Cyclic AMP deficiency, modifier-mutations, and instability of the cr-1 phenotype

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Abstract
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Wild Neurospora isolated from soil.

We earlier reported a technique for isolating Neurospora from soil (Maheshwari and Antony 1974 J. Gen. Microbiol. 81: 505). Using this technique we have recently isolated Neurospora from 18 out of 25 soil samples from cultivated fields of arecanut, cardamom, coconut, coffee, opium, sugarcane and also from natural forests of teak and other species in Southern India. The localities sampled were mostly southwest of Bangalore along a 250 km radius and some place in Tamil Nadu and Kerala. The sites sampled were at least 1-2 km apart and most were 10-15 km apart.

Of the eighteen isolates in pure culture and purified by single-spore isolation, fifteen were identified as N. intermedio bored on the viability of ascospores produced following crosses with tester strains (FGSC #1766 and #1767) or with wild isolates which were identified as N. intermedia. One isolate each from soil from an arecanut and a coffee field behaved as N. sitophila in crosses with tester strains (FGSC #2216 and #2217). N. sitophila was also isolated from a sample of soil collected in Port Blair, Andaman Islands in the Bay of Bengal. It should be mentioned that our earlier isolates designated as N. crassa (Maheshwari and Antony, 1974) have now been identified as N. intermedio. We had previously not taken into account the fact that most of the ascospores were inviable that were produced in crosses to N. crassa testers.

Since our isolation procedure was bored on heat-treatment which activates dormant ascospores and kills conidia, this study suggests that ascospores are prevalent in soil. Both mating types were recovered with approximately equal frequency from some soil sampler.

The isolates differed in growth characteristics, pigmentation and fertility. In our experience crosses with these isolates were uniformly better on Westergaard and Mitchell's medium with filter paper (Whatman #3) rather than sucrose as the carbon source. None of the isolates grew above 42° C in minimal or in rich medium. This study and the collections made by Perkins (Perkins, Turner and Bary 1976 Evolution 30: 281) establish that Southern India is rich in Neurospora.

We thank D. D. Perkins, Stanford University, for advice. Soil from Port Blair, Andaman Islands was collected by Romulus Whitaker. - - - Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India.


Cyclic AMP deficiency, modifier-mutations, and instability of the cr-1 phenotype.

The morphological mutant cr-1 (crisp) of Neurospora crassa is severely deficient in adenyl cyclase activity (Terenzi et al., 1974 Biochem. Biophys. Res. Commun. 58: 990). When cyclic AMP was added to the culture medium of the mutant, it partially restored wild-type morphology (Terenzi et al., 1976 J. Bacteriol. 126: 91); therefore, the enzymatic deficiency may be directly involved in the developmental failure associated with cr-1. Because of these properties, cr-1 mutants represent an interesting system to study the role of cyclic AMP in eucaryotic cells. Earlier work (Gornick and Tatum 1970 Genetics 66: 281) demonstrated that cr-1 cultures accumulate spontaneous mutations which modify the crisp phenotype. There mutations were found in aged cultures and also appeared during vegetative propagation of cultures recently isolated from ascospores. Crisp-modifier mutations had a clear-cut effect on the morphology of homocaryons, but had no visible effect in heterocaryons, until a significant proportion of double mutant nuclei was reached. Thus, the presence of modifier mutations could interfere in onyotempttto characterize the cr-1 mutant biochemically.

In a recent study (Terenzi et al., 1979, in press) we demonstrated that cr-1 mutant strains are "noble to grow on several carbon sources, including glycerol, mannitol and arabinose. This pleiotropic deficiency was overcome by the addition of cyclic AMP to the culture medium. This can be observed in Table 1, where it is shown that the growth yield of the cr-1 strain (FGSC #488) in glycerol supplemented medium was greatly enhanced by cyclic AMP. On the other hand, the nucleotide does not affect the growth of the wild type, or that of the mutant in glucose supplemented medium. Spontaneous mutations were also found to overcome the nutritional deficiencies of the cr-1 mutant (Table 1). These mutations, which occurred at a very high frequency, partially suppressed the abnormal morphology of cr-1, taking advantage of the nutritional differences between cr-1 and wild-type isolates. Therefore, it is possible to establish wild-type lines from cr-1 mutant cultures in glycerol medium by plating on a plate rich in cyclic AMP.

