Neurospora in full bloom

Neurospora, the red bread mould first recognized growing on bread in France (1843), was named as *Monilia sitophila*. An isolate of this fungus from Japan (1923) was named initially as *Oidum aurantiacum*, and later changed to *M. sitophila*. In 1927, Shear and Dodge[1] discovered sexual spores of this fungus. Since it produced ascospores having neuron-like striations resembling neurons, they renamed the genus as *Neurospora*. The fungus is easily recognized in nature by its distinctive colour due to carotenoid pigments in the conidia which are produced in prodigious number (Figure 1). Ramesh Maheshwari's group at the Indian Institute of Science, Bangalore was the first to have systematically unravelled the *Neurospora* life cycle outside the laboratory, in a sugar-cane field[2]. *Neurospora* took centre stage in 1941, with the pioneering work of Beadle and Tautum[3] leading to the famous 'one gene–one enzyme hypothesis' and, in 1958, to the Nobel Prize. This work brought the disciplines of genetics and biochemistry together. Later work on other model organisms such as *Escherichia coli*, *Saccharomyces cerevisiae* and *Drosophila melanogaster*, led to rapid developments of research in modern biology[4]. With the recent report on the near complete genome sequence data of *Neurospora crassa*[5], which is also the first filamentous fungus to be sequenced, once again this organism has come into the limelight. The sequence data have revealed many genes not known in the genomes of bacteria, yeast, *Drosophila* or humans. Also, there are unique features known only to *Neurospora* such as the ability to recognize and silence duplicate genes by a process called RIP (repeat induced point mutation).

We have sighted *Neurospora* bloom on 18 February 2003 at filter-mud (insoluble residue from crude sugar-cane juice generated by the addition of lime) dump site spanning more than 25 acres of sugar-manufacturing industry at Vuyyuru near Vijayawada (Figure 1 *a* and *b*). Surveying this extensive orange *Neurospora* bloom was indeed an amazing experience. Pure cultures were isolated and several of them were crossed with the tester strains of *Neurospora*. The results indicated that most of them were *crassa* species. One such isolate was also sent to Fungal Genetics Stock Center (Kevin McKluskey) Kansas, USA where it was confirmed to be *Neurospora crassa*, mating type 'A' through successful crossing and ejection of black ascospores. It was allotted the FGSC # 9055.

The shear domination of *Neurospora* growing on filter-mud is understandable,

Figure 1. ***a***, Filter-mud dump site; ***b***, a heap of filter mud with *Neurospora*; ***c***, close view of *Neurospora*.

