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https://doi.org/10.5109/22825

出版情報：九州大学大学院農学研究院紀要. 17 (2), pp.137-141, 1973-03. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 17, 137-141 (1973)

Antifungal Substances Produced by *Cephalothecium roseum* Corda

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(Received July 2, 1972)

No antifungal activity could be recognized in the acidic or basic fractions obtained from the culture filtrate of ***Cephalothecium roseum*** Corda, which was not in accordance with Yoshii's description. The active substances were found only in ether soluble neutral fraction. A major antifungal substance of them, $C_{19}H_{24}O_5$ was identified as trichothecin.

From a culture filtrate of ***Cephalothecium roseum*** Corda which was isolated from wheat stems provided as package materials, H. Yoshii obtained, in 1945, a pale yellow crystalline antifungal acidic substance (m. p. 124-6°C) which inhibited the germination of spores of ***Piricularia oryzae, Cochliobolus miyabeanus*** etc. and the development of mycelia of ***126*** phyto-pathogenic fungi, and he named it Cephalothecin (Yoshii, 1957).

At about the same time, Freeman ***et al.*** obtained an antifungal substance, Trichothecin (m. p. 116-8°C) from ***Trichothecium roseum*** Link isolated from diseased tomatoes (Freeman, 1955 ; Freeman and Gill, 1950 ; Freeman, Gill and Waring 1959 ; Freeman and Morrison, 1948, 1949). The chemical structure of trichothecin was finally established by Gutzwiller ***et al.,*** (1964).

According to Freeman, ***Trichothecium roseum*** Link and ***Cephalothecium roseum*** Corda are identical. However, as there are some differences in the descriptions between cephalothecin and trichothecin, the present study was undertaken in order to elucidate the structure of cephalothecin, from a generous supply of the stocked strain of ***Cephalothecium roseum*** Corda.

EXPERIMENTALS

(1) Culture

The culture of ***Cephalothecium roseum*** Corda was carried out according to Yoshii's description. Namely, the culture medium used was half the amount of the Richard-medium type and corn steeped liquor was added to produce a ***0.5*** % solution which was finally adjusted to pH 5.5 with 0.1 N NaOH. After inoculating the strain, it was allowed to stand at ***25°C*** for ***20*** days.

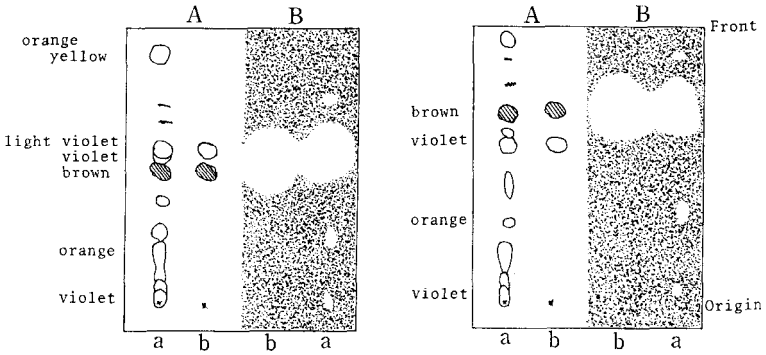
In one week, mycelia covered the surface of the medium; in about 10 days the surface of mycelia became orange owing to the formation of spores; and in 20 days pH of the medium became 8.0-9.0.

(2) **Bioassay**

So as to evaluate the isolation procedure two methods of bioassay were conducted.

(i) Culture method on a slide glass described by Yoshii (1957) : -On a hole slide glass, the sample and spores of fungus to be tested (usually *Aspergillus niger)* were mixed and incubated at **25°C.** In 24 hours the germination of spores was observed microscopically.

(ii) Biochromatography devised by the authors: - One half of a developed and air dried TLC (silica gel) was visualized by a suitable spraying reagent such as vanillin sulfuric acid. The other half of the TLC was sprayed with melted hot culture medium containing agar, then sprayed with spores suspension of testing fungus, and thereafter incubated at 25°C for 48 hours. The positions containing antifungal substances were located as white spots on a black ground due to germination of spores (Fig. 1).



a, Ether extract of the culture filtrate
b, Trichothecin
A, Visualized by vanillin sulfuric acid
B, Agar and spores of *Aspergillus niger* were sprayed

Fig. I. Biochromatograms of antifungal substances produced by *Cephalothecium roseum* Corda.

