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ISOLATION OF ABSCISIC ACID PRODUCING STRAINS OF PHYTOPATHOGENS

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Key Word Index—abscisic acid; phytopathogens; isolation; identification.

Abstract—A screening method has been established to isolate abscisic acid producing strains of four pathogenic fungi from different plant hosts.

INTRODUCTION

The plant hormone abscisic acid (ABA) is produced by certain algae and by several fungi as well [1]. As all ABA-producing fungal strains isolated so far are phytopathogenic, it can be concluded that the ABA produced by these fungi might have a function in phytopathogenesis [2–5]. This paper reports the isolation of ABA-producing strains and gives an effective method to isolate high yielding ABA-producing strains.

RESULTS AND DISCUSSION

In order to investigate the distribution of ABA-producing strains on disease hosts, we selected the typical plants which were easily infected by phytopathogenic genera. These genera, *Botrytis*, *Penicillium*, *Rhizopus* and *Fusarium*, were purely isolated and cultivated on PDA medium at 28°C for 15 days. Samples of ABA were identified by HPLC and mass spectrometry.

Of 26 fungal isolates, 12 produce ABA, when cultured on PDA medium, at concentrations ranging from 0.2 to 39.2 $\mu g\ g^{-1}$ PDA. The strain which produced most ABA (*B. cinerea*, *CW* 04) was isolated from rotten grapes. The results indicate that ABA is produced by a high percentage (87%) of *B. cinerea* isolates. This species is a wide spectrum plant pathogen and causes the hosts to wither. Isolates of other genera, *Penicillium*, *Rhizopus* and *Fusarium* were also screened and only a few of them produced ABA (Table 1). Three, four and six isolates of these genera respectively did not produce enough ABA for it to be detectable. This confirmed that the ABA was produced by the fungal hyphal and was not derived from the potato.

EXPERIMENTAL

Fungal culture

Botrytis cinerea As 3.3789 and *Fusarium culmorum* As 3.4595 were provided by Centre for General Microbiological Culture Collection (CGMCC: Zhongguancun, Haidian, Beijing 100080, China). The other genera were isolated from different plant hosts. The genera were cultured on PDA [6] (PDA: fresh potato 200 g, glucose 5 g, water 1000 ml) at 28°C for 15 days.

Isolation and identification of ABA

The cultured mycelium and the medium were macerated with acetone:water (4:1) and refiltrate was evaporated to an aqueous solution which was extracted (pH 9.0) with EtOAc (1:1) to remove neutral and basic substances. The residual aqueous solution was extracted with EtOAc (residual aqueous solution: EtOAc = 5:1 v/v) at pH 3.0.

There are two methods for identification: (1) The EtOAc extract was separated by TLC (Silica gel, PF_{254}. Solvent system, benzene–EtOAc–HAc = 10:6:1). The spot which was active coincided with the authentic ABA. (2) The ABA in the EtOAc extract was also identified by a colour test [7]. The ABA gave a distinct, transient violet-red colour. In this way the isolate can be easily screened for ABA production.

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Table 1. The relationship between ABA-producing fungi and plant hosts

Organism	Source	ABA content ($\mu g / g^{-1}$ PDA	Culture time (days)
Botrytis cinerea CW01	lettuce	0.2	15
Botrytis cinerea CW02	strawberry	2.3	15
Botrytis cinerea CW03	towelgourd	8.1	15
Botrytis cinerea CW04	grape	39.2	15
Botrytis cinerea CW05	bean	0.8	15
Botrytis cinerea CW06	brassica	1.7	15
Botrytis cinerea CW07	spinach	n.d.	15
Botrytis cinerea As 3.3789	CGMCC	15.1	15
Penicillium sp CW14	grape	0.3	15
Rhizopus sp CW22	lettuce	1.7	15
Rhizopus sp CW23	strawberry	7.2	15
Rhizopus sp CW25	rose	0.3	15
Fusarium sp CW33	bean	3.1	15

ABA quantitation

After the strains were cultured on PDA (pH 6.0) at 28°C for 15 days, the mycelium and culture medium were macerated with acetone solution. Then the aqueous residue was passed through Sep Pak C-18 reverse phase cartridges and then eluted with 10 ml 35% MeOH. The dried MeOH fractions were subjected to HPLC (Bondapak C_{18} Corasil column 3.8×300 mm, linear gradient MeOH 30–70% in 0.1% HOAc, flow rate 2.0 ml min^{-1}), and the UV absorbing peak with the same R_t as standard ABA was collected. Mass spectra of the sample and standard ABA were recorded on a Finnigan FTMS-2000 mass spectrometer by self-chemical ionization at 70 eV, which displayed characteristic major ions at 265 $[M+H]^+$, 247$[M+H-H_2O]^+$, 190, 162, 134 and 91.

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