Turian G., N. Oulevey and F. Tissot, Preliminary stucies on pigmentation and ultrastructure of microconidia of Neurospora crassa.

Parallel to our current studies on macroconidiation in the wild type N. crassa (Turian 1966 Proc. Symp. Colston Res. Soc. 18:61), we have been led to consider the process of microconidiation in a morphological mutant, the fluffy strain (L) A (FGSC#45) which is unable to form macroconidia.

Acetate stimulates macroconidiation in the wild type (Turian 1961 Compt. Rend. 252: 1374) and has also been found to favor the formation of microconidio in the mutant. A slightly modified Westergaard and Mitchell P synthetic medium (1947 Am. J. Bot. 34:573) with either 1% Na acetate replacing half of the normal 2% sucrose (PSA) or just 2% No acetate (PA) as single C source has therefore been used to produce an abundance of microconidio rather than the less well chemically defined enrichment of P formula with casein hydrolysate (Grigg 1965 NN#7: 12) or Vogel's medium with complex extracts (Baylis and DeBusk 1965 NN#7:7).

After 12 days of stationary growth at 25°C on PSA or PA liquid medium (250 ml in 1 liter Fernbach flasks), the fluffy strain forms a dry surface mat which has an orenge-brownish, powdery appearance due to a full cover of arbusculate microconidiophores. Each compartment (cell) of the microconidiophore has a repetitive capacity to produce uninucleate microconidio (Dodge 1935 Mycologia 27:418). For the harvest, the mats were taken out of the flasks with a pair of forceps (fertile surface folded in) and vigorously shaken in a glass-stoppered cylinder in the presence of distilled water containing I-2 drops per liter of a non-ionic detergent. The dense suspension obtained was filtered through a fine nylon net to remove hyphal debris and broken microconidiophorer. Centrifugation (3000 r.p.m.) provided surprisingly dork, blackish-brown pellets which were then dispersed in acetone-water (1:1), united and diluted to a known volume in a glass-stoppered cylinder. Immediately after vigorous shaking, known aliquots were diluted for determining microconidial number in a hemocytometer. The net harvest of filtered microconidio averaged about 10^8-10^9 cells per flask of PSA medium.

After centrifugation, the carotenoids contained in the microconidial pellet were extracted 3 times with pure acetone and then transferred into petroleum ether for further extraction (a), while the remaining dork pellet was kept for melanin pigment extraction (b).

(a) Total carotenoids were estimated spectrophotometrically at 460 mμ (Krzeminski and Quackenbush 1960 Arch. Biochem. Biophys. 88:287) in the petroleum extracts after concentration under nitrogen; they averaged 2 pg per 103 microconidia on PSA and 6 pg per 10^3 conidia on PA. After overnight hydrolysis with an alcoholic KOH solution, the neutral first and subsequently, after HCl addition, the acidic carotenoid (s) could be separately transferred to 'he petroleum ether epiphase and measured at 468 mμ (neutral carotenoids) and 472 mμ (acidic carotenoids); on both media a ratio of 2:1 was obtained in favor of the acidic carotenoid(s) which gave the typical absorption curve of neurosporaxanthin (Zalokar 1957 Arch. Biochem. Biophyr. 70:568). This ratio appears to be higher than that generally obtained from free macroconidia (Zalokar 1954 Arch: Biochem. Biophys. 50: 71).

(b) Melanin pigments were extracted by refluxing twice for 1 hour in 2 N NaOH. The total brownish-black extract gave a decreasing linear absorption slope from 400 to 600 mμ. This is indicative of the melanin nature of this microconidial pigment(s), similar to that previously described in the mycelium of a block mutant (Schaeffer 1953 Arch. Biachem. Biophys. 47:359).

Initial chemical fractionation of walls prepared from lyophilized microconidia first ground in dodecylsulfate in the presence of glass beads and then cleaned according to Mahadevan and Tatum (1965 J. Bacteriol. 90:1073) method has revealed that a major part of the melanin pigment remains associated with the chitin-containing fraction.

For the ultrastructural study of the microconidio, the cells have been fixed in 2% KMn04 for 4 hours and subjected to a post-fixation with 1.5% uranyl acetate in 75% acetone. After dehydration, the specimens were embedded in an Araldite mixture (Durcupan ACM).

Microconidial ultrastructure is characterized by a high nucleo-cytoplasmic ratio expressed as maximal occupancy of the nucleus in the cell. The main features of the cytoplasm are: very few vacuoles, presence of a thin endoplasmic reticulum, small scattered mitochondria which have few cristae, lipid bodies, and dark, supposedly reserve, granules. On the microconidiophores harvested and fixed directly from the powdery surface of the cultures, interesting, lamellated collerettes hove been observed which surround the site of emission of the microconidia budded from their mother cell. - - - Laboratory of Microbiology, Institute of General Botany, University of Geneva, Switzerland.