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PR Toxin Production in Different *Penicillium roqueforti* Strains

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Different *Penicillium roqueforti* strains from the American Type Culture Collection were tested for the production of PR toxin. All four strains were able to produce the toxin on semisynthetic medium at 24°C after certain periods of incubation. The yields were correlated with the pH of the medium. Timing of the harvest influenced both the yield and purification of the toxin.

PR toxin is a toxic secondary metabolite isolated from cultures of *Penicillium roqueforti* in whole-kernel corn or a semisynthetic medium (4). Its chemcial structure has recently been elucidated (2). The toxin is lethal to rats and mice by either oral or intraperitoneal administration (3, 4). Currently, PR toxin has been shown to inhibit in vivo protein synthesis in rat liver (3), probably because the toxin prevents the initiation and elongation steps of transcription (1). This mycotoxin has also revealed strong inhibitory effects on protein, ribonucleic acid, and deoxyribonucleic acid biosynthesis of Ehrlich ascites tumor cells (3).

At first, a strain of *P. roqueforti* isolated from a toxic silage sample of Wisconsin farms was used for production of PR toxin (4; P. E. Still, R. D. Wei, E. B. Smalley, and F. M. Strong, Fed. Proc. **31**:733, 1972). It was later found that *P. roqueforti* NRRL 849 produced much more toxin, and this strain was therefore used for mass production of the toxin (4). This study was undertaken to compare the ability of all *P. roqueforti* strains listed in the American Type Culture Collection Catalogue to produce PR toxin.

MATERIALS AND METHODS

Organisms. Four strains of *P. roqueforti,* ATCC 6987, ATCC 6989, ATCC 9295, and ATCC 10110, were obtained from the American Type Culture Collection, Rockville, Md. The cultures were maintained at 24°C on potato-dextrose agar.

Medium. The basal medium consisted of 2% yeast extract (Difco) and 15% sucrose in demineralized water.

Culture. Fernbach flasks (1 liter) containing 150 ml of medium per flask were stoppered with cotton plugs and autoclaved for 17 min at 121°C. Media were inoculated with about 10^6 to 10^8 spores (0.5 ml) from 8- to 9-day-old cultures of *P. roqueforti* and incubated for 3 to 26 days at 24°C as stationary cultures. Experiments were replicated three times.

Extraction. After a certain period of incubation, the culture was filtered, and the final pH of the filtrate was measured. The culture filtrate was then extracted three times with 100 ml of chloroform. The crude extract was evaporated in vacuo to a thick oil and then dissolved in chloroform to a final volume of 1.0 ml.

Assays. PR toxin assays were done by a thin-layer chromatography procedure (4), except that 2 to 20 μl of the samples was applied to thin-layer plates, using a syringe pipette (Hamilton). PR toxin was visualized on the plate by spraying with 50% sulfuric acid. It appeared immediately as a yellow spot that turned yellowish-brown after charring. The PR toxin was determined quantitatively by visual comparison with an external PR toxin standard.

Isolation of toxin. The chloroform extracts from the cultures of these same *P. roqueforti* strains were pooled, and the PR toxin was isolated into pure form by the previously mentioned method (4).

Spectroscopic measurement. Infrared spectra were determined in a Perkin-Elmer 577 gratting infrared spectrophotometer.

RESULTS AND DISCUSSION

Representative results of PR toxin production in different strains of *P. roqueforti,* as measured by thin-layer chromatography of the chloroform extracts, versus time of culture harvest are shown in Fig. 1. All four strains formed PR toxin with identical infrared spectra (Fig. 2), although the growth appearance, yield of toxin, and the production period were quite different (Table 1). Production of the toxin increased gradually versus time to a maximum and then fell rapidly to zero. The yields correlated with the pH of the medium. The pH of the medium at the maximum yield was between 4.1 to 5.4. As the pH reached 7 or greater, levels of toxin in the medium decreased rapidly. Since levels can fall from a maximum to zero in only 1 to 2 days, timing of the harvest is very critical.

