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SHORT COMMUNICATION

PRODUCTION OF A SIDEROPHORE BY THE FUNGUS *FUSARIUM OXYSPORUM*

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Abstract: The fungal pathogen *Fusarium oxysporum* isolated from banana, when grown under iron limiting conditions in Fries' basal liquid medium produced a siderophore. The production of the siderophore was highest during the early stationary phase. The siderophore was isolated and its structure elucidated, it was an aromatic amide with the formula $C_{16}H_{12}O_7N_2$.

Key words: Fungi, *Fusarium oxysporum*, siderophore.

INTRODUCTION

Although iron is one of the most abundant elements in the earth's crust, the profound insolubility of iron at neutral pH values, limits its availability to soil micro-organisms. Most micro-organisms respond to low iron stress by producing extra-cellular, low molecular weight iron transport agents designated siderophores.[1-3] In an agricultural eco-system, the struggle for iron which is an element essential for growth, is observed not only among micro-organisms, but also between micro-organisms and their host plants. Thus, the production of siderophores provides plant pathogens with a unique and highly specialized way to compete with their hosts.[2] The fungus *Fusarium oxysporum* is a soil borne plant pathogen. Isolates of this fungus cause diseases in many crops such as banana, tomato and chillie.[4] This investigation reports the isolation and purification of an as yet unidentified siderophore produced by *F. oxysporum*.

METHODS AND MATERIALS

Organism: The fungus *F. oxysporum* isolated from infected banana, was obtained from the Plant Pathology Division of the Angunukolapalessa Agricultural Research Centre of the Department of Agriculture. The isolate was maintained at 4°C on potato dextrose agar [PDA] during the period of study.

Growth Medium: The modified Fries' basal liquid medium[2] was used to grow the fungus to detect siderophore production. This medium provides an iron stress environment and it contains, ammonium tartrate [5.0g], NH_4NO_3[1.0g], $MgSO_4.7H_2O$[0.5g], KH_2PO_4 [2.6g], glucose [10.0g], yeast extract [5.0g], micro-nutrient solution [2.0ml] and deionized water [1l]. The micro-nutrient solution had $LiCl_2$[167mg], $CuCl_2.2H_2O$ [107mg], $MnCl_2.4H_2O$[72mg], H_2MoO_4 [34mg] and $CoCl_2.6H_2O$[80mg] in 1l deionized water.

Time Course of Siderophore Production: To determine the time course of siderophore production, 25 ml of Fries' basal liquid medium was dispensed into 150 ml Erlenmeyer flasks. Each flask was, thereafter, inoculated with one 8mm diameter agar disc obtained from the periphery of a 5-day-old culture of *F. oxysporum* growing on PDA plates at room temperature. The inoculated liquid media were incubated without shaking at room temperature.

The cultures were harvested at 3 day intervals for a period of 24 days by filtering through Whatman No. 1 filter paper. The residual mycelium was dried to a constant weight at 80°C and used to assess growth. The filtrate was examined for the presence of any siderophores as described by Letendre and Gibbons,[2] by adding excess $FeCl_3$ followed by measurement of absorption on a Hitachi-Perkin Elmer double beam spectrophotometer at 260nm.

Isolation and Purification of the Siderophore: The fungus was grown in 11 Erlenmeyer flasks each having 250 ml of the Fries' basal liquid medium. Each flask was inoculated with 5, 8.0 mm diameter agar discs obtained from the periphery of a 5-day-old culture of the fungus growing on PDA plates at room temperature. The inoculated media were incubated without shaking and harvested 13 days after inoculation by filtering through Whatman No. 1 filter paper.

The culture filtrate was used to isolate any siderophores as described by Letendre and Gibbons.[2] The culture filtrate was mixed with $FeCl_3$. The resulting brown solution was saturated with $(NH_4)_2SO_4$ with stirring at room temperature for about 2 h. The precipitate formed was removed by centrifugation and the supernatant was extracted with an equal volume of benzyl alcohol. The extract, thereafter, was treated several times with 10 volumes of diethyl ether and 3 volumes of distilled water. The aqueous phase was collected and concentrated [x10] by freeze drying to give 120 mg of the siderophore.

RESULTS AND DISCUSSION

Time Course of Siderophore Production: The fungus *F. oxysporum* produced a siderophore when grown under iron stress conditions. The production was highest around 14 days after inoculation (Fig. 1) i.e. during the early stages of the stationary phase.

Analysis of the Isolated Compound: The isolated siderophore [I] showed the following physical and chemical data:

m.p. 188-190°C; $C_{16}H_{12}O_7N_2$ requires C 55.82%, H 3.51% Found C55.88% H3.42%; UV[H_2O] λ_{max}; nm, [ε_{max}] 262.5 [$9.65x10^2$], 400[$1.8x10^2$] IR[KBr] 3728 and 3700 [NH_2], 3137[broad, OH], 2360 [C-H], 2343[C-H],1631 [broad, C=O], 1401[C-O],1125, 668, 657, 618 [Aromatic ring] cm^{-1}; ^1H NMR [D_2O], δ 6.77 [S,2H], 7.03 [S,2H], 7.29 [S,2H]; MS m/e [relative intensity] 344[22],343[100], 331[21], 325[17], 221[16], 219[30], 218[27] 205[18], 203[16],112[40].

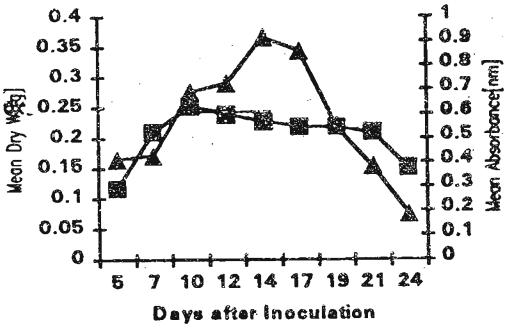
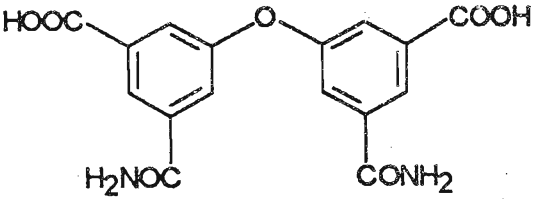


Figure 1: Growth of *Fusarium oxysporum* in Fries' basal liquid medium and absorbance at 260 nm of the culture filtrates obtained at different stages of growth.

The structure of the new siderophore was elucidated as **I** [given below] on the basis of the spectral data. IR spectrum of this compound showed two absorptions at 3728 and 3700 characteristic of a NH_2 group. A strong broad C=O absorption at 1631 was assigned as an amide group and a carboxylic group.

Aromatic absorptions below 700 cm^{-1} were interpreted as a 1,3,5-trisubstituted aromatic ring. ^1H NMR taken in D_2O showed only three singlets at $\delta = 6.77, 7.03$ and 7.29. These three protons were assigned for three aromatic protons in a 1,3,5 trisubstituted ring. COOH and $CONH_2$ protons were not seen in the NMR taken in D_2O and the compound was found to be insoluble in all other common deuterated solvents. Mass spectrum of the compound showed fairly strong molecular iron peak at 344 and the base peak at 343 resulting from loss of one hydrogen. Molecular weight of 344 can be explained only by a dimeric structure with substituted diphenyl ethers. The proposed structure **I** is in agreement with all the physical and spectroscopic data.



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