An Acetyltransferase Conferring Tolerance to Toxic Aromatic Amine Chemicals

MOLECULAR AND FUNCTIONAL STUDIES

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Aromatic amines (AA) are a major class of environmental pollutants that have been shown to have genotoxic and cytotoxic potentials toward most living organisms. Fungi are able to tolerate a diverse range of chemical compounds including certain AA and have long been used as models to understand general biological processes. Deciphering the mechanisms underlying this tolerance may improve our understanding of the adaptation of organisms to stressful environments and pave the way for novel pharmaceutical and/or biotechnological applications. We have identified and characterized two arylamine N-acetyltransferase (NAT) enzymes (PaNAT1 and PaNAT2) from the model fungus Podospora anserina that acetylate a wide range of AA. Targeted gene disruption experiments revealed that PaNAT2 was required for the growth and survival of the fungus in the presence of toxic AA. Functional studies using the knock-out strains and chemically acetylated AA indicated that tolerance of P. anserina to toxic AA was due to the N-acetylation of these chemicals by PaNAT2. Moreover, we provide proof-of-concept remediation experiments where P. anserina, through its PaNAT2 enzyme, is able to detoxify the highly toxic pesticide residue 3,4-dichloroaniline in experimentally contaminated soil samples. Overall, our data show that a single xenobiotic-metabolizing enzyme can mediate tolerance to a major class of pollutants in a eukaryotic species. These findings expand the understanding of the role of xenobiotic-metabolizing enzyme and in particular of NATs in the adaptation of organisms to their chemical environment and provide a basis for new systems for the bioremediation of contaminated soils.

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Aromatic amines (AA) represent one of the most important classes of occupational or environmental pollutants. Many AA are toxic to most living organisms due to their genotoxic or cytotoxic properties (1). AA account for 12% of the 415 chemicals that are either known or strongly suspected to be carcinogenic in humans (2). AA are common by-products of chemical manufacturing (pesticides, dyestuffs, rubbers, or pharmaceuticals), coal and gasoline combustion, or pyrolysis reactions (3). Moreover, the presence of AA in groundwater or soil samples subject to industrial, agricultural, or urban pollution is of increasing concern, particularly for persistent toxic AA contaminants, such as pesticide-derived anilines (4).

The identification of mechanisms by which living organisms can tolerate harmful chemicals, such as AA, is of prime importance to understand their adaptation to stressful environments. In addition, deciphering the molecular mechanisms underlying this tolerance may lead to novel biotechnological and pharmaceutical applications.

Fungi are environmentally ubiquitous and are found with great diversity in both terrestrial and aquatic environments. Fungi are known to tolerate a large range of chemicals of natural or anthropogenic origin by developing mechanisms to act on xenobiotic and natural compounds (5, 6). Fungi are therefore good models to identify and to understand tolerance mechanisms to xenobiotics (7, 8). Moreover, characterization of the mechanisms by which fungi tolerate certain toxic xenobiotics can potentially lead to the identification of new targets for the treatment of fungal infections in vertebrates (7, 8) or plants and to the development of new bioremediation tools for cleaning up contaminated environments (5, 9).

Using the common ascomycete Podospora anserina as a model, we provide here the demonstration that a single enzyme can mediate tolerance to toxic AA chemicals in a eukaryotic species. This enzyme was identified and characterized as an arylamine N-acetyltransferase (NAT), a xenobiotic-metabolizing enzyme that acetylates efficiently several toxic AA. Targeted disruption of this NAT gene led to the complete loss of toxicity in the resulting mutant strain.

3 The abbreviations used are: AA, aromatic amine; NAT, arylamine N-acetyltransferase; 3,4-DCA, 3,4-dichloroaniline; 2-AF, 2-aminofluorene; 4-BOA, 4-butoxyaniline; WT, wild type; HPLC, high pressure liquid chromatography; DMSO, dimethyl sulfoxide.
tolerance to AA, thus confirming that this enzyme enables the fungus to detoxify AA that would otherwise prove toxic. These findings will help to understand the enzymatic mechanisms contributing to adaptation of living organisms to their environment. In particular, our data demonstrate that the NAT-dependent detoxification mechanisms may provide a eukaryotic organism with tolerance to toxic AA. Moreover, we provide proof-of-principle experiments, using soils contaminated with the highly toxic pesticide residue 3,4-dichloroaniline, proving that the fungal NAT-dependent detoxification pathway may represent a novel model with reasonable cost and a low environmental impact for the bioremediation of AA-contaminated environments.

