

SHORT COMMUNICATION

STEROLS OF *SCHIZOPHYLLUM COMMUNE*

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Abstract—The major sterol of two homokaryons and a dikaryon of *Schizophyllum* was identified as ergosterol. No quantitative or qualitative differences were found in the sterols from the homokaryons and the derived dikaryon. Thus it is doubtful whether the sterols play a direct role in sexual morphogenesis of *Schizophyllum*.

INTRODUCTION

SEXUAL reproduction in many of the higher Basidiomycetes entails the interaction between two compatible homokaryotic strains to form a dikaryotic mycelium. A series of morphogenetic events regulated by incompatibility factors lead to the formation of the dikaryotic mycelium.^{1,2}

During studies on alterations of cellular constituents in the morphogenetic process the sterols of the homokaryons and the derived dikaryon were compared. Such comparative studies gain special importance in formulation of a general scheme for the role of fungal steroids in sexual reproduction due to recent findings in various fungi,³⁻⁷ as well as due to the scarcity of information on fungal sterols.

RESULTS

Analysis of the total lipid fraction of the homokaryons and the dikaryon by TLC revealed in all cases an intense sterol band and a few minor components as well as esterified sterols. The intense band cochromatographed with an authentic sample of ergosterol. The minor components did not correspond to any of a number of authentic samples of sterols which included among others β -sitosterol, stigmasterol and cholesterol.

Saponification of the band corresponding to the esterified sterols indicated the band to be uniformly composed of an ester of ergosterol as the unsaponifiable fraction of the ester cochromatographed with ergosterol.

Further support for the identification of the major sterol as ergosterol was obtained by determination of its absorption spectrum after purification by TLC. Maximum absorption of this was at 273, 283 and 294 nm which are the major absorption peaks of ergosterol.

¹ J. R. RAPER, *Genetics of Sexuality in Higher Fungi*, Ronald Press, New York (1966).

² J. R. RAPER and C. A. RAPER, *J. Elisha Mitchell Sci. Soc.* **84**, 367 (1968).

³ R. H. HASKINS, A. P. TULLOCH and R. G. MICETICH, *Can. J. Microbiol.* **10**, 187 (1964).

⁴ J. A. LEAL, J. FRIEND and P. HOLLIDAY, *Nature* **203**, 545 (1964).

⁵ J. W. HENDRIX, *Science* **144**, 1028 (1964).

⁶ C. G. ELLIOTT, *J. Gen. Microbiol.* **56**, 331 (1969).

⁷ T. C. MCMORRIS and A. W. BARKSDALE, *Nature* **215**, 320 (1967).

No qualitative differences in sterol content were detected between the homokaryons and the dikaryon by TLC either in the major or the minor components. Quantitative differences were sought by GLC as well as further confirmation for the identity of the major component and the possible identification of the minor components.

Four sterol peaks were obtained by GLC. The major peak cochromatographed with ergosterol and comprised 88.8% and 94.6% of the total sterols of the two homokaryons and 91.0% of the total sterols of the dikaryon. Three additional peaks were detected in the homokaryons and the dikaryon. One peak was eluted prior to ergosterol and two eluted after ergosterol. The first peak after ergosterol assumes a position near stigmasterol. The other two components did not cochromatograph with cholesterol, β sitosterol, their corresponding esters, progesterone or testosterone. The esterified sterols and sterols were eluted a long time after the sterols of *Schizophyllum*. The relative proportions of the minor components with respect to the ergosterol as well as their absolute proportion of the total sterols did not differ significantly in the homokaryon and the dikaryon. One is led to conclude, therefore, that no quantitative difference related to sexual morphogenesis can be shown. Thus it is doubtful whether the sterols play any direct role in sexual morphogenesis in *Schizophyllum*.

EXPERIMENTAL

Cultures were grown in liquid *Schizophyllum* minimal medium⁸ on a rotary shaker for 48 hr after maceration of the mycelium. The strains used (No. 699 and E 908) were from the collection of Dr. J. R. Raper, Harvard University.

Lipids were extracted by the method of Folch *et al.*⁹ and saponified according to Nishida.¹⁰ Separation into lipid classes was performed on TLC plates.¹¹ The sterols in the nonsaponifiable fraction were determined on a dual column Packard Gas Chromatograph equipped with H₂ flame detector, disc integrator and 1.8 m \times 6 mm glass column packed with 3% OV-17 Gas-Chrom Q 100/120 mesh. Column temp. was 240° and that of the injection port and detector 280°. Nitrogen flowrate 30 ml/min. The absorption spectra of the samples were determined in alcohol by the Zeiss D M.R. 21 double beam spectrophotometer.

⁸ J. R. RAPER and P. J. MILES, *Genetics* **43**, 530 (1958).

⁹ J. FOLCH, M. LEES and G. H. SOLAN STANLEY, *J. Biol. Chem.* **226**, 497 (1957).

¹⁰ T. NISHIDA, F. TAKANAKA and F. A. KUMMEROW, *Circulation Res.* **6**, 194 (1958).

¹¹ E. STAHL, *Thin Layer Chromatography—A Laboratory Handbook*, p. 153, Academic Press, New York (1965).