NOTES

Production of Penicillic Acid and Patulin by an Atypical
Penicillium roqueforti Isolate†

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Simultaneous production of penicillic acid and patulin by an atypical strain of
Penicillium roqueforti isolated from cheddar cheese is reported. Mycotoxin
production was confirmed by thin-layer and gas-liquid chromatography and by
ultraviolet, infrared, and mass spectral analyses. Culture extracts were toxic to
Bacillus megaterium and chicken embryos. Commercial strains of P. roqueforti
used in production of blue-veined cheeses were shown not to produce penicillic
acid and patulin.

In a recent study of the mold flora of cheddar cheese, we reported that a number of mold iso-
lates were capable of producing patulin and pen-
icillic acid in yeast-extract sucrose broth (1). In
subsequent work with one of the isolates design-
nated M-247, it was observed that this organism
appeared to be capable of producing penicillic
acid and patulin simultaneously. Preliminary ex-
amination of the culture indicated that the or-
ganism appeared to be an atypical Penicillium
roqueforti. Since we were unaware of previous
reports of the simultaneous production of peni-
cillic acid and patulin by P. roqueforti, the pur-
pose of the work reported here was to verify the
identity of the organism, confirm the presence
of penicillic acid and patulin in culture extracts,
and to determine whether commercial cultures of
P. roqueforti might also possess this ability.

Upon close examination, it was found that the
culture clearly belonged in the Asymmetrica
section of the genus Penicillium. The correct
placement of the species, however, was some-
what in doubt, even though the classification
systems of Thom (17) and Raper and Thom (12)
were consulted. Initial attempts to identify the
isolate to species level, using the key of Raper
and Thom (12), led to uncertainty as to whether
it was P. cyclopium or P. roqueforti. Culture M-
247 differed from P. cyclopium in that the colony
color was dark green rather than the typical
blue-green, the mycelial mat was very thin, and
the culture lacked the typical strong Actinomy-
cetes-like odor. Reverse color of the isolate was

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Fig. 1. Photomicrograph of M-247 isolate showing two views of the roughened conidiophores and metulae (oil immersion, ×1,000).

Silica Gel G-HR, Brinkmann Instruments Inc.) developed in chloroform–ethyl acetate–90% formic acid (60:30:10) (CEF). The bands corresponding to the $R_f$ of penicillic acid and patulin were previously determined by derivatization with ammonia and phenylhydrazine (14). These bands were collected, and the respective mycotoxin was eluted from the silica gel by refluxing with chloroform for 1 to 2 h in a Mini-Soxhlet apparatus. The fractions were checked by thin-
layer chromatography to confirm the presence of only one of the desired metabolites in each fraction; if the fraction contained both compounds, preparative thin-layer chromatography was repeated until a clean fraction was obtained. The resulting fractions, designated as PA (penicillic acid) and PAT (patulin), were used to confirm the identity of the compounds by determining the various spectral and chromatographic properties of the fractions.

On thin-layer chromatography analysis, the PA fraction had an \( R_f \) of 0.73, and the PAT fraction had an \( R_f \) of 0.64 (Silica Gel G-25, 0.25 mm thick, developed in CEF). When derivatized with ammonia, phenylhydrazine, and \( p \)-anisaldehyde (14), the \( R_f \) values and color reactions were identical to those of standard PA and PAT. Ultraviolet absorption spectra (Beckman model 25) gave results for the PA (241 nm) and PAT (276 nm) fractions identical to those for the PA and PAT standards. The infrared spectra (Beckman model IR-5A) of the two fractions corresponded to those of the two mycotoxin standard compounds, with absorption bands for the PA fraction at 5.7 and 6.1 cm\(^{-1}\) and for the PAT fraction at 5.7, 6.0, and 6.1 cm\(^{-1}\). Gas-liquid chromatography (model 1740 Varian Aerograph with model 30 Varian Aerograph recorder; column conditions: stainless steel, 1.7 m by 3.2 mm OD, 3.0% OV-7 and 1.5% OV-22 on 80- to 100-mesh Chromosorb G, Varian Aerograph; conditioned at 250°C for 24 h; \( N_2 \) carrier gas flow of 25 ml/min; injector block and detector temperatures of 250°C; temperature program of 160 to 250°C in increments of 6°C/min; electrometer range, 10\(^{-10}\) A/mV) was done, comparing both the nondonovatized and the trimethylsilyl-derivatized (‘Tri-Sil-BSA-[N,O-Bis-(trimethylsilyl)-acetamide’; Pierce Chemical Co., Rockford, Ill.) forms of the two fractions to the same forms of the mycotoxin standards (11). Retention times for the PA standard were 6.3 and 5.4 min, and those for the PAT standard were 8.7 and 7.3 min (nondonovatized and derivatized, respectively). The PA and PAT fractions had similar retention times. Finally, the mass spectral patterns of the PA and PAT fractions were compared with authentic PA and PAT standards by using an AE1 MS 50 high-resolution mass spectrometer (source temperature, 200°C; ionizing voltage, 70 eV). It was found that the mass spectral peaks of the fragmentation patterns of the fractions matched those of the authentic compounds. The values presented are in agreement with those reported in the literature (6, 10, 11, 14–16, 18, 20) and confirm that both penicillic acid and patulin were produced by the culture. The culture extract was inhibitory to Bacillus megaterium and toxic to chicken embryos. Both penicillic acid and patulin inhibit the growth of B. megaterium (7), and both are toxic to chicken embryos, in which the mean lethal dose of penicillic acid is 0.85 mg/egg via air sac (3) and that of patulin is 68.7 \( \mu \)g/egg via air sac (4).