EXPERIMENTAL PROCEDURES

Strains, Culture Conditions, Basic Protocols, and Phenotypic Characterization—The S strain of P. anserina was used for all experiments. The culture conditions for this organism have been described elsewhere (10). The methods currently used for genetic analysis, the extraction of nucleic acids and proteins, and genetic transformation are available from the Podospora anserina Genome Project. P. anserina was grown in the well defined M2 synthetic medium providing the wild-type (WT) strain with optimal growth conditions. Phenotypic analyses were performed on WT and mutant (ΔPaNat1, ΔPaNat2 single mutants, and ΔPaNat1/2 double mutants) P. anserina strains. The vegetative part of the life cycle was investigated in laboratory conditions by assessing growth rate and mycelial morphology: that is, the presence or absence of aerial hyphae and accumulation of pigments, as described for other mutants (11). Senescence and other types of cell degeneration were evaluated by measuring life span and investigating “crippled growth,” as described previously (12). Hyphal interference was evaluated by placing P. anserina mycelia in the presence of Penicillium chrysosogenum and Coprinopsis cinerea (13). Completion of the sexual cycle was investigated by measuring male and female fertility, perithecium maturation and content, and the timing of ascospore germination (11).

Chemical Synthesis of N-Acetylated AA—The acetylated forms of 3,4-dichloroaniline (3,4-DCA), 2-aminofluorene (2-AF), and 4-butoxyaniline (4-BOA) were synthesized from 3,4-DCA, 2-AF, and 4-BOA (Sigma) using acetic acid chloride in the presence of triethylamine (base). Column chromatography purification was carried out on silica gel 60 (70–230 mesh American Society for Testing and Materials, Merck). The struc-
different time points for the quantification of 3,4-DCA and acetyl-3,4-DCA by HPLC. For the germination and growth of *L. sativa* seeds in soil, contaminated soil samples (20 g/pot, 80 mg/kg of 3,4-DCA) were inoculated three times (every 24 h) with 0.5 g of WT or Δ*PaNat1/2* strains and incubated for 72 h at 25 °C. Seeds (20) were then sown in soil samples and allowed to germinate and grow at 25 °C for 8 days (illumination for 12 h/day). Controls were carried out with acetyl-3,4-DCA (80 mg/kg) and H₂O.

**Enzyme Assays and Detection of Acetylated Aromatic Amines by HPLC**—NAT activity was measured in the 5,5'-dithiobis-(2-nitrobenzoic acid) assay, as described previously (16). Recombinant enzymes and aromatic amine substrates (500 μM final concentration) in assay buffer (25 mM Tris-HCl, pH 7.5) were incubated for 5 min at 37°C in a 96-well plate. AcCoA (400 μM final concentration) was added, and the plate was incubated at 37°C (for up to 30 min). The reaction (100 μl total volume) was quenched with 25 μl of guanidine hydrochloride solution (6.4 M guanidine/HCl, 0.1 M Tris-HCl, pH 7.5) supplemented with 5 mM 5,5'-dithiobis-(2-nitrobenzoic acid), and absorbance was measured at 405 nm. Kinetic analyses were performed by varying the aromatic amine substrate concentrations. Kinetic constants were determined by non-linear regression analysis, with the Kaleidagraph program (Synergy Software, Reading, PA). The rate of acetylation of aromatic amines by fungal extracts was measured by HPLC, as described previously (17). *N*-Acetylated aromatic amines were detected in samples (M2 liquid medium) or ethanol eluates of soil by HPLC on a C18 column, using 60% sodium perchlorate (20 mM, pH 3) and 40% acetonitrile as the mobile phase. Chemically acetylated AAs (acetylated 3,4-DCA; acetylated 2AF, and acetylated BOA) were synthesized as reported above and used as HPLC standards to identify enzymatically acetylated AAs in samples.

**Statistical Analysis**—Data are expressed as the mean ± S.D. of three independent experiments, with quadruplicate assays for each experiment. Student’s *t* test was used to determine the statistical significance of differences between means. Significance was defined as a *p* ≤ 0.05.

