Nuclear Distribution in the Mycelium of *Claviceps* and the Problem of Strain Selection

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Cytological studies of the developing mycelium of *Claviceps* suggest that genetic uniformity may be obtained in strains derived from single spores.

Reports on variation of morphological characteristics and the production of alkaloids by strains of *Claviceps* appear as a general theme to much of the work on alkaloid production in submerged cultures (e.g., 1-4, 7; A. Mizrahi, Ph.D. Thesis, Hebrew University, Israel, 1968). Most of the studies were performed with no consideration of the genetic background of the tested strains. Even studies on strain selection (1, 4, 7; A. Mizrahi, Ph.D. Thesis, Hebrew University, Israel, 1968) were performed by the isolation of single colonies derived from macerated mycelium which was originally obtained from a sclerotium. However, it has been shown (5; A. Mizrahi, Ph.D. Thesis, Hebrew University, Israel) that the cells of *Claviceps* contain a number of nuclei. Such cells could be either homokaryotic or heterokaryotic depending on the origin of the culture. The basis for any selection must rely on earlier knowledge of the nuclear behavior in the development of the mycelium. Therefore, a cytological study was undertaken to examine ways of obtaining genetically uniform strains.

The distribution of nuclei in the spores and the mycelium of a strain of *C. paspali* ATCC 14591 was followed by staining the nuclei during the development of the mycelium. A spore suspension was sown in a drop of liquid malt extract on glass slides. The slides were kept in a humid petri plate, and at various time intervals a slide was stained with Giemsa by the procedure of Namboodi and Lowry (6) with Carnoy fixative.

The alteration in the number of nuclei in the spores is shown in Table 1. It should be noted that the spores are initially predominantly uninucleate, but binucleate and trinucleate spores can be found. However, in conditions inducive to spore germination, a gradual increase in the number of nuclei per spore follows. Within 24 hr, only a few uninucleate spores can be seen and the majority of the spores contain more than two nuclei. This transition suggests that the origin of the multinucleate spores as well as the binucleate and trinucleate spores is from uninucleate spores.

Germination of the spores was initiated ca. 20

| Time (hr) | 1 | 2 | 3 | >3 |
|-----------|---|---|---|----|
| 0         | 161 | 36 | 1 | 0  |
| 24        | 1  | 10 | 15| 21 |
| 48        | 0  | 1  | 3 | 12 |

**Table 1. Number of nuclei in the spores prior and subsequent to germination**

**Table 2. Nuclear distribution in 16 germlings 48 hr after exposure to conditions inducive to germination**

| Spore | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-------|---|---|---|---|---|---|---|---|---|----|
| 6     | 6 | 2 | 2 | 1 | 2 | 13|
| 4     | 4 | 2 | 4 | 2 | 15|
| 5     | 4 | 5 | 4 | 12|
| 3     | 3 | 4 | 3 | 15|
| 7     | 5 | 4 | 3 | 3 | 3 | 3 | 3 | 15|
| 8     | 4 | 2 | 3 | 3 | 6 | 5 | 14|
| 6     | 2 | 4 | 3 | 3 | 2 | 4 | 17|
| 5     | 2 | 2 | 2 | 3 | 3 | 3 | 2 |
| 1     | 2 | 2 | 2 | 3 | 2 | 3 | 25|
| 8     | 3 | 2 | 3 | 2 | 5 | 11|
| 2     | 3 | 3 | 2 | 2 | 2 | 2 | 3 | 15|
| 3     | 3 | 1 | 3 | 2 | 2 | 3 | 2 | 13|
| 6     | 2 | 1 | 2 | 13|
| 3     | 2 | 2 | 12|
| 5     | 3 | 8 | 2 | 6 | 4|
| 4     | 6 | 5 | 7 | 5 | 12|
hr after the spores were sown on the slide. The 24-hr sample contained spores with a germ tube and ungerminated spores. Some of the germ tubes were already septated. Both the septated and non-septated germ tubes contained from four to eight nuclei.

After 48 hr, the germlings consisted of 2 to 10 cells. The nuclear distribution from the terminal cell to the spore was determined (Table 2); with few exceptions the cells were multinucleate. The terminal cell in most cases contained more than 10 nuclei. This observation, together with the information on the germ tubes, suggests that, once a separation between the spore and the germ tube is attained, rapid nuclear division takes place within the terminal cell, unless septation in the subterminal cells obscures the divisions occurring in those cells. The nuclear distribution recorded after 48 hr is maintained in samples examined after 96 hr.

The results suggest that selection of strains derived from single spores should constitute the means to obtain genetically uniform strains. Although such strains will eventually accumulate new mutated nuclei, these can be separated from a heterogenous population via single-spore isolation. Attainment of genetic uniformity by isolation of hyphal fragments is only a matter of chance.

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