Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizosphere of pine (*Pinus silvestris* L.)*

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Received 2 December 1983. Revised March 1984

Key words Auxins Gibberellin-like substances *Pinus silvestris* Mycorrhizosphere flora Mycorrhizal fungi

Summary Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from the mycorrhizosphere of Scots pine was studied. Chromatography and bioassays were used.

Most of the organisms required tryptophan for auxins production. The highest biological activity exhibited substances located at Rf 0.2–0.4.

The organisms produced minute amounts of gibberellin-like substances which appeared at different Rf values. It was stated that auxins production is much more common among the root zone organisms of pine than the production of gibberellin-like substances.

Introduction

It is well known that microorganisms exert marked influence on growth of plants and are themselves responsive to the environmental conditions imposed by the plant root12,21. Results from controlled experiments have indicated either stimulation or inhibition of plant growth by microorganisms3,8,21. The mechanisms of such action are little understood. However recognition of the dependence of plants upon growth regulators for growth and development has led to suggestions that they might benefit from external supply of such substances.

Bacteria are known to affect morphology of plant roots in the same way as gibberellic acid and indoleacetic acid (IAA)3. It has been stated that epiphytic bacteria may increase the IAA content in plants14,16. The direct uptake by plants of IAA produced by bacteria has been also observed15.

Plant growth regulators may affect not only the plant but they are also of importance in mycorrhizal relationships. They seem to play

*This research was carried out under problem MR.II.16 coordinated by the Institute of Dendrology, Polish Academy of Sciences.
a key role in the establishment of mycorrhizas in forest trees\textsuperscript{19,23,28}.

Despite the important role that external plant growth regulators seem to play in forest trees, surprisingly little work has been done on the synthesis of these substances by the root zone microorganisms of these trees other than mycorrhizal fungi\textsuperscript{9,10,11}. To our knowledge nothing is known about production of these compounds by microorganisms of the mycorrhizosphere (surface of mycorrhizae). Most studies concerning this problem were performed with the rhizosphere or epiphytic organisms of cultivated plants\textsuperscript{1,3,4,7}.

This paper presents results of a study conducted to determine the relation between plant growth regulators, plants and associated microorganisms.

Materials and methods

Microorganisms

Five mycorrhizal fungi (\textit{Amanita muscaria}, \textit{Paxillus involutus}, \textit{Suillus luteus}, \textit{S. bovinus}, \textit{Rhizopogon luteolus}), 10 bacterial isolates (5 obtained from soil and 5 from the mycorrhizosphere) and 7 actinomycetes isolated from the mycorrhizosphere were used in our studies. Only two bacterial isolates from soil were spore-formers belonging to the species \textit{Bacillus circulans}, the remaining were pleomorphic types resembling \textit{Arthrobacter} sp. All the actinomycetes belonged to the genus \textit{Streptomyces}.

Culturing

The mycorrhizal fungi were grown in modified Melin-Rama Das\textsuperscript{18} medium of the following composition: glucose 20.0 g, ammonium tartrate 0.5 g, K\textsubscript{2}HPO\textsubscript{4} 1.0 g, MgSO\textsubscript{4}.7H\textsubscript{2}O 0.5 g, Malt Extract Broth (Oxoid) 5.0 g, ferrous citrate 0.5 ml of 1\% aqueous solution, zinc sulphate 0.5 ml of a 1:500 aqueous solution, thiamin 100 \mu g, biotin 5 \mu g, pyridoxine 100 \mu g, nicotinic acid amide 0.5 mg, H\textsubscript{2}O dist. 1 l, pH 5.8. The media (300 ml in 1 l erlenmeyer flasks) were inoculated with 3 discs (1 cm \phi) of fungi grown for 14–21 days at 25\degree C. The mycelium was separated from the medium by filtration through filter paper.

Bacteria were grown in modified 'B' medium (Lochhead and Chase\textsuperscript{17}) composed of: glucose 5.0 g; K\textsubscript{2}HPO\textsubscript{4} 1.0 g; NH\textsubscript{4}NO\textsubscript{3} 0.4 g; MgSO\textsubscript{4}.7H\textsubscript{2}O 0.4 g; CaCl\textsubscript{2} 0.2 g; NaCl 0.1 g; FeCl\textsubscript{3} trace, H\textsubscript{2}O dist. 1 l; pH 6.8–7.2. The media (300 ml in 1 l flasks) were inoculated with 2 ml of bacterial suspensions prepared from 72 h old slant cultures washed off with 5 ml of sterile water. The bacteria were separated from the medium by centrifugation.

Actinomycetes were grown in the medium of Shirling and Gottlieb\textsuperscript{22} and 300 ml portions of this medium (in 11 Erlenmeyer flasks) were inoculated with 2 ml of a suspension obtained by washing off from a 7 days old slant cultures. After 7 days of growth at 26\degree C the actinomycetes were separated from the medium by filtration on filter paper.

Auxins production was studied in tryptophan-free and L-tryptophan (0.2 g/l, filter sterilized Millipore, 0.2 \mu m pore size) supplemented media.

Extraction of auxins and gibberellin like substances

The post culture liquids obtained from cultures grown without tryptophan were acidified to pH 2.0–3.0 with 1\% HCl and extracted twice with 100 ml of peroxide free ethyl ether. The ether extracts were evaporated at 40–45\degree C to dryness, the residue was dissolved in 2 ml of methanol and used for determination of auxins.

The post culture liquids obtained from cultures grown with tryptophan were additionally extracted twice with 100 ml of ethyl acetate. The combined ether-ethyl acetate fractions were evaporated at 40–45\degree C to dryness in vacuo and the residue was dissolved in 2 ml of methanol. The amount of auxins and gibberellin like substances was determined in such methanolic solutions.