**FIGURE 1.** Functional characterization of *PaNat1* and *PaNat2*. *a*, schematic representation of the NAT-dependent acetylation of three aromatic amines: 3,4-DCA, 4-BOA, and 2-AF. *b*, detailed Michaelis-Menten kinetic characterization of *PaNat1* (black lines) and *PaNat2* (dotted lines) with 3,4-DCA, 4-BOA, 2-AF, 5-aminosalicylate (5-AS), and hydralazine (HDZ). All assays were performed in quadruplicate. *c*, comparison of catalytic efficiencies, as estimated from ratios of kinetic parameters (*k*₅/*K*₉), expressed in M⁻¹·s⁻¹. Error bars indicate S.E.
Identification and Characterization of P. anserina NAT Enzymes and Their Activity toward Aromatic Amine Substrates—AA are an important class of chemicals that are used in the manufacturing of pesticides, dyes, and pharmaceuticals. AA are also found in tobacco smoke and food pyrolysis products (18). Many AA are toxic for living organisms, and several AA are recognized as carcinogens. It has been suggested that certain soil fungi can detoxify and tolerate toxic AA (19). Therefore, fungal models are of interest to identify and understand mechanisms responsible for tolerance to toxic AA in eukaryotic organisms (7, 8).

Four well-known fungal species (Fusarium graminearum, Phycomyces blakesleeanus, P. anserina, and Rhizopus oryzae) from different ecosystems, for which complete genome sequences were available, were screened for radial growth in the presence of three toxic AA: 3,4-DCA (pesticide residue), 2-AF (carcinogen), and 4-BOA (chemical intermediate) (14). The presence of three toxic AA: 3,4-DCA (pesticide residue), 2-AF (carcinogen), and 4-BOA (chemical intermediate) (14). The growth of R. oryzae and P. blakesleeanus was almost completely abolished by these three AA at concentrations of 100–250 μM in standard minimal growth medium. In the same conditions, little effect on growth was observed in the other two species studied (P. anserina and F. graminearum) (data not shown), suggesting that these two species have mechanisms of tolerance to AA. We aimed to decipher the mechanisms underlying fungal survival in AA-contaminated environments.

Tolerance to potentially toxic xenobiotics often depends on specific biotransformation pathways catalyzed by endogenous enzymes, and in particular, xenobiotic-metabolizing enzyme, such as cytochrome P-450 varieties or glutathione S-transferases. AA may be biotransformed via several routes involving different types of xenobiotic-metabolizing enzyme, in particular, arylamine NATs (20). In eukaryotes, NAT enzymes have long been known to biotransform AA (20). However, it is still unclear what role NAT enzymes actually play in either preventing or enhancing toxic response to AA (21). So far, the studies on the knock-out NAT mouse models (21–23) have not demonstrated clearly the relevance of this pathway to AA tolerance in living organisms.

BLAST analysis indicated that P. anserina had two putative NAT enzymes and that F. graminearum had three such enzymes, whereas the two sensitive fungi had no genes encoding NAT enzymes (see supplemental Table 1 and supplemental Fig. 2). A broader BLAST screening of the complete genome sequences of eumycete fungi showed that many of these fungi had NAT genes (see supplemental Table 1 and supplemental Fig. 2). We investigated the possible role of these genes in AA tolerance, focusing on the filamentous ascomycete P. anserina, which is highly tolerant to 2-AF, 3,4-DCA, and 4-BOA and amenable to reverse genetics techniques. The two NAT genes present in the genome of P. anserina (24) encode two putative NAT enzymes, PaNat1 and PaNat2 (Coding Sequence Regions (CDS) numbers: Pa_2_13150 and Pa_4_4860, respectively). These genes are expressed as an expressed sequence tag was identified for each of these genes in the data base (Podospora anserina Genome Project). The P. anserina PaNat1 and PaNat2 genes encode two polypeptides, which, at 333 and 303 amino acids in length, are larger than any previously described NAT enzyme (20). The P. anserina NATs contain all the known NAT-specific functional motifs (25). Sequence analyses (see supplemental Fig. 2) showed the percentage of identity between the P. anserina NAT isoforms (32%) to be lower than that between NAT isoforms in any other eukaryotic species (67–94%). This unusually low level of identity between two paralogous eukaryotic NAT enzymes may reflect functional divergence (26). The percentage of identity between the two P. anserina NAT and the other predicted fungal NAT proteins were found to range from 15% (with Batrachochytrium dendrobatidis NAT) to 55% (with Chaetomium globosum NAT1 and NAT2) (supplemental Fig. 2). PaNat1 and PaNat2 were found to share around 30% identity with a newly characterized NAT isoform (called FDB2) from Fusarium verticillioides, which is involved in benzoazoxinone metabolism (27). When compared with characterized mammalian NAT enzymes such as human NAT1 and NAT2, identities were found to be around 25–30% (data not shown). Protein sequence identities between PaNat1 and PaNat2 and known bacterial NAT enzymes such as the Mycobacterium smegmatis NAT-specific functional motifs (25). Sequence analyses (see supplemental Fig. 2) showed the percentage of identity between the P. anserina NAT isoforms (32%) to be lower than that between NAT isoforms in any other eukaryotic species (67–94%). This unusually low level of identity between two paralogous eukaryotic NAT enzymes may reflect functional divergence (26). The percentage of identity between the two P. anserina NAT and the other predicted fungal NAT proteins were found to range from 15% (with Batrachochytrium dendrobatidis NAT) to 55% (with Chaetomium globosum NAT1 and NAT2) (supplemental Fig. 2). PaNat1 and PaNat2 were found to share around 30% identity with a newly characterized NAT isoform (called FDB2) from Fusarium verticillioides, which is involved in benzoazoxinone metabolism (27). When compared with characterized mammalian NAT enzymes such as human NAT1 and NAT2, identities were found to be around 25–30% (data not shown). Protein sequence identities between PaNat1 and PaNat2 and known bacterial NAT enzymes such as the Mycobacterium smegmatis or S. typhimurium NAT isoforms were around 15–20% (data not shown). Predicted fungal NAT enzymes and the mammalian and bacterial isoforms also differ in their protein sequence lengths (supplemental Fig. 2). Almost all mammalian and bacterial NAT enzymes identified so far are less than 295 amino acids long (28). On the contrary, all fungal NAT identified in this study (including PaNat1 and PaNat2) range between 303 and 387 amino acids (supplemental Fig. 2). So far, no NAT enzyme has been identified in plants.