(3) **Thin layer chromatography (TLC)**

TLC was carried out by using silica gel. Benzene: ethylacetate (5: 1) and petroleum ether : ether (1: 2) were suitable as the developing solvent, and chromatograms were visualized by spraying with vanillin sulfuric acid or alkaline permanganate solution.

(4) **Isolation and Identification**

The extraction and purification were at first performed according to Yoshii's description. However, no antifungal activity could be recognized in the acidic or basic fractions of the culture filtrate and mycelia. The active substance(s) was found only in ether soluble neutral fraction (Fig. 2).

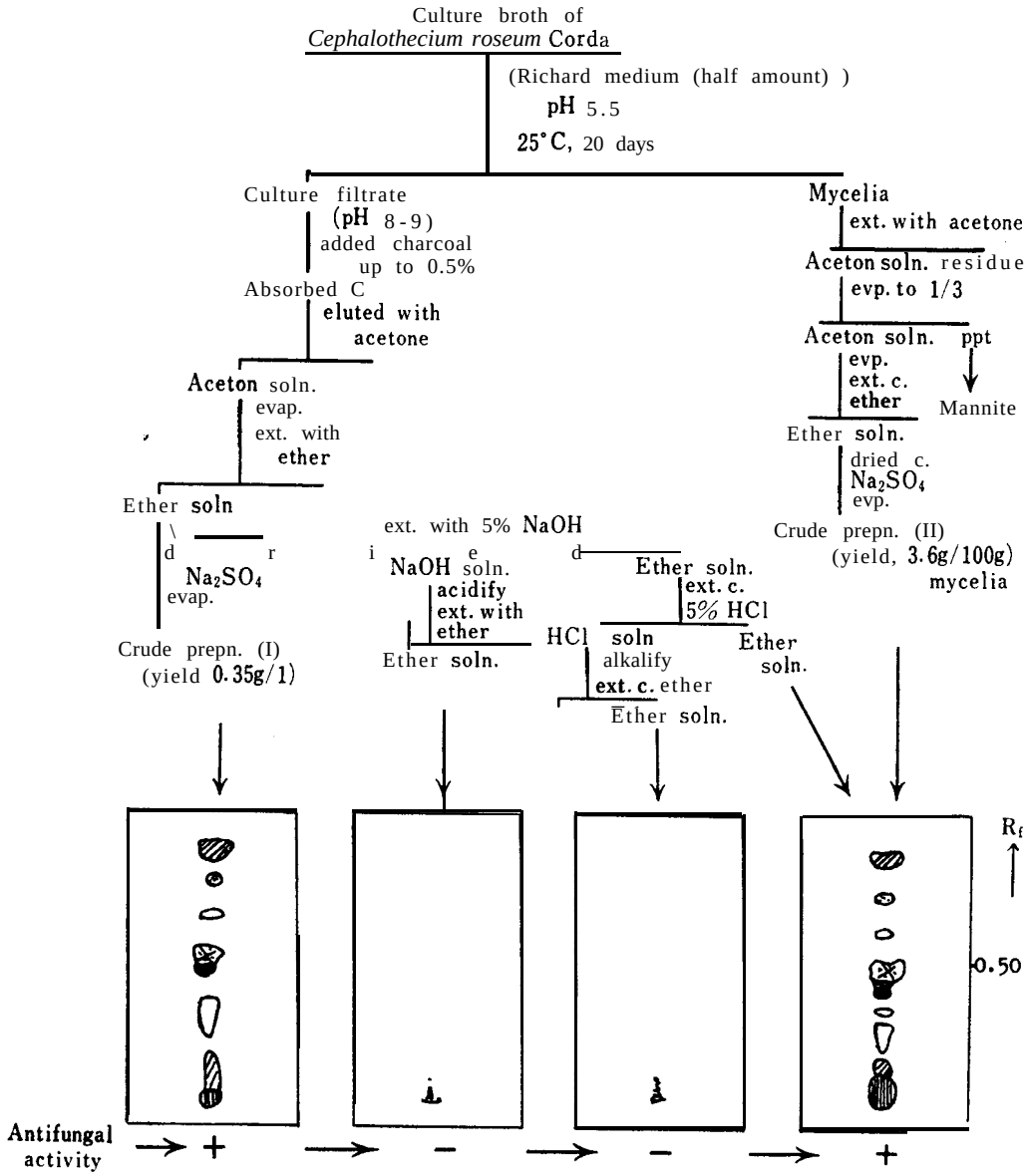


Fig. 2. Isolation procedure of the antifungal metabolite from *Cephalothecium roseum* Corda.

