.2–0.5
Rhizopogon luteolus	0	–	18.7	0.3–0.5
	Pyruvic acid			
Amanita muscaria	0	–	5.5	0.3–0.5
Suillus bovinus No. 7	trace	0.4–0.5	121.5	0.3–0.5
Suillus bovinus No. 12	32.7	0.2–0.5	79.8	0.3–0.5
Rhizopogon luteolus	0	–	0	–
	α-ketoglutaric acid			
Amanita muscaria	0	–	3.8	0.4–0.5
Suillus bovinus No. 7	trace	0.4–0.5	17.7	0.4–0.5
Suillus bovinus No. 12	0	–	19.7	0.3–0.5
Rhizopogon luteolus	trace	0.4–0.5	0	–

acid inhibited this process completely. It should be mentioned however that *R. luteolus* produced the best growth in all media and excreted large amounts of dark pigment. All three organic acids retarded also the production of ALS in *A. muscaria* (Fig. 2).

Among the organic acids used, α-ketoglutaric acid exhibited the strongest inhibitory effect in the fungi studies (Figs. 1–3). In general fumaric acid was also inhibitory. On the other hand, pyruvic acid markedly enhanced the production of ALS in *S. bovinus* No. 7 and No. 12 (Table 1).

One substance active in the Avena coleoptile test with R_f value 0.3–0.5 was extracted from the culture liquid of all the fungi. Comparative experiments with pure IAA suggested this to be IAA and this was confirmed by the gas chromatography analyses (Fig. 4).

The post culture liquids of *A. muscaria* grown with fumaric acid and of *S. bovinus* No. 12 grown with pyruvic acid contained substances inhibiting the Avena coleoptile test (Fig. 1). These not closer identified compounds were located on the chromatograms at R_f 0.8–1.0.

4. Discussion

Plant growth regulators are considered to play a key role in the establishment and functioning of mycorrhizae in forest trees (Slankis, 1973; Meyer, 1974; Tomaszewski and Wojciechowska, 1974). Among these substances auxins and cytokinins are of special interest. Auxins were found long ago to be of importance in plant-mycorrhiza relationships. They are known to affect root morphology (stunting, dichotomy), stimulate the synthesis of nucleic acids, proteins, cellulolytic and pectolytic enzymes and cell division. They may also be responsible for translocation of soluble sugar to mycorrhizal roots (Haselwandter, 1973; Slankis, 1973; Meyer, 1974; Tomaszewski and Wojciechowska, 1974). The ability of ectomycorrhizal fungi to synthesize auxins if tryptophan is provided has been established by numerous workers (Moser, 1959; Ulrich, 1969; Strzelczyk et al., 1977; Strzelczyk and Pokojska-Burdziej, 1984). Tryptophan is however not a common constituent of root exudates of crop plants (Rovira, 1965) and forest trees (Smith, 1976, 1977). It may be assumed therefore that microorganisms releasing tryptophan into the environment are providing the auxin producing organisms with this precursor. It has in fact been reported that certain ectomycorrhizal fungi are capable of synthesizing auxins from tryptophan precursors (Moser, 1959; Haselwandter, 1973).

The quality and quantity of organic substances excreted by roots is affected by different ecological factors (Rovira, 1959; Rovira and Harris, 1961; Bowen, 1969). The same factors affect the exudation of different organic substances by microorganisms (Lee et al., 1970; Pokojska-Burdziej, 1981; Kampert and Strzelczyk, 1984). Certain compounds in the root exudates and those excreted by microorganisms living in the root zone are likely to affect mycorrhizal fungi (Malyshkin, 1955; Barea, 1986).

Organic acis constitute a significant part of root exudates of forest trees (Smith, 1969, 1976). They occur in the exudates of different *Pinus* species in amounts of 46.2–382.2 μg plant/week (Smith, 1977), and are released in larger amounts than other organic substances like sugars or amino acids (Smith, 1977). Organic acids play an important role in cell metabolism and affect rhizosphere pH and microbial activity (Curl and Truelove, 1986).

On the basis of the results obtained in this work it can be assumed that organic acids affect important processes in mycorrhizal fungi — the production of auxins. The mechanism of such an action requires further studies in which other organic acids should also be considered.

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