Neth. J. Pl. Path. 79 (1973) 282–284 SHORT COMMUNICATION

Extraction, purification and identification of 3-indoleacetic acid (IAA) from culture filtrates of Pythium sylvaticum

A. C. POSTHUMUS

Centre for Plant Physiological Research (CPO), Wageningen

Accepted 5 October 1973

In addition to the evidence obtained by Blok (1973) for the presence of a growth-regulating substance in culturefi ltrates of *Pythium sylvaticum* Campbell & Hendrix, a physical and chemical approach was used to investigate the production of 3-indoleacetic acid (IAA) by this fungus.

Culture filtrates of *Pythium sylvaticum* of 12 days old shake cultures in Czapek-Dox solution were prepared as described by Blok (1973). The filtrates were concentrated and partitioned twice with equal volumes of diethyl ether at pH 8 to eliminate fats and waxes and subsequently twice with equal volumes of ether after acidification with orthophosphoric acid to pH 3. The acid ether fractions were collected, concentrated under reduced pressure and partitioned twice with equal volumes of a 5% sodium bicarbonate solution. These solutions were collected, acidified to pH 3 and extracted twice with ether. The last ether fractions were combined, dried over anhydrous sodium sulphate and evaporated to dryness. The residues were solved in a small volume of 96% ethanol and applied as a streak on a rolled sheet of filter paper (Whatman No. 1) according to the method of Posthumus (1967). The chromatograms were developed by ascending chromatography in isopropanol:ammonia:water (10:1:1 v/v/v) during 15 hours. After drying the chromatograms were scanned under u.v. irradiation (254 nm) to localize the IAA reference spots and to check the IAA regions on fluorescence. Strips of 3 cm in width with a Rf value equal to that of IAA were cut out of the chromatograms, eluted during 30 hours with 96% ethanol and the eluates were concentrated and rechromatographed on Whatman No. 1 paper in ethylacetate:isopropanol:ammonia (45:35:20 v/v/v) during 15 hours. If large volumes (circa 500 ml) of culture filtrate were used, the residues were chromatographed twice in the two different solvents to ascertain a sufficient purity of the IAA samples.

The chromatograms finally obtained, showed clear fluorescent bands with Rf values similar to that of IAA, and some other vague bands. Blok (1973) found that in strips cut from these chromatograms biological activity in the flax root bio-assay was only present in zones with an Rf value equal to or slightly higher than that of IAA. Physical and chemical characterization of the active compound was achieved subsequently. The ethanolic eluates of the active bands of the ultimate chromatograms contained a rather pure sample of IAA, as was demonstrated by the combination of fluorescence characteristics, u.v. absorption spectrum and the colour reaction with the Salkowski reagent. As to the fluorescence characteristics, excitation and emission spectra were

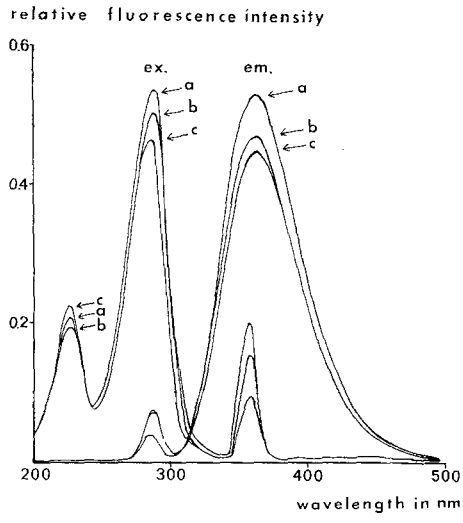

Fig. 1. Excitation (ex.) and emission (em.) spectra of synthetic IAA (curve c) and of two purified IAA samples of culture filtrates of *P. sylvaticum* (curves a and b) in 0.01 M phosphate buffer pH 5.9.

Fig. 1. Excitatie- en emissiespectra (ex. en em.) van synthetisch IAZ (curve c) en van twee gezuiverde IAZ-preparaten van cultuurfiltraten van P. sylvaticum (curven a en b) in 0,01 M fosfaatbuffer pH 5,9.

determined in 0.01 M phosphate buffer pH 5.9. As can be seen in Fig. 1 (curve c), the excitation maximum for IAA is 285 nm, while a lower peak is present at 225 nm, and the emission maximum is 355 nm. The given spectra of the eluates of the chromatograms of two culture filtrates (Fig. 1, curves a and b) are in good agreement with the postulated presence of IAA. The u.v. absorption spectra of the eluates showing a maximum at 280 nm and the positive colour reaction with the reagent of Salkowski according to the method of Gordon and Weber (1951) were in accordance herewith. Based on fluorimetric measurements several hundreds of μg's of IAA per liter of culture filtrate were found. In conclusion it may be stated that the combination of data mentioned above makes it clear that IAA is produced by *Pythium sylvaticum*.

Samenvatting

Extractie, zuivering en identificatie van 3-indolylazijnzuur (IAZ) uit cultuurfiltraten van Pythium sylvaticum

De aanwezigheid van 3-indolylazijnzuur (IAZ) in cultuurfiltraten van *Phythium sylvaticum* werd aangetoond. Hiertoe werden geconcentreerde cultuurfiltraten geextraheerd met ether en de IAZ bevattende fracties door middel van herhaalde papierchromatografie verder gezuiverd. Na elutie van de in Rf-waarde met IAZ overeenkomende stroken van de laatste chromatogrammen, werden voor deze eluaten dezelfde fluorescentiekarakteristieken (Fig. 1), U.V. absorptiespectrum en kleurreactie met Salkowski's reagens gevonden als voor IAZ.

Acknowledgments

I wish to thank Miss Ida Blok for providing the culture filtrates of *Phytium sylvaticum* and for carrying out the bio-assays on chromatogram segments. Also the competent technical assistance of Mr G. W. H. Laurens is gratefully acknowledged.

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Address

Centrum voor Plantenfysiologisch Onderzoek (CPO), Bornsesteeg 47, Wageningen, the Netherlands.