We purified recombinant P. anserina NAT isoforms and showed that they were readily detected with an anti-NAT antibody (supplemental Fig. 3). We further characterized the puri-
Effects of the Targeted Disruption of NAT Enzymes on the Tolerance of P. anserina to Toxic AA—We investigated the functions of the proteins encoded by the PaNat1 and PaNat2 genes in P. anserina, focusing particularly on their contribution to AA tolerance, by carrying out targeted gene disruption (see “Experimental Procedures” and supplemental Fig. 1). The phenotypes of mutants lacking either one (∆PaNat1 or ∆PaNat2) or both (∆PaNat1/2) NAT genes were compared with that of the WT strain (11). No obvious differences in key biological features, including growth, differentiation, defense against competitors, aging, sexual reproduction, and ascospore germination, were observed (data not shown). We then evaluated the AA tolerance of P. anserina WT and mutant strains. We first assessed AA tolerance in a minimal medium to which selected aromatic compounds (3,4-DCA, 2-AF, 4-BOA) were added. All the fungal isolates (WT and mutants) were screened by assessing radial growth for 3 days. The growth of strains lacking PaNat2 was strongly impaired in the presence of 2-AF, 3,4-DCA, or 4-BOA, whereas strains lacking PaNat1 were less affected and grew similarly to the WT (Fig. 2a). Sensitivity to AA cosegregated in crosses with the phleomycin resistance marker gene used to inactivate PaNat2, and the introduction of a PaNat2 gene by cotransformation with a hygromycin B resistance marker restored WT levels of growth in the ∆PaNat2 strain (11). No obvious differences in key biological features, including growth, reproduction, and ascospore germination, were observed (data not shown). We then evaluated the AA tolerance of P. anserina WT and mutant strains. We first assessed AA tolerance in a minimal medium to which selected aromatic compounds (3,4-DCA, 2-AF, 4-BOA) were added. All the fungal isolates (WT and mutants) were screened by assessing radial growth for 3 days. The growth of strains lacking PaNat2 was strongly impaired in the presence of 2-AF, 3,4-DCA, or 4-BOA, whereas strains lacking PaNat1 were less affected and grew similarly to the WT (Fig. 2a). Sensitivity to AA cosegregated in crosses with the phleomycin resistance marker gene used to inactivate PaNat2, and the introduction of a PaNat2 gene by cotransformation with a hygromycin B resistance marker restored WT levels of growth in the ∆PaNat2 mutants. Hypersensitivity to AA therefore resulted from PaNat2 deletion. The WT and mutant strains grew similarly in the presence of chemically synthesized N-acetylated forms from the plant symbiotic bacterium Mesorhizobium loti, the catalytic efficiency toward 3,4-DCA by PaNAT2 (k_{cat}/K_m = 17,400 M^{-1} s^{-1}) was 220 and 120 times higher than that of M. loti NAT1 and M. loti NAT2, respectively (14).