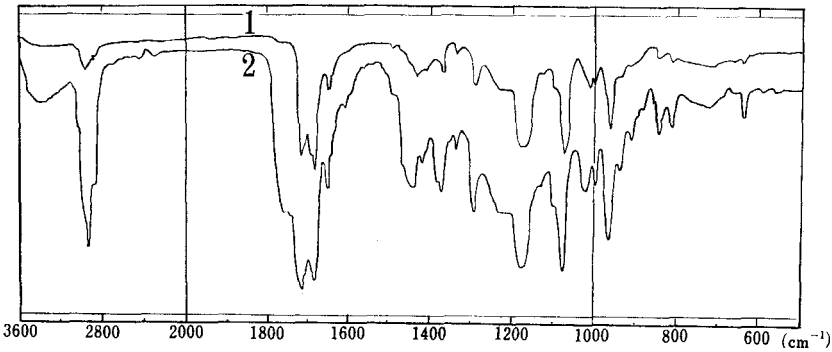
In order to confirm this fact the crude ether extract was repeatedly washed with 5 % NaOH aq. solution. Nevertheless, the active substance did not transfer to the alkaline solution.

On the whole no acidic antifungal substances could be found. This result

might be ascribed to the fact that the strain mutated during long storage (over 15 years) or to some other facts.

Therefore, the investigation for the ether soluble fraction was continued. A major antifungal substance (Rf 0.53 on silica gel TLC ; solvent, benzene :ethylacetate=5 :1) was repeatedly purified by TLC and finally a crystalline substance of m. p. 118°C and of $C_{19}H_{24}O_5$ was obtained.

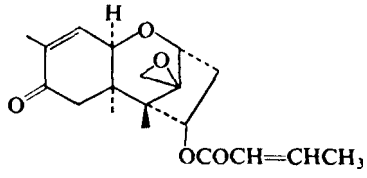
$C_{19}H_{24}O_5$: Calcd. C, 68.65 % H, 7.28%
Found C, 68.32 % H, 7.42 %



1. Trichothecin[18], *2.* Isolated preparation

Fig. 3. IR spectra of the preparation isolated from *Cephalothecium roseum* Corda and of trichothecin.

This substance was identified as trichothecin by co-chromatography (Rf, activity and brown coloration by vanillin-sulfuric acid), IR-spectrum (Fig. 3) (Freeman, Gill and Waring, 1959), (Yamamoto, Henmi and Yamano 1969) and mixed melting point.



Trichothecin

As shown in Fig. 1, there are two minor antifugal substances (Rf 0.75 and 0.25 on TLC, by solvent, benzene: ethylacetate=5: 1).

When ***Trichothecium roseum*** was grown on surface culture on a Czapek-Dox-ammonium tartrate: corn steeped liquor medium for 4 weeks, the major metabolites were rosenonolactone (Harris, Robertson and Whalley, 1958), (Robertson, Smithies and Tittensor, 1949) and rosololactone [Harris, Robertson and Whalley, 1958). Desoxyrosenonolactone (Whalley, Green, Arigoni, Britt and Djerassi, 1959), 6β-hydroxyrosenolactone (Allison, Connolly and Overton, 1968), (Holzapfel and Steyn, 1968) and trichothecin (Fishman, Jones, Lowe and Whiting, 1960), (Freeman, Gill and Waring, 1959), (Godtfredsen and Vangedal, 1964), (Jones and Lowe, 1960) were isolated in smaller amounts.

Furthermore, B. Achilladelis et *al.* (1969) isolated rosenololactone and crotocin. Similarly, it had already been reported that several related antifungal compounds were isolated.

Two minor substances observed on TLC remained for a further elucidation.

ACKNOWLEDGEMENT

The authors are indebted to Prof. Dr. T. Asada, Ehime University for kindly supplying the stocked strain, and to Dr. K. Nakazawa, Takeda Pharmaceutical Co. for a sample of trichothecin.

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