STIMULATION OF SCLEROTIAL GERMINATION OF SCLEROTIUM CEPIVORUM BY HOST-PLANT EXTRACT

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SUMMARY

Extract from onion bulbs and diffusate from roots of onion seedlings were fractionated by column chromatography. The stimulatory effects of the different fractions of onion extract on sclerotial germination of Sclerotium cepivorum were studied. The sugar fraction was the most stimulatory, whereas, the amino acid fraction was not effective. Paper chromatographic analysis revealed the presence of glucose, fructose and no amino acids in the root diffusate. These two sugars and 13 amino acids were identified in the onion extract. When various sugars and amino acids were supplied individually to autoclaved soil, only glucose, fructose, mannose and maltose effectively induced sclerotial germination. Partial stimulation occurred in nonsterile soil amended with high glucose concentrations.

Studies on the antibiotic effect of the different fractions against some soil fungi by the spore germination method showed that, the sugar fraction inhibits completely the spore germination of all the fungi, tested, whereas, the amino acid fraction was non-inhibitory. Both fractions did not show antibiotic activity when tested by the filter paper disc method.

Attempts to extract inhibitory substances from soil which inhibit sclerotial germination were unsuccessful.

It was suggested that onion extract plays a twofold role in stimulating sclerotial germination in natural soil: (a) a direct nutritional influence; (b) an antibiotic effect on soil mycoflora which reduces competition for nutrients.

INTRODUCTION

Most natural soils are known to inhibit spore germination and mycelial growth of many pathogenic fungi \(^7\ ^8\ ^9\ ^{10}\). Despite a great deal of attention, there is no clear explanation of this phenomenon, \textit{i.e.} soil fungistasis, but there is little doubt of its widespread occurrence and significance in the survival of fungi in soil.

Sclerotia of Sclerotium cepivorum Berk., the causal organism of
white rot in onions, can remain viable in natural soil for some
years. They are specifically stimulated to germinate in nonsterile
soil by the presence of host plants or by their water extracts. The
cause of the specific reversal of soil fungistatic effect on sclerotial
ermination by host plants, or their extracts, is still not fully under-
stood. It has been suggested that they may exert their influence
through an antibiotic effect on the soil microflora, however no
convincing evidence has been presented to support this hypothesis.
Allium extracts were shown to have antibiotic effects on soil fungi
and bacteria in culture media, but whether such antibiotic influ-
ence plays a role in the stimulatory mechanism in soil is not known.

In this study an attempt was made to determine which component
of onion (Allium cepa L.) extract, and root diffusate obtained from
onion seedlings, stimulate the germination of sclerotia of S. cepi-
vorum.

MATERIAL AND METHODS

The pathogen

Sclerotia were obtained from 6-week old sand-cornmeal cultures of S.
cepivorum. Sclerotia were washed several times with distilled water, air dried,
and subjected to alternating freezing and thawing for one week to break
dormancy. They were surface sterilized before use by immersing them in a
0.1% mercuric chloride solution for 1 minute, then washed several times with
sterile distilled water.

Preparation and fractionation of onion extract

Onion extract was prepared by squeezing crushed onion bulbs through a
cheesecloth. The freshly expressed juice was filtered and centrifuged. The
clear filtrate (50 ml) was extracted 3 times with 25-ml portions of distilled
erther. The combined ether extracts were distilled under vacuo to yield an oil
(ether-soluble fraction). The ether-insoluble fraction was then passed through
Amberlite IR-120 cation-exchange resin in the H+ form (column 2 × 13 cm)
at a rate of 1 ml/min. The solution passing through, containing mainly sugars,
organic acids, phosphate esters and nucleotides is hereby referred to as a sugar
fraction. The column was washed with 25 ml of distilled water. The amino
acids were then eluted with 50 ml of 1 N ammonium hydroxide solution. The
sugar and amino acid fractions were concentrated each to the last 5 ml in
vacuo at 60°C. One ml of each of the concentrated fractions was removed for
identification of sugars and amino acids by paper chromatography. The re-
mainning 4 ml of each concentrate were diluted with distilled water to a
volume of 40 ml, then sterilized by Seitz filtration and kept in refrigerator.