Undecanoic acid (UDA) is a fatty acid that has been used in the treatment of dermatophytoses in humans. Formulations containing UDA have been used in the treatment of superficial mycoses, such as athlete’s foot (tinea pedis) and keratomycoses (Chretien et al., 1980). It was reported that high concentrations of UDA inhibit the ubiquitous dermatophyte *Trichophyton rubrum* (Horsfall, 1956), but the exact mode of action is still not known.

*Aspergillus nidulans* is an ascomycete fungus of considerable biological interest (Rao et al., 2003) and is an appropriate experimental model for investigating drug resistance in fungi (Rocha et al., 2002), but no study has yet been made regarding the resistance to UDA in this species.

One strategy to evaluate the biological effect of a drug in fungi is to induce mutations that lead to drug resistance and then to determine the pattern and the genetic basis of this response. Here, we describe the identification and the linkage mapping of two mutations that cause resistance to UDA in *A. nidulans* and compare the responses of two filamentous fungi, *A. nidulans* and *T. rubrum*, to this compound.

Two *A. nidulans* strains were used: *pabaA1* (p-aminobenzoic acid auxotroph) and Master Strain F (MSF), which are FGSC strains A610 and A283, respectively. MSF strain contains multiple markers in all eight linkage groups—*suA1adE20* (I), *ya2* (I), *ade20* (I), *acrA1* (II), *galA1* (III), *pyroA4* (IV), *facA303* (V), *sBo3* (VI), *nicB8* (VII), and *riboB2* (VIII)—and was used for gene mapping. *T. rubrum* H6 is a clinical isolate obtained from University Hospital in Ribeirão Preto, Brazil, which has a UDA-response similar to that previously described for the *T. rubrum* strain *udas* (Das and Banerjee, 1982). *T. rubrum* was used as a reference species in this study.

The culture medium for *A. nidulans* (CM) was that described by Cove (1966). *T. rubrum* was cultured on Sabouraud Glucose Agar (SGA; 2% peptone [Sigma, St. Louis, MO, USA], 1% glucose [Mallinkrodt, Paris, KY, USA], w/v; pH 5.7).

A spore suspension (10⁸ conidia·ml⁻¹) from each fungal strain (*A. nidulans pabaA1* strain and *T. rubrum H6* strain) was treated with a G15T8 germicidal lamp until 95% of the conidia were killed (Cuadros et al., 1999). Through this method, resistant mutants were recovered on CM or SGA dishes containing UDA at concentrations higher than those required to kill the...
wild-type strains of these fungi.

Two strains (udaA1 and udaA2) showing resistance to UDA were isolated after UV light mutagenesis of the pabaA1 strain of A. nidulans. One T. rubrum mutant was selected after UV-treatment of strain H6 and named udar-2.

The susceptibility of the A. nidulans and T. rubrum mutants to UDA was determined by estimating the minimal inhibitory concentration (MIC) making an agar dilution assay (Mock et al., 1998). The UDA concentrations tested were 0 to 300 µg·ml⁻¹ for A. nidulans strains and 0 to 50 µg·ml⁻¹ for T. rubrum strains. The A. nidulans strains MSF and pabaA1 showed similar MICs of UDA, which were 10-fold higher than that of T. rubrum strain H6 (Table 1), what suggests a structural difference in the cellular wall of these fungi. In addition, drug extrusion pumps would be also different between the two fungi.

Conidia germination was impaired in the A. nidulans strains pabaA1, udaA1 and udaA2 grown in the presence of sub-inhibitory concentrations UDA. A similar effect was observed in the T. rubrum strains H6 and udar-2. These findings indicate a common mechanism of action for UDA in these two filamentous fungi.

The linkage mapping of A. nidulans mutations conferring resistance to UDA was performed using standard genetic techniques according to Pontecorvo et al. (1953), and mutations were assigned to their linkage groups according to McCully and Forbes (1965). Two strains were inoculated on a CM dish and their mycelia were allowed to overlap. During the parasexual cycle of A. nidulans, a heterokaryon can originate, leading to the formation of a diploid nucleus. Diploid strains are selected for the colony pigmentation, the diameter of the conidia and their ability to grow on minimal medium. However, the diploid nucleus is unstable and can spontaneously haploidize. The extremely low frequency of recombination during this event is technically positive, because genes located in the same chromosome will segregate together, including those that would freely recombine during meiosis. In our experimental procedure, haploidization was facilitated by the use of benlate (Hastie, 1970) on CM dishes.

For the mapping of genes assigned to the same linkage group, the sexual cycle of A. nidulans was used. Each resistant strain (udaA1 or udaA2) was crossed with MSF. The resulting diploid strains were incubated under semi-anoxia conditions for 10 to 15 days to induce the formation of cleistothecia. Hybrid cleistothe-

Table 1. Minimal inhibitory concentration (MIC) of undecanoic acid for A. nidulans and T. rubrum strains as measured with an agar dilution method (Mock et al., 1998).

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>MIC (µg·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. rubrum</td>
<td>H6</td>
<td>28</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>udas</td>
<td>30ᵃ</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>udar-2</td>
<td>40</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>MSF</td>
<td>300</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>pabaA1</td>
<td>300</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>udaA1 pabaA1</td>
<td>350</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>udaA2 pabaA1</td>
<td>340</td>
</tr>
</tbody>
</table>

ᵃDas and Banerjee (1977).

Table 2. Meiotic analysis of the crosses between strains udaA1 and MSF and between strains udaA2 and MSF.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>udaA1</th>
<th>udaA2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Sensible</td>
</tr>
<tr>
<td>riboB²</td>
<td>59</td>
<td>13</td>
</tr>
<tr>
<td>riboB²</td>
<td>3</td>
<td>47</td>
</tr>
</tbody>
</table>

The numbers represent the combinations observed between the genes udaA and riboB in all analyzed segregants. The number of recombinant segregants is in boldface.
measuring the diameter of colonies grown for 72 h on CM agar supplemented with UDA (0 to 300 \( \mu \text{g} \cdot \text{ml}^{-1} \)).

The indicated values are the means of three independent experiments. Standard deviations were lower than 0.2.

The udaA1 diploid was found to be resistant to UDA (Fig. 1), indicating a dominant character of the udaA1 mutation over the wild-type gene. It was observed that MSF and pabaA1 strains have similar concentration-responses to UDA.

To our knowledge, this is the first report of a gene involved in the resistance of A. nidulans to UDA. Efforts are under way to identify the biochemical nature of the UDA resistance in A. nidulans and the physical map proposed here will be useful for designing cloning strategies for the udaA gene.

Acknowledgments

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References