***P. roqueforti***, the essential fungus used in the manufacture of Roquefort and other varieties of blue cheeses, has been shown to produce other toxic compounds. These include PR toxin (19) and roquefortine, a neurotoxic alkaloid, which has been detected in blue cheese samples from seven different countries (13). Orth (9) reported finding PR-toxin-producing *P. roqueforti* isolates in nut ice cream, a dessert cream, and on a red wine bottle cork. However, the significance of these toxins in terms of human health is unclear, particularly because blue-veined cheeses have been consumed for centuries without apparent ill effects.

The potential carcinogenic nature of penicillic acid and patulin (5) possibly makes them a cause for concern. However, work that we have reported elsewhere with M-247 has shown that these toxins may not be produced in proteinaceous substrates, such as cheese (Olivigni and Bullerman, J. Food Sci., in press). Also, it is possible that commercial cultures of *P. roqueforti* used in blue cheese production do not produce penicillic acid and patulin. This latter point was studied in our laboratory, using cultures obtained from commercial suppliers of *P. roqueforti* cultures (Dairyland Food Laboratories Inc., Waukesha, Wis., and Laboratorium Visby, Tönder, Denmark) and cultures isolated directly from blue-veined cheeses. Three commercial strains of *P. roqueforti* and seven isolates obtained from domestic and imported blue, Roquefort, and Gorgonzola cheeses were tested for their ability to produce penicillic acid and patulin. Each culture was grown on 50 ml each of 2% yeast extract–15% sucrose and potato-dextrose (8) broth in 250-ml Erlenmeyer flasks at 12°C for 14 days. In addition, culture M-247 and a patulin-producing strain of *P. patulum*, also isolated from cheese, were grown under the same conditions and used as controls. All cultures were separated into mold mat and broth fractions, and each was extracted separately. The yeast extract-sucrose broth and corresponding mold mats were each extracted with two 50-ml volumes of chloroform. The potato-dextrose broth and corresponding mold mats were each extracted with two 50-ml volumes of ethyl acetate. Samples were concentrated and examined by thin-layer chromatography, as previously described. All commercial cultures and isolates of *P. roqueforti* obtained from the blue-veined cheeses were found negative for both penicillic
acid and patulin. However, control culture M-247 produced detectable amounts of patulin (75 \(\mu g/ml\) on potato-dextrose broth and both penicillic acid and patulin (100 and 35 \(\mu g/ml\), respectively) on yeast extract-sucrose broth. *P. patulum* likewise produced detectable amounts of patulin in both potato-dextrose (2.8 mg/ml) and yeast extract-sucrose (1.4 mg/ml) broths. Thus, whereas the known toxin-producing controls produced easily detectable amounts of penicillic acid and patulin under the conditions of this test, the commercial strains of *P. roqueforti* did not.

Several species of *Penicillium* and *Aspergillus* have been reported to produce either patulin or penicillic acid (2). Ciegler (2) previously listed *P. roqueforti* as capable of producing penicillic acid, but no reference has been found associating it with patulin production. Also, to our knowledge this is the first report of any isolate producing both mycotoxins.

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LITERATURE CITED