Isolation and Characterization of Respiration-Deficient Mutants in *Aspergillus ochraceus*

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ABSTRACT. Two respiration-deficient mutants (*rd*) were isolated from the acetate-nonutilizing mutants (*acu*) induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in *Aspergillus ochraceus*. A complementation analysis of the two *rd* mutants indicated that MNNG had caused a mutation at a single locus. The diameter of the two *rd* mutant colonies in glucose medium was found to be small in comparison to that of the wild type and the other *acu* mutants; the diameter of the isolated mutant colonies in acetate medium was very small. The grown zone of *rd* mutants remained colorless up to 20 h incubation in 2,3,5-triphenyltetrazolium-overlaid solid Czapek–Dox medium and it turned pink after prolonged incubation, whereas the wild type and the other *acu* mutants became pink within 30 min in the same medium. The *rd* mutants were further characterized by measuring the respiratory activities of intact mycelia in the presence of glucose.

Respiration-deficient mutants (*rd*) have been extensively used as a genetic tool for examining the role of membranes in energy metabolism (Lloyd 1974; Mandal et al. 1978; Johansson and Jan-Eric 1984; Dubreucq et al. 1990; Jean-Paul et al. 1990) and in molecular biology (Goddard and Cummings 1975; Mironova et al. 1986; Yoshiko et al. 1989) of mitochondria of some microorganisms, such as yeasts and *Neurospora crassa*. The present authors have isolated *rd* mutants in *Aspergillus ochraceus*, a filamentous fungus of the ascomycete group; their characterization is presented here.

MATERIALS AND METHODS

*Aspergillus ochraceus* strain IMI297011 was used for the isolation of acetate-nonutilizing (*acu*) mutants and *rd* mutants. Czapek–Dox (CD) medium was basically that of Clutterbuck *et al.* (1932). This contained (in g/L): glucose 40, NaNO₃ 2, MgSO₄·7H₂O 0.5, KCl 0.5, KH₂PO₄ 1.0, FeSO₄·7H₂O 0.01, ZnSO₄·7H₂O 0.01. The pH of the medium was adjusted to 6.8 before autoclaving. The medium for plates and slants was solidified with 2 % (W/V) agar. In the acetate medium (AC) all the ingredients remained the same except 0.8 % acetate replaced glucose as carbon source.

The *rd* mutants (*rd1* and *rd2*) were grown in the same medium containing glucose as carbon source.

Conidia of *rd* mutants (*rd1* and *rd2*) were point-inoculated side by side on solid CD medium and incubated at 30 °C for 3 d. Then a small sector of agar containing the growing mycelia of both mutants was cut and mounted onto solid AC medium and incubated at 30 °C for 5 d for the examination of heterokaryotic growth.
The uptake of O₂ was measured polarographically at 30 °C with Clark-type O₂ electrode (YS Model 55) in 3 mL of reaction medium consisting of glucose (80 mmol/L), MgCl₂ (5 mmol/L), sodium phosphate buffer (20 mmol/L, pH 7.0) and intact mycelial suspension of wild type and rd mutant.

RESULTS AND DISCUSSION

The highest frequency of acu mutants (0.49 %) was achieved with 100 mg MNNG/L, that of r mutants at 200 and 300 mg MNNG/L (roughly 0.1 %) in Tris maleate buffer (pH 5.2) for 25 min incubation at 30 °C (Table I). The isolation of different auxotrophs (Herman and Griffin 1968; Sen et al. 1976; Das and Sen 1983a) using the same mutagen required different pH and temperature. Failure to isolate acu and rd mutants at alkaline pH suggests that acidic pH is favorable for isolation of the mutants as reported for Neurospora crassa (Scott 1970), E. coli (Adelberg et al. 1965) and Aspergillus sp. (Mandal et al. 1978; Das and Sen 1983a). It was also reported that MNNG decomposes under acidic and alkaline conditions to yield nitrous acid and diazomethane, respectively (Mandell and Greenberg 1960), and the nascent nitrous acid, a potent chemical mutagen, may cause the mutation by changing the amino group linked to DNA base to a hydroxyl group. MNNG can cause mutation under different conditions, depending on the microorganism and its physiological state (Adelberg et al. 1965; Delic et al. 1970; Das and Sen 1983b; Saha and Das 1992).

Table I. Effect of MNNG mutant formation in A. ochraceus

<table>
<thead>
<tr>
<th>Dose of MNNG mg/L</th>
<th>Survival %</th>
<th>Number of coloniesa</th>
<th>Number of mutantsb</th>
<th>Frequency of the acu mutantsc</th>
<th>Number of the rd mutantsd</th>
<th>Frequency of the rd mutantsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>12.5</td>
<td>1,020</td>
<td>5</td>
<td>0.49</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>200</td>
<td>8.0</td>
<td>800</td>
<td>3</td>
<td>0.37</td>
<td>1</td>
<td>0.12</td>
</tr>
<tr>
<td>300</td>
<td>3.5</td>
<td>900</td>
<td>2</td>
<td>0.22</td>
<td>1</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*aExaminad after filtrate enrichment technique.
*bFailed to grow on AC medium.
*cCalculated for the number of examined mutagenized spores.
*dFailed to change the color of TTC to pink.

Fig. 1. Growth of A. ochraceus rd1 and rd2 mutants on CD solid media containing glucose and acetate, respectively, as carbon source.