

## Formation of a large number of conidia

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The addition of cotton to the medium in flasks can result in a significant increase in the number of conidia from *Neurospora crassa* strains (wild type and mutants).

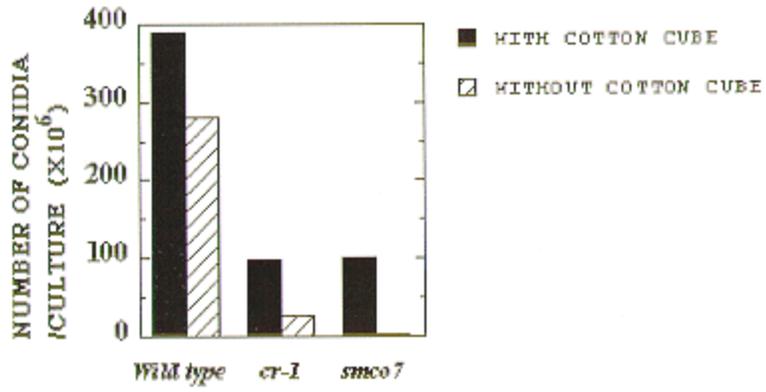
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In *Neurospora crassa*, a large number of conidia are needed for transformation experiments, or cultivation of mycelia for DNA/RNA isolation. However, it is often difficult to obtain them from some morphological mutants which form few conidia (e.g. *smco7*). Sometimes, not enough conidia are formed for various experiments even in the wild type. We have developed the easy and simple cotton method which consistently yields a large number of conidia. This protocol simply involves the addition of a piece of cotton to the medium.

After 15 ml of Fries minimal medium containing 1.5% saccharose (sucrose) was poured into a 100 ml flask containing 0.2 g agar (final concentration 1.5%), 0.5 g cotton cube was added to the center of the medium. As a control, the same medium without a cotton cube was prepared. The flask with the cotton plug must be placed horizontally in the autoclave to avoid the medium and cotton cube inclining to one side of the flask.

Two hundred microliters of a conidial suspension ( $1 \times 10^6$ /ml) from the wild type (74-OR23-1A, FGSC2489) and two morphological mutants, *cr-1* (FGSC# 4009) and *smco7* (FGSC# 4253) were inoculated on the surface of the medium. DO NOT inoculate conidia on the cotton. After these cultures were grown for seven days at room temperature, 30 ml of H<sub>2</sub>O was poured into each culture and the cotton cube was squeezed using a spatula. The number of conidia in each suspension was counted using a hemocytometer and the average of three cultures for each strain is shown in the Figure 1. The ratios of the number of conidia with cotton cube versus without a cotton cube were 1.4 ( $3.9 \times 10^8 / 2.8 \times 10^8$ ) in the wild type, 3.8 ( $9.8 \times 10^7 / 2.6 \times 10^7$ ) in *cr-1* and 59 ( $1 \times 10^8 / 1.7 \times 10^6$ )

in *smco7*. In morphological mutants such as *cr-1* or *smco7* which form few conidia, this method has proved to be consistently effective. In a culture of the wild type grown on medium which contained just cotton cube as the sole carbon source, no conidia were detected. We propose that the cotton is not a carbon source but act as a support for the growth of aerial hyphae.



**Figure 1.** The number of conidia formed in the medium with cotton cube and without cotton cube.

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