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Neurospora as a laboratory contaminant.

R. W. Barratt

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Abstract

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Barratt, R. W. Neurospora as a laboratory contaminant.

It may seem inappropriate in an issue of the Newsletter devoted largely to laboratory experiments using Neurospora to include a note on its control. Yet many biologists have refused even to have Neurospora cultures around the laboratory, to say nothing about permitting students

to work with them. Such an attitude has the weight of the testimony of many investigators who in a moment of weakness let down the bars just once, only to suffer widespread contamination of valuable microbial stocks by that "dirty orange bread mold". (This attitude has even been known to work against the employment of Neurosporologists.) The difficulty arises from the ease with which Neurospora macroconidia are dispersed aerially, their longevity under laboratory conditions, the high rate of mycelial growth (4-5 mm/hr) once established on suitable media, and the fact that the fungus will grow readily on many common laboratory media. When then can be done to circumvent these real drawbacks to the use of Neurospora in class experiments?

Several years ago when I was teaching a course in "fungal genetics of filamentous fungi" with E. Kafer at the Cold Spring Harbor laboratory and simultaneously using Neurospora crassa, with its spreading growth habit, and Aspergillus nidulans , with its restricted growth habit, we were faced with this problem in an acute form. Other than the obvious standard microbiological sanitary procedures, including wiping the laboratory bench down with a disinfectant (Lysol or dilute Chlorox solution) prior to and after use, and spraying the air with propylene glycol to settle conidia, we found that very effective control was accomplished by increasing to between 39 and 40C the temperature of incubators being used for other organisms. Ryan, Beadle and Tatum (1943 Am. J. Botany 30:784) reported that "at a temperature above 40C the rate of growth (of mycelia) progressively slowed down and eventually reached zero." Neurospora has a sharp temperature optimum at 34-35C. They further showed that Neurospora has a pH optimum of 5-6 with a marked decrease in growth rate above 6.5. Their experiments were carried out in growth tubes on Fries minimal medium (which is poorly buffered) which showed a decrease in pH as the mycelial front progressed. Thus, well-buffered medium at pH 7 or above provides a poor environment for the growth of Neurospora, though it may not prevent its growth completely.

More recently, two additional methods have become available which help to avoid the contamination problem. Aspergillus workers frequently add sodium desoxycholate (800 mg/liter) to restrict colony size and to enhance conidiation. Many other fungi (e.g., Penicillia) are also resistant to desoxycholate. Neurospora, on the other hand, is completely inhibited by low concentrations of desoxycholate.

In many experiments and experimental techniques, strains of Neurospora with restricted growth rates (colonials), aconidial strains (fluffy), or microconidiating strains can be substituted for wild type macroconidiating strains. Colonial strains have much lower growth rates and are no more of a laboratory hazard than normal fungal aerial contaminants (Penicillium, Aspergillus, Fusarium, Alternaria). Perkins employs aconidial (fluffy) stocks as mating type testers. Microconidia are short-lived, are not borne on long hyphal filaments and conidiophores, and are thus much less of a source of aerial contamination.

Lastly, and as a general practice, whenever practical, students should be provided with aqueous suspensions of Neurospora conidia rather than making any transfers of dry conidia. Further, any petri plate contaminated by Neurospora should be destroyed by autoclaving immediately upon its discovery, and students should be forewarned of the contamination problem. Each of us has his pet story about Neurospora contamination, but one more note of caution is worth mentioning: never discard agar or other suitable substrata into a wastebasket. Neurospora will find it and conidiate abundantly within 2 days. Paranoid individuals should employ only suitably tagged strains (e.g., albino) to avoid the unjustified wrath of their colleagues.

Other Neurospora workers may have developed methods to reduce the risk of contaminating a laboratory with Neurospora. If so, I encourage them to communicate with the Newsletter editor. To end on a note of optimism, we have never, to the best of our knowledge, cross-contaminated any of the over 1,200 stocks of Neurospora in the FGSC. So the problem is not insurmountable.