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Uptake of bacteriophages by *Neurospora crassa* during heterocaryosis.

Neurospora crassa cells are capable of taking up exogenous DNA (Szabo and Schablik 1973 Neurospora Newsl. 20: 27). Here we report that uptake of viruses in Neurospora can occur during heterocaryosis.

The experiments were carried out with the Neurospora strains *trp-1;his-1 A* and *ad-4;his-1 A* as recipients of the *E. coli* phage, Φ 80. The uptake of phage was followed by their plaque forming ability on *E.coli* after reisolation from the Neurospora cells.

Liquid Vogel's minimal medium (5 ml) containing 1.5% sucrose, 0.01M MgS04, and 100 µg/ml histidine, was inoculated with both *trp-1;his-1 A* and *ad-4;his-1 A* conidia and with phage (final concentration, 2×10^8 plaque-forming units/ml). To force heterocaryosis, neither tryptophan nor adenine was added. Incubation was carried out at 25°C for 24 h when the heterocaryotic mycelia were collected on a filter and exhaustively washed with distilled water. To inactive any residual extracellular phage, 1 ml Φ 80-antiserum was added for 60 min. After the antiserum treatment, no plaque-forming phage could be detected in the medium. The mycelia were washed again with distilled water, and then were disrupted to look for presumed intracellular phages. The Neurospora cell wall was enzymatically digested with 10% glucuronidase in sorbitol buffer, pH 5.7 (1.3M sorbitol, 0.02M maleate, 2mM Tris, 2mM EDTA), for 120 min at 25X. The protoplasts were disrupted by addition of distilled water and ultra sonication. To check for plaque-forming phage, samples of the cell homogenate were mixed with *E.coli* cells and plated on TB agar (1% tryptone, 0.5% NaCl, 1.2% agar). By this method we detected a total of 3 X 104 plaque-forming phages in the homogenate, which corresponds to about 0.003% of the plaque-forming units added to the incubation mixture. Because an unknown quantity of phage might have lost their plaque-forming ability after their uptake in the Neurospora cells, we cannot be certain of the intracellular phage titer.

TABLE 1

Cultivation of phage-infected mycelia in phage-free medium (days)	0	1	3	5
% plaque forming phages in the homogenate	100	20	5	2

Mycelia of one incubation were divided into four equal parts by wet weight after the antiserum treatment.

When phage-infected mycelia were treated with Φ 80 antiserum and then cultivated in phage-free medium for 1, 3 or 5 days before the cells were disrupted, the number of plaque-forming phages observed in the homogenate decreased continuously (Table 1). The decrease could be due to the degradation of the phage coats by Neurospora proteases.

The method described here offers a possibile way in which to transfer genetic information from unrelated species to *Neurospora crassa*.

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