**FIGURE 2. Contribution of PaNAT enzymes to tolerance to the toxic aromatic amines 2-AF, 3,4-DCA, and 4-BOA in P. anserina.** a, 8-cm Petri dish containing the indicated strains grown on M2 agar medium with 2-AF, 3,4-DCA, and 4-BOA or their acetylated forms at the indicated final concentrations. Photographs were taken after 3 days of growth at 27 °C. DMSO (0.25% final concentration) in solidified M2 medium was used as a control and had no effect on growth (data not shown). The data presented are representative of three independent experiments. *, *p < 0.01 versus WT and ∆PaNat1 strains. The data presented are representative of three independent experiments. Error bars indicate S.E.

We tested a series of different substrates, including a carcinogen (2-AF), drugs (SMX, SMZ, 4-AS, 5-AS, INH, HDZ; definitions are available in Table 1), industrial chemical intermediates (4-BOA, 4-EOA, 4-PD, 4-ANS, 4-AMV; definitions are available in Table 1), and pesticide residues (4-BOA, 4-IA, 3,4-DCA; definitions are available in Table 1). Kinetic parameters (V_m, k_{cat}, and K_m) were estimated for aromatic NAT substrates (14) (Fig. 1, b and c). P. anserina NAT enzymes were highly active against most of the AA substrates tested, with PaNat2 systemically more active than PaNat1 (Table 1 and Fig. 1, b and c). The catalytic efficiency (k_{cat}/K_m) of PaNat2 was up to 80 times higher than that of PaNat1 (Fig. 1c). The catalytic efficiency of PaNat1 with 3,4-DCA was 2.5 times higher and that of PaNat2 was five times higher than that of the Pseudomonas aeruginosa NAT, which is currently considered to be the most efficient NAT enzyme ever described, particularly with 3,4-DCA (29). When compared with the two NAT isoforms from the plant symbiotic bacterium Mesorhizobium loti, the catalytic efficiency toward 3,4-DCA by PaNAT2 (k_{cat}/K_m = 17,400 M^{-1} s^{-1}) was 220 and 120 times higher than that of M. loti NAT1 and M. loti NAT2, respectively (14).
N-acetylated principally by PaNat2 (Fig. 2b). A low level of AA acetylation was detected with the ΔPaNat1/2 extracts (Figs. 2b and 3a). This is likely due to background acetylation by non-NAT acetyltransferases present in *P. anserina*.

**Detoxification of the Highly Toxic Pesticide Residue 3,4-DCA and Bioremediation Applications Using Experimentally Polluted Soils**—We characterized the role of PaNat2 in tolerance to AA in more detail, focusing on 3,4-DCA, a highly toxic pesticide-derived AA persistent in soil, surface water, and groundwater. This compound is the major breakdown product of the phenylamide herbicides diuron, linuron, and propanil (30). The N-acetylated form of 3,4-DCA has been shown to be much less toxic than the parental compound (19). We therefore investigated whether 3,4-DCA was acetylated *in vivo* by WT and mutant *P. anserina* strains. Similar amounts of the N-acetylated form of 3,4-DCA were detected by HPLC in the liquid medium of WT and ΔPaNat1 strains grown in the presence of a toxic dose of 3,4-DCA (250 μM; Fig. 3a). By contrast, very low levels of acetyl-3,4-DCA were found in the media of strains lacking PaNat2, mainly due to the very poor growth of these strains in the presence of 3,4-DCA. Acetyl-3,4-DCA generation was time-dependent. After 3 days of incubation, 45% of the 3,4-DCA had been biotransformed into its acetylated product, 3,4-dichloroacetanilide, in WT cultures, versus 38% in ΔPaNat1 cultures and only 5% in ΔPaNat2 cultures (no significant differences were found between ΔPaNat2 and ΔPaNat1/2; Fig. 3a). No other 3,4-DCA metabolite was detected, suggesting that the PaNat2-dependent N-acetylation of 3,4-DCA was the main biotransformation pathway *in vivo*. Lettuce (*L. sativa*) seeds have been shown to be highly sensitive to 3,4-DCA (at concentrations >10 mg/kg of soil), with the complete abolition of germination and growth (31). After 7 days (Fig. 3b), no seed germination was observed in 3,4-DCA-contaminated medium (200 μM) or in 3,4-DCA-contaminated medium previously incubated with the ΔPaNat1/2 strain. Conversely, seeds were able to germinate and grow in contaminated medium previously incubated with WT *P. anserina*. Thus, PaNat2 is sufficient for the detoxification of 3,4-DCA *in vivo*. These data also suggest that no toxic (at least for the seeds) fungal compound was released by *P. anserina* strains.

