Ornithine synthesis by an ornithine-deficient triple mutant

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B. Ng

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Abstract
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<table>
<thead>
<tr>
<th>Mutant</th>
<th>uninduced</th>
<th>NO$_3^-$ induced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nitrate reductase, sp. act.</td>
<td>nitrate reductase, sp. act.</td>
</tr>
<tr>
<td></td>
<td>r Car/time</td>
<td>r Car/time</td>
</tr>
<tr>
<td></td>
<td>NADPH MVH</td>
<td>NADPH MVH</td>
</tr>
<tr>
<td>nada</td>
<td>0.33 0.12 0.90±.05</td>
<td>3.05 2.83 0.99±.11</td>
</tr>
<tr>
<td>nit-1</td>
<td>0.30 0.10 0.80±.09</td>
<td>0.38 0.15 0.80±.10</td>
</tr>
<tr>
<td>nit-2</td>
<td>0.0 0.0 0.85±.09</td>
<td>0.0 0.0 0.83±.10</td>
</tr>
<tr>
<td>nit-3</td>
<td>0.0 0.0 0.96±14</td>
<td>0.0 15.70 0.83±.09</td>
</tr>
</tbody>
</table>

Specific activity of nitrate reductase is expressed in nmole NO$_2^-$.min$^{-1}$.mg protein$^{-1}$; the rate of carotenoid accumulation in the light, r Car/time, is expressed in arbitrary units.

Thus, nitrate reductase can hardly be regarded as a key element of the photoreceptor system. The photoregulation mechanism may be associated with some NADH-dependent redox processes.

*The journal Applied Biochemistry and Microbiology (Prikladnaya Biokhimia i Mikrobiologija) is translated from Russian into English by Plenum Publishing Corporation and is available in the libraries in the United States and other countries. – – – A. N. Bach Institute of Biochemistry, Moscow USSR.

Charlanq, G. W. and B. Ng

Ornithine synthesis by an ornithine-deficient triple mutant.

Siderophores, small molecules that function in iron transport are produced by many microorganisms. Although there is much structural variability, most siderophores are either hydroxamates or phenolates-catecholates. Both major siderophores produced by Neurospora belong to the hydroxamate group and contain ornithine. Coprogen is secreted into the medium to scavenge for iron. Ferricorcin is an intracellular siderophore, whose probable function is iron storage.

Siderophore synthesis is regulated by a feedback system and is repressed if iron is present in the growth medium. This repression is not total, however, since we can detect both siderophores when Neurospora is grown in Vogel's minimal medium (MM) containing the usual amount of iron.

A mutant blocked in all pathways of ornithine synthesis should be unable to make siderophores. Such a mutant has been constructed by Rowland Davis (1970 J. Bacterial. 102: 799). The triple mutant, arg-5 ota, aga (FGSC #2744) lacks enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Microbiol. 88: 49).

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml) added to the autoclaved medium), the conidia of the triple mutant still contain ferricorcin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e., growing in medium without added iron (Kappner et al. 1977 Arch. Microbiol. 115: 323) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricorcin (Table 1). Dry weights of siderophores in the Table are post- XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga under iron starvation conditions, of nearly 6 nmol of ornithine/mg dry weight in spermidine supplemented medium (about 3.5 nmol/mg dry weight in putrescine supplemented medium) suggests the following possible explanations: (a) an alternative pathway to ornithine synthesis exists which is derepressed under iron starvation conditions. (b) The mutant may be leaky. Under ordinary conditions this would not be detectable, but when stressed for iron, siderophore synthesis is so derepressed that enough of the enzymes are mobilized for production to occur. The tremendous derepression of siderophore synthesis that is possible is illustrated by examples cited by Emery (1971 Adv. Enzymol. 35: 125): organisms that produce siderophores in quantities that exceed the dry weight of the cells under deficient growth conditions.
### TABLE 1
Siderophore production by arg-5, ota, aga and wild-type under iron-stressed conditions

<table>
<thead>
<tr>
<th>Strains added to medium</th>
<th>Growth (gm wet wt)</th>
<th>Total siderophores (mg)</th>
<th>Total siderophore ornithine (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>arg-5, ota, aga putrescine (100 µg/ml)</td>
<td>3.9</td>
<td>4.3</td>
<td>1.3</td>
</tr>
<tr>
<td>arg-5, ota, aga spermidie (100 µg/ml)</td>
<td>11.2</td>
<td>9.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Wild-type (74A)</td>
<td>13.3</td>
<td>116.1</td>
<td>18.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strains added to medium</th>
<th>Growth (gm wet wt)</th>
<th>Total siderophores (mg)</th>
<th>Total siderophore ornithine (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>arginine (50 µg/ml)</td>
<td>11.2</td>
<td>9.7</td>
<td>3.1</td>
</tr>
<tr>
<td>spermidine (100 µg/ml)</td>
<td>13.3</td>
<td>116.1</td>
<td>18.0</td>
</tr>
</tbody>
</table>

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a Arginine (recrystallized) and polyamines were filter sterilized and added to autoclaved medium.
b Mycelial wet wt of cultures grown in 500 ml medium for four days at 25° with shaking.
c Post XAD-2 column chromatography siderophores. Amounts are per 500 ml culture.
d Siderophores were hydrolyzed reductively with HI, and ornithine determined with a Beckman amino acid analyzer.

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Grindle, M and W Temple

Fungicide-resistant os mutants of Neurospora crassa

We are investigating mutants of Batrytis cinerea and N. crassa that are resistant to various antifungal chemicals. Mutants selected on media containing the agricultural fungicide, vinclozolin, are cross-resistant to other "dicarboximide" and "aromatic hydrocarbon" fungicides. Most of the mutants (which we have designated Vm) grow poorly on Vogel's minimal medium MM and on media supplemented with chemicals that cause a marked increase in the osmolarity and/or the supply of ammonium ion.

The sensitivity of vin mutants to high osmotic pressure is paralleled by the osmotic mutants described by Mays (Genetics 1969, 63: 781) and by Murayama and Ishikawa (J. Bacteriology 1973, 115: 796). We have characterized a selection of these osmotic mutants to determine whether they are resistant to some dicarboximide (procymidone, iprodione and vinclozolin) and aromatic hydrocarbon (chloroneb, dicloran and quintozene) fungicides. The data (Table 1) show that os-1, os-2, os-4, os-5 film 1 (=os-1 and film 2 (= os-4) mutants are resistant to the fungicides, but cut and gla mutants are not. The phenotypes (i.e. rates of growth on MM and supplemented MM and levels of resistance to the fungicides) of the os mutants are as variable as those of our vin mutants. With a few exceptions, os-1 mutants can be distinguished from os-2, os-4, and os-5 mutants by their lower growth on MM, their higher resistance to the fungicides, and their greater sensitivity to media supplemented with amino acids or NaCl.

Preliminary genetic studies of our vinclozolin-resistant mutants of N. crassa indicate that this phenotypic character is specified by numerous genes; most of the Vin genes seem to be located on chr 1, but we have detected few Vin mutants that are clearly allelic with os-1 os-4 or os-5.

Osmotic mutants can be isolated and identified with ease on media containing any of the fungicides listed in Table 1. We use commercial fungicides which are supplied as wettable powders (e.g. "Ronilan", supplied by BASF United Kingdom Ltd., contains 50% vinclozolin; the fungicides are added to cool, sterilized media.