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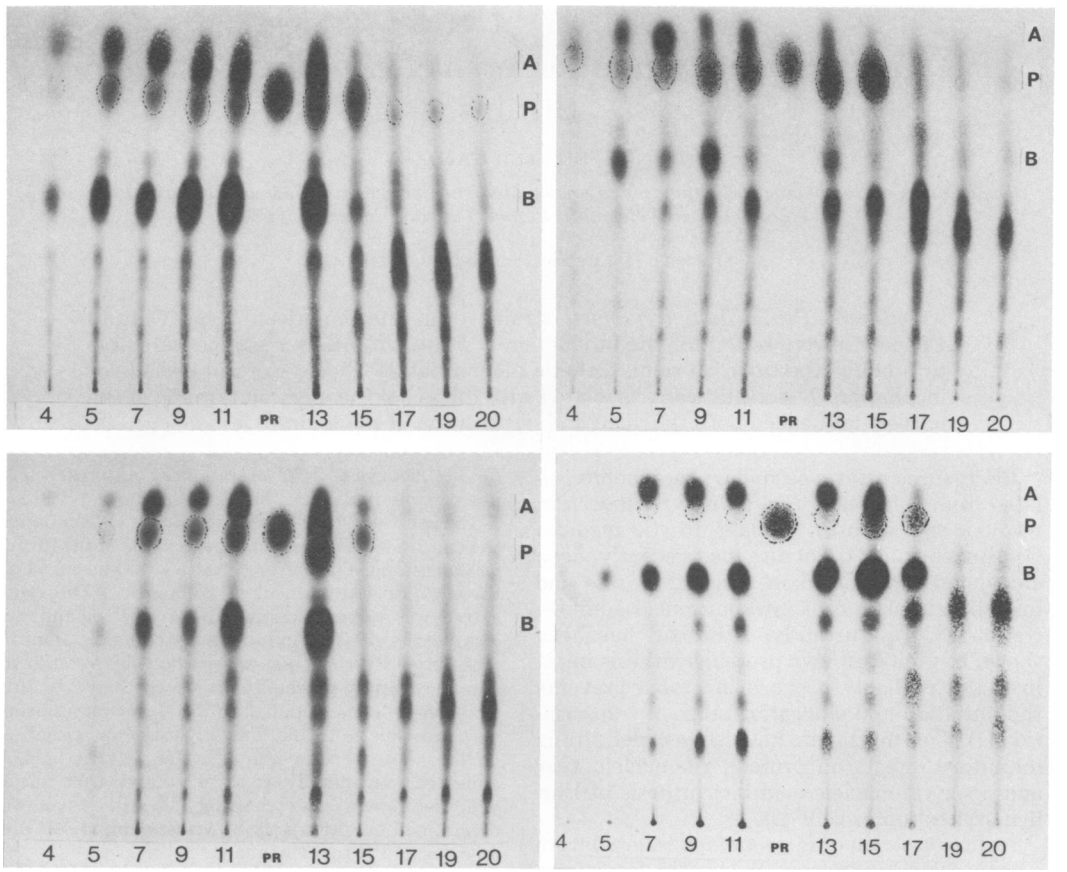

FIG. 1. *Thin-layer chromatograms of chloroform extracts of P. roqueforti culture broths. (Upper left) Strain ATCC 6987; (upper right) ATCC 6989; (lower left) ATCC 9295; (lower right) ATCC 10110. Numbers indicate the day of harvest. Each spot represents 2/1,000 parts (2 μl) of the total extract. PR or P is PR toxin; A and B are impurities of unknown composition. The plates were developed with MeOH-$CHCl_3$ (4:96, vol/vol) and colored by charring with H_2SO_4 and heat.*

TABLE 1. *Comparative production of PR toxin by different P. roqueforti strains at 24°C*

Strain	Medium color[a]	Toxin production period (days of incubation)	Maximal yield of toxin		
			Days	pH	mg/100 ml of medium[b]
ATCC 6987	Brown	4–20	12–15	4.5–5.1	29 (25–31)[c]
ATCC 6989	Deep brown	4–15	13	4.1	30 (29–31)
ATCC 9295	Yellowish green	5–15	12–14	5.0–5.4	24 (21–28)
ATCC 10110	Brown	7–17	15	5.2	21 (19–25)

[a] Medium color was judged after 12 days of incubation.
[b] Values are the means of three experiments.
[c] Ranges of yields are shown in parentheses.

