Graham, J. D. The use of malachite green to determine disruption of conidia by sonification.

Our laboratory has been studying the effects of ultraviolet light upon the ribonucleic and deoxyribonucleic acids of Neurospora crassa. The effects of UV on the rote of synthesis of DNA and its effects on the nucleosides and nucleotides are being explored. The most difficult barrier to this work has been the resistance of the conidiol case to most simple methods of disruption. We found that normal acid hydmlysir had little or no effect on the strain under study, to the extent that at least 20% of the conidia were viable after a 10-minute hydrolysis with concentrated HCl. Homogenization war also unsuccessful. The criterion for a successful conidial disruption method in this study was 100% disruption of the conidia without destruction of the primary structure of the DNA molecule and the individual nucleosides and nucleotides. Cellular viability and integrity (as opposed to conidiol) was not essential. The choice of ultrasound over liquid nitrogen or carbon dioxide freezing was made on the basis of ease of handling.

Disruption was produced with a microprobe sonifier (Branson Instruments, Inc.). The conidia were suspended in 10 ml of water (5 x 10^6 cells per ml) in a thick glass tube, which was immersed in ice water. The microprobe of the sonifier was placed in the solution to a depth of 1 1/2 cm. The sonifier was tuned to intensity level 7 and the current to between 4 and 5 amperes. The conidiol suspension was sonified for 60 seconds.

In order to determine the success of the disruption technique, a malachite green stain was used. The conidiol suspension was smeared on a slide, cleaned with chromic acid solution before use. The slide was air-dried and the cells fixed to the slide by passing it over a flame several times. The fixed slide was placed on top of a boiling water bath and covered with a piece of filter paper. A 5% malachite green solution was dripped onto the filter paper, keeping the paper thoroughly moistened for the five-minute staining period. The slide was rinsed with distilled water. Counterstaining, if desired, was done with 0.4 molar safranin for 45 seconds. Staining by this method was strong and quite acceptable for purposes of visualizing the extent of conidial disruption. Absence of intact conidio and the presence of malachite green-stained frogmen's were used as indications of conidial disruption. If intact conidio were present, disruption was less than complete. In repeated trials, it was found to be more than 96% complete.

Further studies showed that the DNA isolated was highly polymerized. Also, nucleotide ond nucleoside spectra and Rf'r corresponded well with the literature standards. One of the primary advantages of this method was the speed and simplicity of its use. It was possible to prepare the conidiol suspension, sonify, and prepare and stain the slides all within 15 minutes.

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