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Introduction

Nowadays, pollution is a major concern. Human activities have resulted in xenobiotics accumulation in natural environments, particularly soil contamination by pesticides, industrial chemicals and their derivatives. Living species use several xenobiotic metabolic pathways to protect themselves against the toxic effects of these pollutants.

Arylamine *N*-acetyltransferase (NAT) proteins are xenobiotic-metabolizing enzymes (XME) which catalyze the transfer of an acetyl group from acetyl-coA (AcCoA) to aromatic amines and N-hydroxylated metabolites. The N-acetylation of these chemicals has been shown to detoxify them.

Previous studies have identified and studied two homologous NAT genes from the filamentous ascomycetes soil fungus *Podospora anserina*. Here, we present the characterization of two new ascomycetes (*Trichoderma virens* and *Trichoderma reesei*) NAT enzyme. *Trichoderma* are fungi commonly found in soils, where they are the most prevalent culturable fungi.

Functional characterization of TreeNAT2 and TvirNAT2

Materials and Methods : The recombinant TvirNAT2 and TreeNAT2 activities were measured by the DTNB test. This test quantifies the hydrolysis rate of acCoA (400µM) in presence of aromatic substrates (up to 2000 µM). Apparent Michaelis-Menten parameters Vmax and Km were determined by direct curve-fitting to Michaelis-Menten equation (non linear regression).

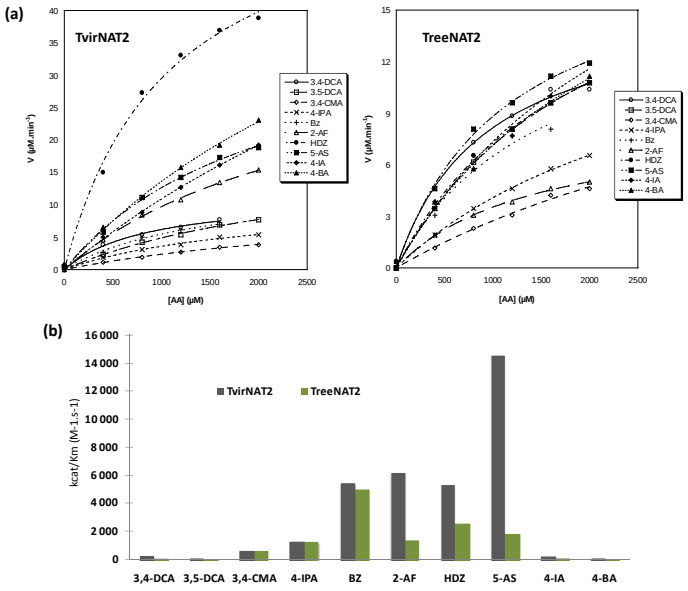

Figure 1 : Functional characterization of TvirNAT2 and TreeNAT2. Specific activities were determined with 5.5'-dithio-bis-nitrobenzoic acid (DTNB) assay. (a) Michaelis-Menten kinetic characterization of *T. virNAT2* and *T. reeNAT2* with typical substrates, (b) Comparaison of catalytic efficiencies, as estimated from ratios of kinetic parameters (k_{cat}/K_m), expressed in $M^{-1}.s^{-1}$.

TvirNAT2 demonstrates higher activity for almost all the substrates tested here, compared to TreeNAT2. The substrates tested are drugs (5AS, HDZ), industrial chemical intermediates (BZ) and pesticide residues (34-DCA, 4-IA, 4-IPA, 4-BA,35-DCA).

Tolerance assays to aromatic amines

Materials and Methods : 8-cm Petri dish containing the indicated strains grown on M2 agar medium 4-IPA, BZ and 3,4-DCA 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).

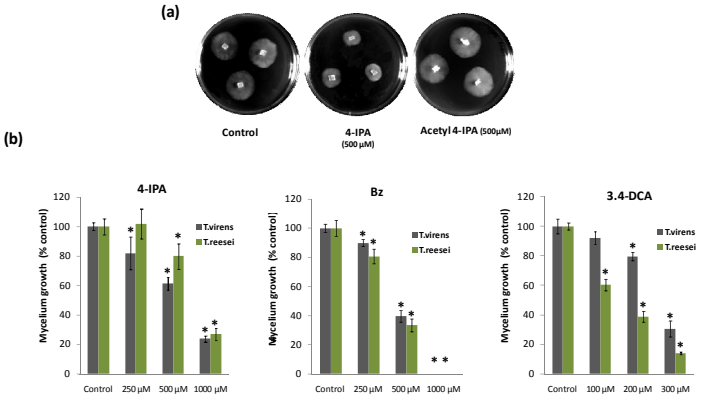

Figure 2 : Contribution of *T. ree* et *T. vir* NAT enzymes to tolerance to the toxic aromatic amines 4-IPA, BZ and 3,4-DCA.

The fungal tolerance to AA or acetyl-AA, was quantified by determination of radial growth (Fig.2a). When 250 µM of 4-IPA or Bz were added, the growth of strains was poorly impaired (Fig.2b). However, sensitivity was increased in the presence of 3.4-DCA especially for *T. reesei*.

In vivo acetylation assays

Materials and Methods : *T.reesei* and *T. virens* (0.25 g of fungal dry mass) were grown in M_2 liquid medium in the presence of 250 µM Bz. At different time points, acetyl-Bz and Bz were detected in the growth medium and quantified by HPLC. Data were normalised with the final fungal dry mass.

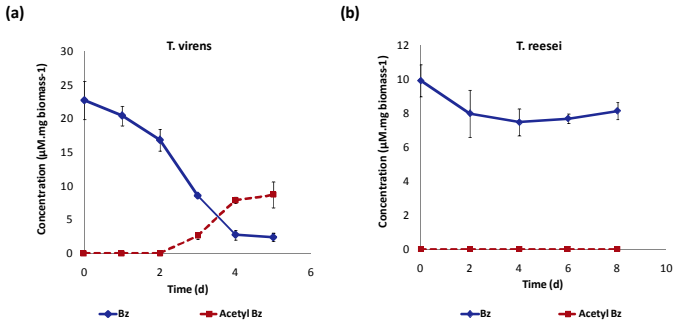

Figure 3 : *In vivo* acetylation of Bz by *T. reesei* et *T. virens*.

Cultures of T. virens and T. reesei were performed to evaluate their capacity to acetylate aromatic amines. After 5 days, 38% of Bz had been biotransformed into acetyl-Bz, in T. virens cultures (Fig.3a). In T. reesei cultures, no acetylated product was detected in the medium (Fig.3b).

Therefore, the *in vivo* *N*-acetylation pathway appears more efficient in *T. virens* to detoxify aromatic amines.

Conclusion

NAT enzymes are XME that play an important role in the detoxification of many therapeutic drugs and pollutants such as chemical intermediates or by-products of additives, pharmaceuticals, dyes and pesticides. We reported here the presence of two functional NAT enzyme in *T. virens* and *T. reesei*. Enzymatic properties were tested against a variety of common anthropic substrates. TvirNAT2 and TreeNAT2 activities shows to be very similar.

These new fungal NAT enzymes showed the same substrate specificity as *Podospora anserina* enzyme but with lower catalytic efficiency. However, *Trichoderma* sp. are common in soils and on plant roots. They are also among the most abundant culturable fungi in many soils. Therefore, these species may be good candidates for use in bioremediation.

Silar P, Dairou J, Cocaign A, Busi F, Rodrigues-Lima F, Dupret JM, Nat. Rev. Microbiol. 2011;9(6):477