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GUEST COMMENTARY

Evidence for Safety of *Neurospora* Species for Academic and Commercial Uses

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Fungi of the genus *Neurospora* inspired the development of microbial genetics and initiated the molecular revolution in biology by demonstrating that genes encode enzymes. Because of its useful biological attributes, *Neurospora crassa* has become a favored organism for research in a variety of biological problems and a basic model organism among the filamentous fungi. A vast store of information has been acquired on the organism during 75 years of research. Over 1,000 loci have been mapped on the chromosomes. Genome sequencing, now in progress, is expected to be completed within a year.

Fungi of the genus *Neurospora* have been known in the scientific literature since 1843 (23). The species *N. crassa* has been used intensively in many laboratories since 1941 (5, 24, 25). Generations of investigators in hundreds of laboratories have used the organism, with results reported in thousands of research papers. (The bibliography of reference 25, for example, contains 2,300 references.)

The conidiating *Neurospora* species *N. crassa*, *N. sitophila*, *N. intermedia*, *N. tetrasperma*, and *N. discreta* are conspicuous in nature because of their distinctive orange color, rapid growth, and profuse production of powdery conidia. Extensive observations have been made of the occurrence of *Neurospora* outside the laboratory on natural and artificial substrates throughout the world (26, 37). The organism is typically found in moist tropical or subtropical climates. Because dormant ascospores are activated by heat, blooms occur on burned vegetation.

Never in more than a century of observation and experimentation has the genus been implicated in human disease or observed to cause disease in animals or plants. Sequencing of the *Neurospora* genome, soon to be completed, is expected to stimulate increased use of the organism in academic and commercial settings. Certification may be requested that *Neurospora* is not pathogenic or hazardous. Questions of safety will be raised and documentation will be required by regulatory agencies. For example, the U.S. Department of Agriculture Animal and Plant Health Inspection Service issues regulations for import and shipping of living organisms. This agency and others concerned with transport of living organisms or with workplace safety may require application for a permit certifying that *Neurospora* is not a pest or pathogen. One object of the present communication is to provide such documentation.

***Neurospora* species are apparently incapable of causing disease in animals.** Certain innate characteristics make it unlikely that *Neurospora* will have adverse effects on animals. Unlike yeasts, *Neurospora* species are obligate aerobes, unable to grow

in the gut or bladder, in tissues, or systemically. Aside from its use in the laboratory, *Neurospora* has long been known to occur in close association with humans in contaminated bakeries (23, 32, 39), lumber yards, and plywood factories; on steamed logs (30); on the stubble of burned sugar cane fields (20); and on burned grass along railways and roads (26). (Different conidiating *Neurospora* species are indistinguishable on the basis of gross appearance.) Some conidiating blooms are enormous. For example, sugar cane bagasse baled for use in manufacturing fiberboard may become blanketed with the orange mold. Dense growth and conidiation may occur over many acres when filter mud from sugar refineries is used to fertilize fields (29). *Neurospora* appears in orange blooms following volcanic eruptions (3, 31 [and references therein]), urban and suburban fires (12), and slash-and-burn clearing of rain forest (26). Conidiating colonies are found on cooked corncocks discarded in tropical marketplaces and on temple offerings in Bali (26). Despite the many opportunities for human exposure to the powdery airborne conidia, no evidence has been obtained that *Neurospora* is the causal agent of any disease or infection.

Despite its abundant, readily airborne conidia, *Neurospora* does not appear from the medical literature to be a significant allergen. Medical textbooks on allergy either fail to mention *Neurospora* (e.g., see references 7, 11, 17, and 22) or refer to the vegetative spores (under the anamorph name *Monilia sitophila* or *Chrysonilia sitophila*) as components of airborne spore flora, hence possible allergens. One text (10) refers to *Monilia sitophila* as a possible aeroallergen, prominent in volumetric collections of airborne spores in tropical areas but favoring bakeries and flour mills in cooler regions. Another text (2) reports a weak skin test reaction to *Monilia sitophila*, which is stated to be associated with the milling and baking trades. Two of 526 allergy patients in one study showed positive skin and nasal tests in response to *Neurospora* (1). One case of allergic alveolitis has been reported following the use of a heated, enclosed swimming pool where both *Neurospora* and a thermophilic actinomycete were abundant (19).

Most textbooks of medical mycology attribute no significant pathogenic effects to *Neurospora*, either failing entirely to mention it (e.g., see references 14 and 15) or simply listing it as a potential laboratory contaminant. However, two texts (14, 34) cite a 1962 report of *Neurospora* growing in the inner eye following cataract surgery (36). The report also cites adventitious eye infections by other nonparasitic fungi following surgery and stresses the importance of ensuring that operating rooms are free of mold spores. There has been no report of *Neurospora* forming a “fungus ball” in the lungs as *Aspergillus fumigatus* is known to do.