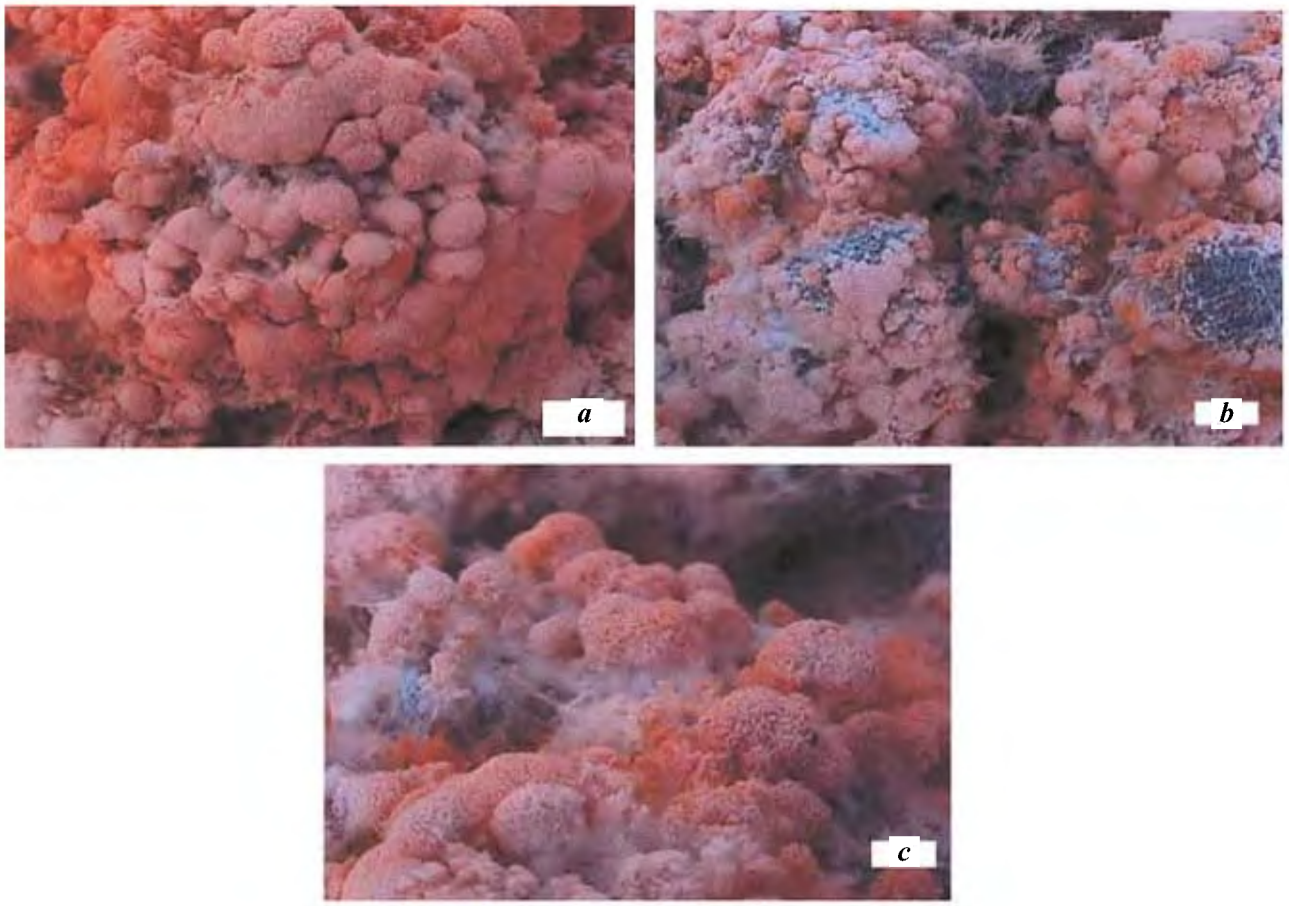

Figure 2 ***a–c.*** *Neurospora* growing with other fungi.

based on the sugar manufacturing technology. The sexual cycle of *Neurospora* on sugarcane could provide ascospores as the initial inoculum. Large quantities of filter-mud (250–300 tonnes/day) are generated at the initial step of refining, wherein crude cane juice is subjected to sulphur dioxide bubbling and heating (70°C) for 1 h and precipitation of turbid particulate material with lime. The heating step eliminates most of the microorganisms, whereas the black ascospores of *Neurospora* are resistant to this treatment. In addition, some furfural generated during the process would help the ascospores to germinate. Our observations revealed that the bloom continues for 3–4 weeks at any one point, after which *Neurospora* is overpowered by other fungal species, predominantly *Trichoderma* species (Figure 2 *a–c*). Some *Aspergillus* and *Penicillium* species were also noticed, though to a lesser extent. *Trichoderma* species are well-known producers of cell-wall lytic enzymes, especially those which are commercially used for preparation of *Neurospora* or fungal protoplasts (Novozyme-product of Novo Biolabs).

David Perkins (Stanford University) made global collections of *Neurospora* species, mostly from burned vegetation, and developed a method of identifying the wild collected strains based on unambiguous fertility tests with specially developed tester strains. The Perkins collection of over 4000 cultures of wild *Neurosporas* is being maintained at Stanford and at the Fungal Genetics Stock Center as a valuable resource for population genetics studies[6,7]. It is hoped that the present report will alert mycologists in India and simulate them to collect, identify and undertake investigations on natural biology of this organism.

Neurospora has been observed in many countries, growing commonly on vegetation scorched by fire, on corn cobs and in sugar-cane fields[2,4,8]. The first extensive bloom of this fungus was observed on 1 September 1923, when Tokyo was struck by a massive earthquake followed by fire. A pink–orange growth covered almost all burnt vegetation. *Neurospora* blooms on filter-mud from sugar mills have been described in Australia in studies by Shaw and Robertson[9], who observed honey bees foraging on conidiospores. Recently, a group at New Mexico, Berkeley and Stanford (USA) has found that scorched sugar-cane stems (due to natural fire) in the Everglades in Florida had as many as 3–4 pustules on a single cane sampling and DNA-sequencing of the *frq*, *het* loci, showed polymorphism. This observation has been interpreted to suggest that 'infection in nature comes from sexually produced ascospores and not due to airborne asexually produced conidia'. Both mating types of *N. crassa* ('A' and 'a') were observed in our isolates from the bloom which lends support to the above suggestion.

The filter-mud dump site described here could provide the basis for develop-

ing a '*Neurospora* farm' for the extraction of industrially useful enzymes (invertase, cellulases, proteases, etc.). This is the real field model of a solid-state fermentation, which could yield valuable products. Such natural or man-made blooms could be of great value for future research.

