

ULTRASTRUCTURE OF A CAFFEINE-RESISTANT MUTANT OF ASPERGILLUS PARASITICUS  
DURING AFLATOXIN PRODUCTION

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Caffeine (1,3,7-trimethylxanthine) inhibits growth and aflatoxin synthesis in A. parasiticus (1). A caffeine-resistant mutant (BCR1) of A. parasiticus NRRL 2999 was isolated (2) in which sporulation and aflatoxin production occurred only in the presence of caffeine. Further, when cultured in glucose-mineral salts (GMS) (3), BCR1 required amino acids in addition to caffeine in order to produce aflatoxin (4). A system for ultrastructure study was thus available in which aflatoxin synthesis was controlled by supplementation with amino acids. Experiments were conducted to identify morphology that could be correlated with aflatoxin production in the mutant.

Cultures were conidia-initiated in GMS at 28C with agitation, with 6% peptone as amino acid source. Caffeine at 4 mg/ml permitted full growth and stimulated maximal aflatoxin accumulation (4). Fungal pellets were retrieved at 4 days and portions of the periphery were fixed in 3% glutaraldehyde in 80 mM Na-cacodylate, pH 7.0, for 3 hr. After 3 rinses in the vehicle buffer, specimens were postfixed in 1% OsO<sub>4</sub> in the same buffer for 4 hr. Specimens were dehydrated in graded acetone and embedded in Spurr's resin. Sections stained with uranyl acetate and lead citrate were observed in a Zeiss EM 10 transmission electron microscope at 60 kV.

A. parasiticus BCR1 from unsupplemented GMS (Fig. 1) had moderately contracted mitochondria and abundant pinocytotic activity contributing to vacuole growth. Light microscopy of lum epoxy sections showed small, complex, randomly spaced vacuoles. With peptone supplement, growth was enhanced but cells displayed less pinocytosis and smaller cell vacuoles (Fig. 2). Mitochondria were more expanded when amino acids were supplied. In caffeine-supplemented cells, with (Fig. 3) or without (Fig. 4) peptone, extreme vacuolization caused cytoplasm and organelles to be compressed into bands. Pinocytotic vesicles were swollen, and mitochondrial matrix compartments were expanded. Caffeine and peptone together produced the largest mitochondria, but without obvious organelle damage.

The caffeine-induced morphological changes most clearly documented were observed both in aflatoxin-competent and -incompetent BCR1 and in the wild type (5) and so appear to be effects coincidental to the role of caffeine in aflatoxin production in A. parasiticus. Mitochondrial compression is normally observed as A. parasiticus changes from exponential to stationary growth. The present results and previous work with NRRL 2999 (5) indicate that caffeine prevents or reverses this compression, but that caffeine also produces swelling of other osmosensitive subcellular compartments and may not be specific in its effect on mitochondria. Ultrastructure unique to the aflatoxin-competent BCR1 cells supplemented with both caffeine and peptone was not observed. Biosynthetic pathways opened in the mutant by amino acids with caffeine appear silent with respect to cell structure (6).

