

Differences in the Sterol Synthesizing Pathways of Sterol-Producing and Non-Sterol-Producing Fungi

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Accepted for publication 27 February 1978.

ABSTRACT

GOTTLIEB, D., R. J. KNAUS, and S. G. WOOD. 1978. Differences in the Sterol synthesizing pathways of sterol-producing and non-sterol-producing fungi. *Phytopathology* 68: 1168-1169.

The mycelia of several sterol-producing and non-sterol-producing fungi were analyzed for the presence of squalene, squalene epoxide, lanosterol, and ergosterol. The sterol-producing fungi contained all four compounds, whereas only

squalene was detected in the non-sterol-producing fungi. The block in the synthesis of ergosterol by the latter organisms is presumed to occur at the stage at which squalene is usually oxidized to squalene epoxide.

Additional key word: Pythiaceae.

Most fungi produce sterols, primarily ergosterol; however, one family, the Pythiaceae, is unique in that its members do not make detectable amounts of such compounds. The characteristics and general nature of these fungi have been discussed in previous papers (2, 3). These earlier studies involved one sterol-producing species, *Rhizoctonia solani*, and a non-sterol-producing species, *Phytophthora cinnamomi*, and revealed differences in the extent of the biosynthetic pathways of the two fungi. *Rhizoctonia solani* had enzyme activities for all conversion steps of the pathway from acetate to ergosterol. In contrast, *P. cinnamomi* appeared to have only the enzymes that converted these substrates to squalene and not further. The absent enzymes were those

required: (i) to convert squalene to its epoxide, (ii) to cyclize the epoxide to lanosterol, and (iii) to catalyze at least some of the steps in the transformation of lanosterol to ergosterol. The first and key block in the system was at the epoxidase step. Whether or not the differences found between these two species are representative of the differences between sterol-producing and non-sterol-producing fungi in general needed elucidation and is the subject of this communication.

MATERIALS AND METHODS

The materials and methods used in the present study were the same as those used in our earlier studies (2). The

TABLE 1. Sterol pathway components present in 3-day-old mycelia of several sterol-producing and non-sterol-producing fungi

Fungus	Squalene ($\mu\text{g/g}$ dry wt)	Squalene epoxide ($\mu\text{g/g}$ dry wt)	Lanosterol ($\mu\text{g/g}$ dry wt)	Ergosterol (mg/g dry wt)
Sterol-producing:				
<i>Rhizoctonia solani</i>	7.8 ^a	6.5	11.9	2.2
<i>Aspergillus flavus</i>	9.4	8.5	14.9	1.0
<i>A. fumigatus</i>	7.3	6.1	11.0	4.0
<i>Penicillium atrovenerum</i>	6.1	5.5	16.6	3.2
Non-sterol-producing:				
<i>Phytophthora cinnamomi</i>	6.9	— ^b	—	—
<i>P. cactorum</i> (#39)	5.6	—	—	—
<i>P. cactorum</i> (IMI 21168)	4.0	—	—	—
<i>Pythium graminicola</i>	5.5	—	—	—
<i>P. ultimum</i>	4.2	(\pm) ^c	—	—

^aEach value represents the average of multiple determinations.

^bThe symbol — = none detected; the limits of detection by gas chromatography were 2 ng of squalene, 5 ng of squalene epoxide or ergosterol, and 10 ng of lanosterol per injected sample; these values represent, respectively, 2 μg , 5 μg , and 10 μg of a 1-g sample of dried mycelium.

^cIn the gas chromatographic scans of 27% of the extracts of this organism, a small peak with a retention time similar to that of squalene epoxide was evident; no further attempt was made to identify this peak.

current data were compiled from two series of replicated experiments performed separately by two of us with an interval of more than 1 yr between series. Experiments were carried out with 3-day-old mycelia grown in shake cultures in sterol-free liquid medium. The mycelia were analyzed by gas chromatography for the presence of four key compounds in the sterol pathway: squalene, squalene epoxide, lanosterol, and ergosterol. Thin-layer chromatographic- and mass spectrometric analyses were included for at least one sterol-producing species and for all species of the non-sterol-producing fungi where information on the presence or absence of squalene was especially important. The non-sterol-producing fungi included in the study were *Pythium graminicola* Subramanian, *P. ultimum* Trow, *Phytophthora cinnamomi* Rands, and two isolates of *P. cactorum* (Lebert et Cohn) Schroeter (#39 and IMI 21168). Two isolates of the latter species were included because one (IMI 21168) had been reported as unable to produce squalene (1). The sterol-producing fungi were *Rhizoctonia solani* Kühn (Illinois strain), *Aspergillus flavus* Link ex Fries, *A. fumigatus* Fresenius, and *Penicillium atrovenerium* Smith.

RESULTS AND DISCUSSION

In the current study, the sterol-producing fungi all contained squalene, squalene epoxide, lanosterol, and ergosterol (Table 1). In contrast, although all the non-sterol-producers also contained squalene, they did not

contain squalene epoxide, lanosterol, or ergosterol in detectable quantities. Thus, the first effective block in the synthesis of ergosterol by these organisms occurred at the stage at which squalene is usually oxidized to squalene epoxide. Even if the epoxide were made, it is doubtful whether ergosterol would be synthesized because, based on our previous studies with *Phytophthora cinnamomi*, the rest of these non-sterol-producing fungi probably can neither cyclize the epoxide to lanosterol nor convert lanosterol to ergosterol (2, 3).

We propose that the prime reason members of the Pythiaceae do not synthesize sterols is the absence of a mechanism to convert squalene to squalene epoxide. The data presented in this report on four sterol-producing and five non-sterol-producing fungi are consistent with this concept.

LITERATURE CITED

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