A Selective Technique for the Isolation of *Neurospora crassa* from Soil

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INTRODUCTION

Neurospora is among the most studied of all eukaryotes. It is a convenient organism for the study of genetic polymorphism through analysis of isozymes (Yu, Garrett & Sussman, 1971; Reddy & Threlkeld, 1971, 1972), and the possibility of population studies with *Neurospora* has been demonstrated by Perkins (1970) who collected isolates from over 60 localities in the tropics. However, Perkins' collection was derived from visibly growing colonies on vegetation or upon the soil surface in burned areas, so selection of certain forms may have occurred. To be more representative of the potential genetic variability in nature, collections should include samples from a diversity of soil habitats. We report here on a technique found effective for the isolation of *Neurospora crassa* from soils in India.

METHODS

The soil samples analysed were collected from over 30 localities in Mysore, Goa and Delhi during 1972–3. About 500 g of soil from a 30 × 30 cm (1 × 1 ft) area were removed from the upper 7.5 to 10 cm layer and packaged in polythene bags. These were stored at 7 °C or at room temperature until use, one to six months later. Soil (3 to 5 g) was suspended in 10 ml 10⁻³ M-furfural and heated to 60 °C for 30 min. The liquid was then removed by low-speed centrifugation and portions of soil were plated in several Petri dishes containing Vogel's N medium (Davis & de Serres, 1970) acidified to pH 4.0 immediately before use with 1 N-HCl to prevent bacterial growth. The Petri dishes were incubated under fluorescent light in a chamber at room temperature which ranged from 20 to 30 °C.

Crosses were made in test tubes on Westergaard and Mitchell's crossing medium (Davis & de Serres, 1970) containing filter paper strips as the carbon source. The protoperithecial parent was inoculated 5 to 7 days before fertilization by conidia from the other parent. For compatibility and fertility tests, the standard strains shown in Table 1 (obtained from Fungal Genetics Stock Center) were used as protoperithecial parents.

RESULTS AND DISCUSSION

The isolation procedure described yielded *Neurospora crassa* from two only of over 30 soil samples screened. One of these was collected from a coconut plantation having an undergrowth of grass and *Mimosa* sp., situated on the banks of a river in Goa, and it was dark brown and clayey with a pH of 5.0. The other sample, which was black, clay loam rich in humus, with a pH of 7.0, was collected from the shady slopes of a hill near Bangalore covered with a thick growth of shrubs, at an altitude of 1200 m.

Table 1. *Standard strains used in compatibility and fertility tests*

FGSC no.	Locus	Allele (isolation no.)	Mating type	Linkage group
987	w.t. (St Lawrence)	74-OR23-IA	A	—
988	w.t. (St Lawrence)	74-OR8-1a	a	—
45	fl	L	A	IIR
46	fl	L	a	IIR
1838	fl	fl ^p	A	IIR
1690	fl	fl ^p	a	IIR

w.t. = wild type; fl = fluffy; fl^p = fluffy, pH-sensitive allele.

Table 2. *Germination of ascospores from crosses of wild Neurospora crassa isolates from soil*

Spores were germinated in distilled water on a reciprocal shaker for 24 h at room temperature. Germination counts were based on at least 100 spores.

Treatment	Germination (%)
Control	Nil
Heat-shock at 60 °C for 30 min in water	80
Heat-shock at 60 °C for 30 min in 10 ⁻³ M-furfural	81
10 ⁻³ M-Furfural for 30 min	76
65 % ethanol for 15 min	12
As above + heat-shock at 60 °C for 30 min in water	24

When present, *Neurospora crassa* spread over other fungi and completely covered the plates in 48 to 72 h. It was readily identified by its pink masses of loose conidia on hyphae growing away from the agar and by its tendency to creep out of the Petri dish. These isolates resembled wild-type strains of *N. crassa* morphologically and their identity was confirmed by crossing with the standard strains of *N. crassa*. Crosses with the fl^p isolates produced the most perithecia. Both mating types were found with equal frequency in colonies isolated from the same soil sample. This is to be expected if *Neurospora* reproduces sexually in nature and colonies are derived from randomly mixed ascospores. The isolates from nature (wild isolates) were not equally fertile as judged by the number of perithecia produced when crossed with the standard strains. Crosses among wild isolates also differed in fertility, but produced viable ascospores which germinated only when heat-shocked or treated with furfural or ethanol.

The alcohol-treatment technique ('soil pasteurization') devised by Warcup & Baker (1963) and used in Wisconsin, U.S.A. to isolate some new species of *Neurospora* from soil (Nelson, Novak & Backus, 1964; Mahoney, Huang & Backus, 1969) was also tested. We found treatment of soil with 65 % (v/v) ethanol for 15 min followed by heating to 60 °C for 30 min in water or in 10⁻³ M-furfural more effective than alcohol treatment alone for obtaining *N. crassa*, but again only from the two soils mentioned above. When these soils containing *N. crassa* were used to compare the relative effectiveness of the alcohol, alcohol + heat, or furfural + heat treatments, we found the last yielded *Neurospora* more frequently. Thus, *Neurospora* appeared in Petri dishes with a frequency of 0 to 5 % with alcohol treated soil, 10 to 20 % with alcohol + heat treated soil, and 20 to 40 % with furfural + heat treated soil. These results are corroborated by tests with ascospores (Table 2). When soil was used untreated or was heated to 60 °C in a moist chamber, *Neurospora* was not obtained even from those soils known to contain it. Although we generally used Vogel's medium, trials with

some soils were repeated with Czapek's medium supplemented with biotin, with corn meal agar, or with a glucose-asparagine medium (Gochenaour, 1964) used by workers in Wisconsin to isolate *Neurospora* from tropical soils. From the results, availability of ingredients, and convenience in preparation of the media, we consider Vogel's medium is better.

Hitherto there has been no evidence that *N. crassa* reproduces sexually in nature (Prakash, 1965; and cf. *Neurospora Newsletter* No. 16, 1970, p. 9). The fact that we could isolate compatible mating types in equal frequency from soil removed from a single site by using treatments known specifically to activate dormant ascospores of *Neurospora* (Sussman, 1969) suggests that this species reproduces sexually under natural conditions.

This is the first time *Neurospora* has been isolated from soils in India, although Perkins (1970) had collected this organism from visibly growing colonies on burnt vegetation in India and a new species was recently described from soil in Pakistan (Frederich, Vecker & Benjamin, 1969).

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