These reports all involve *Neurospora* from sources outside

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the laboratory. Exposure to airborne spores would be negligible in any well-regulated laboratory or industrial establishment. The medical literature can be taken to indicate that *Neurospora* is almost wholly benign in terms of allergenic or infectious potential.

Rather than being avoided, *Neurospora* has in fact been put to use in several human societies, either as a food or in processing foods and beverages. It is a major constituent of onchom, a highly nutritious soybean-based presscake produced for human consumption as a cottage industry in west Java, where it is distributed daily in marketplaces and shops (16, 33, 38). It also is used in preparing oriental foods such as koji (9, 18 [and references therein], 33). Indigenous tribesmen in Brazil use *Neurospora* to process cassava in preparing a fermented beverage (21). In Borneo, the Iban people collect the orange fungus called kulat amau from burnt-over hill rice fields and use it as food (28). *Neurospora* was present regularly in Roquefort cheese prepared by traditional methods in southern France (6). These informal tests of human populations, lacking any safety safeguards and with no evidence of toxicity or other disagreeable consequences, add an important dimension to our knowledge of the safety of *Neurospora*.

Other fungi considered safe for food include not only *Saccharomyces cerevisiae* but also *Penicillium camemberti*, *Penicillium roqueforti*, *Aspergillus oryzae* (= domesticated *Aspergillus flavus*), and *Fusarium venenatum*. These four filamentous fungi either are capable of producing mycotoxins or are close relatives of toxigenic fungi, whereas *Neurospora* is not. No dangerous secondary metabolites are known to be produced by strains of any *Neurospora* species. Thus, *N. crassa* and other conidiating species of the genus *Neurospora* are better qualified than the others to be recognized under Food and Drug Administration (FDA) regulations as a GRAS ("generally recognized as safe") organism (see the FDA website [http://vm.cfsan.fda.gov/~dms/opa-micr.html]).

***Neurospora* is not a plant pathogen or plant pest.** Burned or scorched vegetation is a natural substrate, but *Neurospora* has never been observed to invade living plant tissue or to cause disease in a plant. In 1989, an official of the U.S. Department of Agriculture Animal and Plant Health Inspection Service ruled that *Neurospora* species "are not subject to Federal Plant Pest Act regulations" (U. S. Department of Agriculture Animal and Plant Health Inspection Service PPQ Form 526, *Permit to Move Live Plant Pests*, no. 4432, issued by B. P. Singh to D. D. Perkins, 14 June 1989).

Contamination by *Neurospora* is readily controlled. Because of its high growth rate (3 to 5 mm/h) and the ease with which the powdery conidia become airborne, *Neurospora* has gained a reputation in some quarters as a laboratory contaminant. This reputation is largely undeserved. Cross-contamination or overgrowth of slowly growing cultures by *Neurospora* is not a problem in any well-ordered research laboratory. Good laboratory practice includes avoidance of drafts, attention to cleanliness, autoclaving of discarded cultures and contaminated glassware before dishes are washed, and care not to incubate cultures in closed containers in which humidity approaches 100%. Thousands of *Neurospora* strains are maintained in pure culture, without problems of contamination, in leading research laboratories and at the Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City.

Outside the laboratory, *Neurospora* can occasionally be a nuisance. Contamination of bakery products has sometimes been a problem, not because of any hazard but because of spoilage. Bakery contamination is now largely controlled by improved sanitation and by the use of Food preservative chemicals such as propionate. *Neurospora* may overgrow cultured

mushroom beds (18 [and references therein]), and it has been known to invade optical equipment in the humid tropics (8). None of these transgressions can be considered dangerous.

Summary and conclusions. *N. crassa* is the preeminent model organism among filamentous fungi, with a long history of seminal contributions, a wealth of basic information, and prospects for expanded use as the complete genome sequence becomes available. Seventy-five years of use in research laboratories and a long history of association with humans and their activities have failed to reveal any cause for concern regarding the safety of *Neurospora*, either as a pathogen or as a toxin producer.

Although it is impossible to prove the negative assertion that *Neurospora* is harmless, experience clearly places the burden of proof on those who would claim that the fungus is unsafe. In absence of any indication that the organism is hazardous, we conclude that *N. crassa* and other *Neurospora* species qualify for exemption from restrictive regulation, equivalent to that granted the brewers' and bakers' yeast *Saccharomyces cerevisiae*.

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