During the early stages and the period of PR toxin production, two major spots (A, at higher, and B, at lower, R_f values) (Fig. 1) were closely associated with the toxin. The lower-R_f compounds were easily removed from the toxin by chromatographing on a short column of Silica Gel G eluted with chloroform (4). These compounds were retained on the column, whereas compound A and PR toxin were almost totally recovered. The higher-R_f compound, A, which overlapped with PR toxin in the chromatograms, was separated from the toxin by chromatographing on Sephadex LH-20 eluted with Skellysolve B-chloroform (40:60, vol/vol).

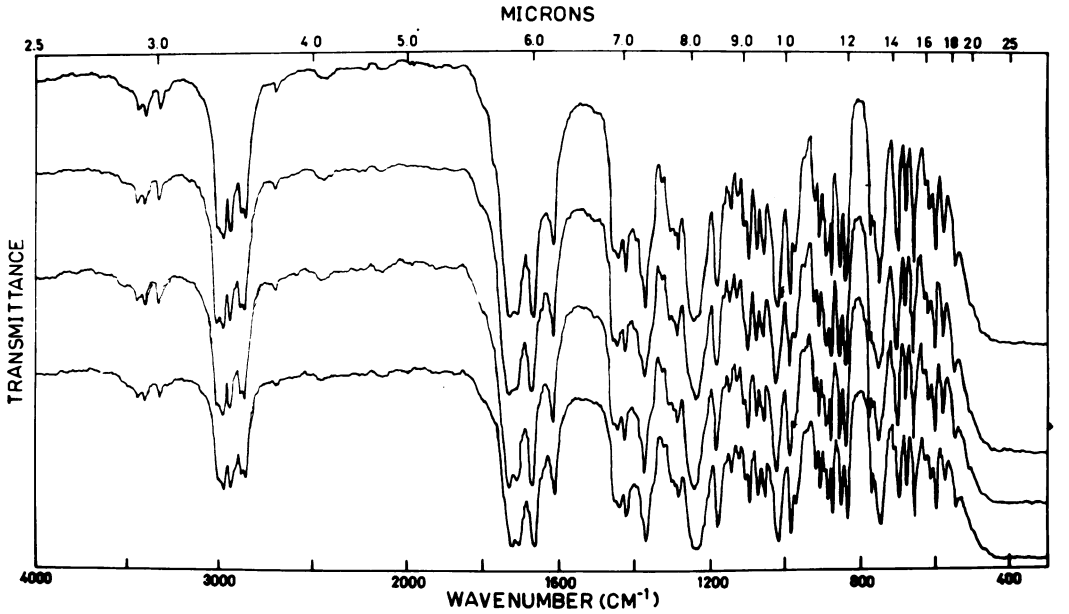

FIG. 2. *Infrared spectra of PR toxin isolated from different P. roqueforti strains. The samples were dissolved in $CHCl_3$ and deposited on demountable cells as thin film by solvent evaporation. Spectra from the top to the bottom are samples from strains ATCC 6987, ATCC 6989, ATCC 9295, and ATCC 10110.*

In a further examination of the chromatograms (Fig. 1), there was always a short period in which the yield of PR toxin was still maintained at the maximal level while compounds A and B fell almost to zero. The cultures harvested at such an optimal time gave higher yields of the toxin, and the further purification process was also much easier. For instance, in the purification of the extract from the broth of a 14-day culture of ATCC 6989, a single step of the short-column chromatography (4) was sufficient to obtain the pure toxin.

ACKNOWLEDGMENT

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