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## Excretion of low molecular weight, folin-positive metabolites by the female receptor mycelium, in response to mating.

In a previous communication (Cruz and Terenzi, 1981 Neurospora Newsletter 28: 8) we described an experimental procedure which permits the observation of biochemical phenomena associated with sexual development in Neurospora. In brief, it consists of growing a "female receptor" mycelium of an extra-fertile strain in liquid Westergaard and Mitchell crossing medium for seven days at 25° C without agitation. After this time the mycelial mat is covered with a heavy conidial suspension from a strain of the opposite mating type. This treatment rapidly promotes marked biochemical changes in the femalereceptor mycelium, such as synthesis of tyrosinase and L-amino acid oxidase, a decrease in the level of low molecular weight sulfhydryl metabolites (Prade and Terenzi, 1982 Biochem. Genetics 20: 1235) and the excretion of a brown-yellowish substance which reacts as phenol with the Folin reagent. The present report concerns this latter phenomenon.

In the experiment shown in Figure 1 A and 8, the female receptor mycelium was submitted to mating (A) or to starvation in phosphate buffer (B). Both treatments stimulated tyrosinase synthesis, which occurred earlier in the mated cultures. Release of Folin-positive material into the culture medium was observed in mated but not in the starved cultures, suggesting that the excreted material was not a product of endogenous tyrosinase activity.

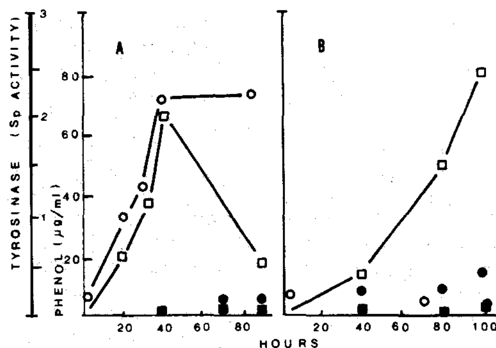


Figure 1. -- Cultures of the extra-fertile strain BAT 9-4 were prepared in standard petri dishes containing 15 ml of Westergaard and Mitchell crossing medium, and incubated for seven days at 25° C without agitation. At that time the cultures were treated as follows: (A) a heavy suspension of conidia from the opposite mating type was evenly distributed on the surface of the mycelial mat. (B) the culture medium was removed by aspiration, the mycelium was rinsed twice with sterile distilled water and was resuspended in 10 ml of phosphate buffer 0.1 M, pH 6.0. At the indicated times sample cultures were taken and tyrosinase activity was determined in the crude mycelial extracts (Fling et al., 1963 J. Biol. Chem. 238: 2045). Folin-positive material was quantified in the culture medium. Symbols: £, I: tyrosinase activity; O,~ Folin-positive material. Open symbols correspond to treated cultures and closed symbols to untreated controls.

Figure 2 A shows the absorbance profile of the culture medium before and 30 hours after mating. A shoulder can be observed between 200 and 350 nm in the spectrum of the culture medium after mating. In the same figure is shown the spectrum of material extracted with n-butanol (B) and with ethyl acetate (C). As can be seen, ethyl acetate extraction removed from the culture medium of the mated mycelia a material absorbing at 345, 275 and 217 nm This material was not present in the medium of unmated cultures. Recovery data (not shown) indicated that ethyl acetate extraction removed from the mated culture medium 71% of the Folin-positive material and an equivalent amount of the material with the characteristic absorbance profile shown in Figure 2 C. A sample of the ethyl acetate extract was analyzed by thin layer chromatography in silica gel using n-butanol:ethanol:water (4:1:5) as solvent. A major and two minor untraviolet-absorbing spots were visualized, with Rf of 0.70, 0.57 and 0.50 respectively. The absorbance spectrum of the major component, that is, the material with Rf of 0.70 was closely similar to that of the ethyl acetate shown in Figure 2 C.

In a preliminary study it was concluded that, according to spectral and chromatographic properties, the material with Rf 0.70 was not tyrosine, phenylalanine, tryptophan, phenylpyruvic acid, p-OH phenylpyruvic acid, anthranilic acid, shikimic acid, chorismic acid, prephrenic acid or p-aminobenzoic acid. The biological origin and chemical nature of this substance (substances?) seems interesting because it appears to be related to a physiological response triggered by a specific cell-cell interaction. We are at the present trying to identify the chemical nature of this substance as a preliminary step toward the clarification of its metabolic origin and its relationship with the processess of sexual development.

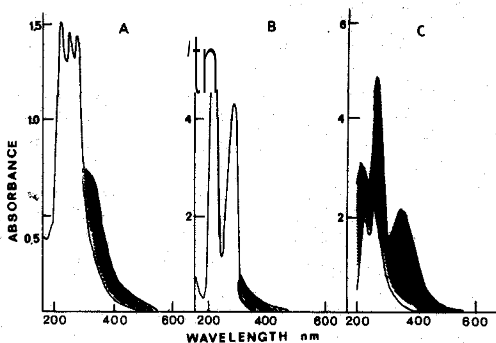


Figure 2. -- Absorbance profile of the culture medium of mated and unmated cultures. Equal volumes of medium from unmated and 30 h mated cultures were lyophilised and (A) redissolved in 0.1 N HCL, (B) redissolved in 0.1 N HCl and extracted three times with three volumes of n-butanol, evaporated and redissolved in 0.1 N HCl, (C) same as B but using ethyl acetate for extraction. The spectra were determined against 0.1 N HCl. The regions of the profile of the mated culture medium different from that of the unmated culture medium are shown shadowed.

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