1. Shear, C. L. and Dodge, B. O., *J. Agric. Res.*, 1927, **34**, 1019–1042.
2. Pandit, A. and Maheswari, R., *J. Biosci.*, 1996, **21**, 57–59.
3. Beadle, G. W. and Tautum, E. L., *Proc. Natl. Acad. Sci. USA*, 1941, **27**, 499–506.
4. Perkins, D. D., *Genetics*, 1992, **130**, 687–701.
5. Galagan, J. E. *et al.*, *Nature*, 2003, **422**, 859–868.
6. Turner, B. C., Raju, N. B. and Perkins, D. D., *Genome*, 1988, **30**, 298.
7. Turner, B. C., Perkins, D. D. and Fairfield, A., *Fungal Genet. Biol.*, 2001, **32**, 67–92.
8. Perkins, D. D., Turner, B. C. and Barry, E. G., *Evolution*, 1976, **30**, 281–313.
9. Shaw, D. E. and Robertson, D. F., *Mycology*, 1998, **12**, 154–158.

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Colonization of cruciferous plants by *Piriformospora indica*

This correspondence provides evidence for a positive interaction of *Piriformospora indica*, a symbiotic fungus with several members of Cruciferae. The evidence is based on *in vitro* and *in vivo* studies. *P. indica* was characterized by Verma *et al.*[1] and subsequently reported for its various positive relations with plants and also being opted as a bio-fertilizer, -protector, -regulator, -fungicide and -pesticide[2–7]. This fungus also serves as an agent for biological hardening of tissue-culture-raised plants[8–10]. Interaction with the terrestrial orchids was also demonstrated[10–14]. 18s rDNA and 28s-rDNA analysis assigned this fungus to Basidiomycetes. *P. indica* is cultivable on several defined synthetic media[15,16] and can colonize the root of a large number of hosts. Earlier, we have reported a non-interaction of the fungus with myc$^-$ mutants of soyabean (*Glycine max*) and pea (*Pisum sativum*)[10].

Seeds of *Brassica oleracea* var. *capitata* (cabbage), *Spinacia oleracea* (spinach) and *Brassica juncea* (mustard) were surface-sterilized with 0.02% $HgCl_2$ and placed on 0.7% water agar petri plates for germination in the dark. After 7–10 days when the plumule and radicle appeared, they were transferred to pre-fungus inoculated agar slants in glass tubes containing 1/10 concentration of MMN medium[17]. Plants alone (control) or in co-culture with fungus were allowed to grow for 60 days in the tissue-culture laboratory maintaining 16 h photoperiod, 1000 lux at 25 ± 2°C. Root infections were checked after 45 days. Root colonization was evaluated by grid intersect method followed by staining about 100 roots segments (1 cm each) with Trypan blue or Chlorozal black E. Root per cent colonization was calculated using the standard formula[18].

Figure 1 *a* shows better cabbage growth as a result of interaction with *P. indica*. An overall enhancement of plant biomass was recorded when the fungus interacted with other members like *S. oleracea*, *B. oleracea* var. *capitata* and *B. juncea* (Table 1). Chlamydospores were produced on the root surface and scattered away from the root, extramatrically. Profuse root colonization by the fungus was observed at inter- and intracellular levels and extramatrically (Figure 1 *b*, left). Figure 1 *b* (right) shows an enlarged view of the chlamydospores (arrows). At no stage did fungal hyphae invade the stellar tissue and aerial portions. An identical positive interaction was also recorded for *S. oleracea*, *B. oleracea* and *B. juncea*.

This observation was repeated in a glasshouse using sterile soil : sand mixture as substratum (3 : 1). The substratum was sterilized and filled in pre-washed and sterile earthenware pots (1 kg). The fungus was allowed to grow on Kaefer broth medium under constant shaking condition at 25 ± 2°C for 7 days (Figure 2 *a*, top). Small and large colonies giving an appearance of 'corals' (arrows) were washed to remove the adhering chemicals. Inoculum (1% w/v) was thoroughly mixed with soil/sand mixture. Pre-germinated sterile seedlings (four in number) were placed in each pot. Efforts were made to keep the root system in direct contact with the fungal inoculum[19]. Plants were grown in an environmentally controlled greenhouse maintained at 25 ± 2°C, 16 h light/8 h dark with fluorescent light intensity 1000 lux and relative humidity 70%. Plants were fertilized on alternate weeks with 1/10 diluted Hoagland solution[20] and irrigated with autoclaved tap-water on alternate days to maintain about 70% soil moisture. Plants were photographed after 45 days. Treated plants were superior in growth (Figure 2 *b*, right) leading to early flowering (see close arrows) and fruiting (open arrows). Roots were heavily colonized and produced a large number of chlamydospores as recorded for *in vitro* conditions.

Results demonstrate that this fungus colonizes a large number of photosymbionts of importance in agriculture, horticulture and forestry. It is capable of interacting and also promoting the overall development of members of Cruciferae,