*P. anserina* is found on herbivore dung in nature but can grow in soil in the presence of plant debris. This species reproduces by sexual means only, with mating occurring only between partners of opposite mating types (32). The spread of this non-pathogenic fungus is therefore easy to control, making it an attractive candidate for safe bioremediation. As proof of principle, we assessed the capacity of the NAT-dependent acetylation pathway of *P. anserina* to N-acetylate 3,4-DCA present in soil samples. For this purpose, we inoculated soils highly contaminated with 3,4-DCA (final concentration 25 mg/kg of soil) with WT or ΔPaNat1/2 *P. anserina* strains and incubated the mixtures at 25°C for 2 days. We then extracted 3,4-DCA and its acetylated metabolites for detection by HPLC (Fig. 4a). Acetylated 3,4-DCA was readily detected in soil samples incubated with WT *P. anserina*. The amount of acetyl-3,4-DCA was found to depend on the amount of fungus used for soil inoculation (Fig. 4a, black and dotted lines). We found that 40% of the 3,4-DCA present could be N-acetylated within 48 h (Fig. 4b). In the same conditions, no acetylation of 3,4-DCA was detected with ΔPaNat1/2 *P. anserina* (Fig. 4a, dashed line, and 4b). We analyzed *L. sativa* seed germination and growth in 3,4-DCA-contaminated (80 mg/kg of soil) soils after incubation with WT or ΔPaNat1/2 *P. anserina* strains for 72 h (Fig. 4c). *L. sativa* seed germination and early growth were completely abolished in soils contaminated with 3,4-DCA. No seed germination or growth was observed with 3,4-DCA-contaminated soil inoculated with the ΔPaNat1/2 strain (Fig. 4c). Conversely, in contaminated soil treated with WT *P. anserina*, seed germination and growth were restored to the levels observed with soil treated with chemically acetylated 3,4-DCA (Fig. 4c). Thus, the inoculation of a 3,4-DCA-contaminated soil with a *P. anserina* strain harboring a functional NAT-dependent acetylation pathways leads, *in situ*, to significant detoxification of this toxic compound, making possible the germination of *L. sativa* seeds and the early growth of the seedlings. Concentrations around 100 μg/kg of 3,4-DCA have been reported in contaminated soils (33). Our study shows that even at higher 3,4-DCA con-
Podospora anserina Arylamine N-Acetyltransferases

centrations (25 mg/kg), P. anserina mediates efficient bioremediation of this compound in soil.

These results pave the way for use of the fungal NAT metabolic pathway in the bioremediation of AA pollution in soils. The potential of fungal metabolic pathways for the bioremediation of AA pollution, particularly in soils contaminated with aniline pesticide residues such as 3,4-DCA, has been little studied. Certain fungi and bacteria have nonetheless been shown to biotransform 3,4-DCA to its acetylated form (19, 34). In the bacterium M. loti, a NAT isoform has been shown to acetylate 3,4-DCA (14). In plants, glucosylation of 3,4-DCA was described, but this pathway was considered as non-effective at detoxifying this AA (35). Our results should facilitate prospective studies of AA bioremediation and the rationalization of future strategies based on the fungal NAT pathway for AA detoxification. In addition, our findings further emphasize that certain well characterized fungi may constitute a more efficient alternative model, with a reasonable cost and a low environmental impact.

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Studies on model fungi have provided much of our understanding of biological processes. Such models also help to understand the general mechanisms by which living organisms protect themselves against potentially toxic effects of natural products or xenobiotics present in their environment (7, 8). The NAT-dependent xenobiotic biotransformation pathway is found in many organisms ranging from bacteria to humans (26). However, the relevance of this pathway to AA tolerance in living organisms has remained unclear. So far, studies done on knock-out NAT mouse models have not demonstrated a role for NAT enzymes in preventing AA toxicity (21). Our P. anserina model provides the first clear molecular and functional evidence indicating that the NAT-dependent xenobiotic-biotransformation pathway can afford complete tolerance toward toxic AA in a eukaryotic organism. In addition to the existing knock-out mouse model, our P. anserina model should be helpful to uncover the potential endogenous functions of NAT enzymes. Overall, our data underline the role of certain xenobiotic-metab-
olizing enzymes in the adaptation of organisms to their chemical environment and emphasize the potential biotechnological applications of such enzymatic pathways.

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Podospora anserina Arylamine N-Acetyltransferases