TABLE 1

<table>
<thead>
<tr>
<th>Medium</th>
<th>cAMP (1 mM)</th>
<th>Growth of cultures (mg total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>St. L. 74A</td>
</tr>
<tr>
<td>2% glucose</td>
<td>=</td>
<td>17.1</td>
</tr>
<tr>
<td>2% glucose</td>
<td>=</td>
<td>18.0</td>
</tr>
<tr>
<td>1% glycerol</td>
<td>=</td>
<td>10.8</td>
</tr>
<tr>
<td>1% glycerol</td>
<td>=</td>
<td>8.9</td>
</tr>
</tbody>
</table>

(a) Cultures were grown on 10 ml of Vogel's liquid medium supplemented as indicated. Incubations carried out at 30°C for 48 hr. Mycella collected and precipitated with cold 10% TCA. After centrifugation the mycelial pellet was extracted with 1 N NaOH at 100°C, and recentrifuged. Protein was determined in the supernatant by the method of Lowry, et al. (1951, J. Biol. Chem. 193: 265).

(b) This strain was isolated from a cr-1 (8123) culture (FGSC #488) grown in glycerol medium and reisolated several times by plating on glycerol supplemented medium.
All cultures were prepared, using standard petri dishes, in Vogel's medium supplemented with glucose (2%), or glycerol (1%). The \( cr^{-1} \) cultures employed were established from ascospores of a cross of \( cr^{-1} \) (FGSC 488, allele 8123) X St. L 74A wild-type. The presence of modifier mutations was tested for by plating a conveniently diluted conidial suspension on minimal medium supplemented with glucose or with glycerol. Colonies were counted after 48 hr at 32° C. The number of colonies developing in glycerol-supplemented medium, expressed as a percent of the viable population (No. colonies in glycerol/No. colonies in glucose \( \times 100 \)), was regarded as the frequency of crisp-modifiers present in the culture. As Figure 1 shows, the proportion of glycerol-utilizing conidia increased dramatically in aging cultures. In that experiment, the cultures were all inoculated simultaneously and were from a single \( cr^{-1} \) isolate. In a different experiment we studied fifty-two separate \( cr^{-1} \) isolates obtained from the cross \( cr^{-1} \) x wild-type. At different times, conidial samples from each culture were tested in glucose and in glycerol media. Seven days after isolation, growth in glycerol was negative for all the cultures; after fifteen days, twelve cultures (23%) gave a positive response. This number increased to twenty-one (40%) at the 22nd day, and, after a month all cultures produced conidia able to develop in glycerol medium. We conclude that old cultures of the \( cr^{-1} \) mutant inevitably contain modifier mutations.

Figure 1.-- Increase in the proportion of glycerol-utilizing conidia in aging \( cr^{-1} \) cultures. Each experimental point represents one slant from a group of five, which had been simultaneously inoculated with 0.05 ml of a single conidial suspension.

Figure 2 shows the exponential increase in the proportion of glycerol-utilizing conidia in a \( cr^{-1} \) strain which was propagated by repeated transfers. According to Garnjobst and Tatum, spontaneous \( cr^{-1} \)-modifiers were not observed in wild-type strains, and, although we have occasionally observed \( w^{-1} \)-modified phenotypes among the progeny of \( cr^{-1} \) x wild-type crosses, they were not very common. Therefore, we suspected that the extremely fast appearance of the modifier in \( cr^{-1} \) cultures might be related to the mutant deficiency of adenyl cyclase activity. In support of this view, it was observed that when the \( cr^{-1} \) strain was propagated in cyclic AMP-supplemented medium, the proportion of glycerol-utilizing conidia did not increase (Figure 2). This effect of cyclic AMP was observed at both a low (0.001%) and a high (1%) proportion of modifier in the heterocaryon. When cyclic AMP was withdrawn, a rapid increase in the number of glycerol-utilizing conidia occurred.

The nutritional advantages provided to the \( cr^{-1} \) mutant by the modifier mutation do not seem to contribute to the rapid selection of the latter in any obvious way; i.e., \( cr^{-1} \) cultures were propagated in glucose-supplemented medium, in which wild type, \( cr^{-1} \), and modified-\( w^{-1} \) growth rates are the same (Table 1). Moreover, the effects of the modifier on \( cr^{-1} \) morphology only became apparent when a high proportion (over 10%) of nuclei contained the modifier. Nevertheless, the rate of increase of modifiers during the vegetative propagation of a \( cr^{-1} \) strain (Figure 2), was linear over four orders of magnitude. Garnjobst and Tatum found that the spontaneous crisp-modifiers represented at least five different loci. It remains to be established whether the \( cr^{-1} \) modifiers can be selected on the basis of the nutritional requirements of a single or several genetic loci.

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