References

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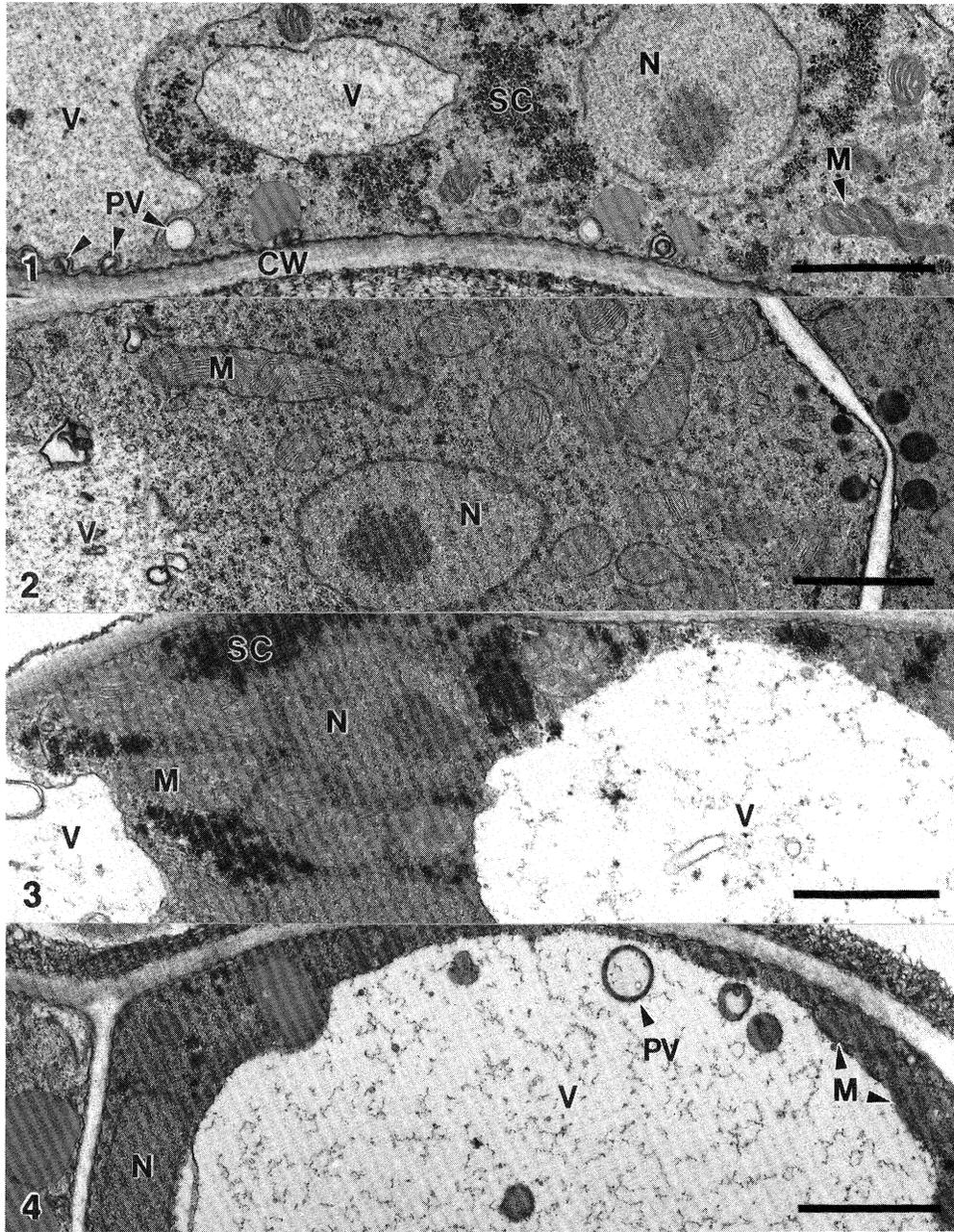


FIG. 1.--Section from hypha of *Aspergillus parasiticus* cultured 4 days with agitation in GMS medium. CW = cell wall; SC = storage carbohydrate; M = mitochondrion; N = nucleus; PV = pinocytotic vesicle; V = vacuole. Bar = lum.

FIG. 2.--*A. parasiticus* cultured as above in GMS + 6% peptone. M = mitochondrion; N = nucleus; V = vacuole. Bar = lum.

FIG. 3.--*A. parasiticus* cultured as above in GMS + 4 mg/ml caffeine + 6% peptone. M = mitochondrion; SC = storage carbohydrate; V = vacuole; N = nucleus. Bar = lum.

FIG. 4.--*A. parasiticus* cultured as above in GMS + 4 mg/ml caffeine. M = mitochondrion; N = nucleus; PV = pinocytotic vesicle; V = vacuole. Bar = lum.