Biotransformation des amines aromatiques par Trichoderma spp

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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 (AA). 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 fungus *Podospora anserina*[1]. Here, we present the characterization of two new fungal NAT enzyme. *Trichoderma virens* (teleomorph *Hypocrea virens*) and *Trichoderma reesei* (teleomorph *Hypocrea jecorina*) are fungi commonly found in soils, where they are the most prevalent culturable fungi. Both species have already industrial applications : *T. reesei* and *T. virens* are used respectively as cellulases source and biocontrol agent in plant protection.

Tolerance assays to aromatic amines

- *T. virens* is most resistant than *T. reesei* to 3,4-DCA (pesticide residue).
- Acetylation increases AA tolerance in *Trichoderma* spp.
- Growth inhibition is dependent on AA concentrations.
- Hypothesis : acetylation of AA by fungal NAT enzymes may detoxify them.

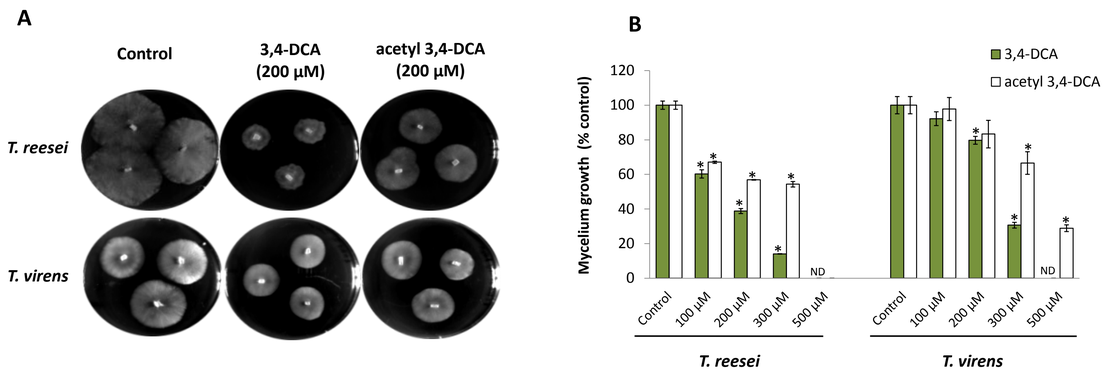

Figure 1 : *T. reesei* and *T. virens* tolerance to the toxic aromatic amine 3,4-DCA (3,4-dichloroaniline). A) The indicated strains grown on M2 agar 3,4-DCA or acetyl 3,4-DCA. Photographs were taken after 3 days of growth at 27 °C. **B)** Rate of growth of *T. virens* and *T. reesei* on M2 agar medium in the presence of 3,4-DCA or acetyl 3,4-DCA, at the indicated final concentration. Data are presented as mean ± SD of three independent experiments. *p < 0.05 compared with control. ND: not detectable.

Biotransformation assays

- NAT activity was detected in fungal extract -> NAT enzymes are expressed in both species. **(Fig. 3A)**
- In contaminated liquid medium : ≈ 80% of 3,4-DCA were metabolized whose 2% were biotransformed by *N*-acetylation in *T. reesei* culture. **(Fig. 3B)**
- In contaminated soil : ≈ 62% of 3,4-DCA were metabolized whose 34% were acetylated in presence of *T. reesei*. **(Fig. 3C)**
- Both species biotransform AA but only *T. reesei* uses the *N*-acetylation pathway to detoxify them.

A

Strain	NAT activity (pmol.min^{-1}.mg^{-1})
T. reesei	61.8 ± 2.5
T. virens	98.7 ± 7.4

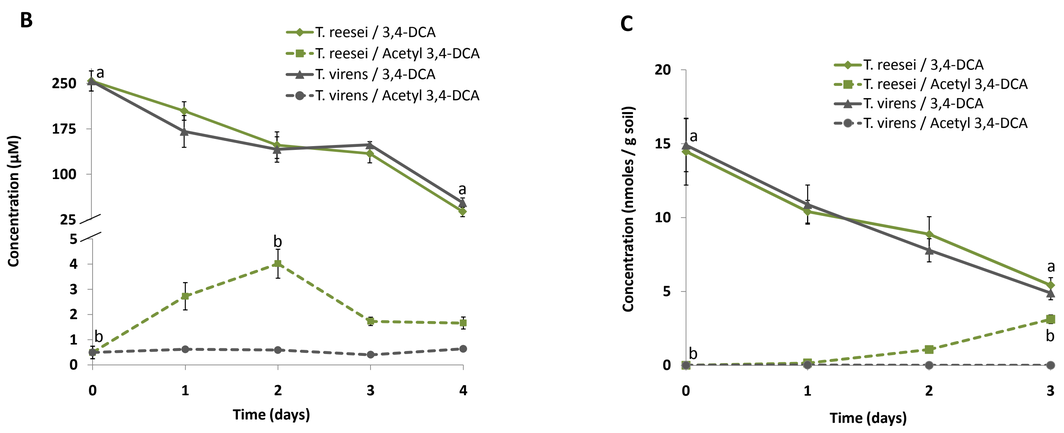

Figure 3 *: In vivo* acetylation of AA by *T. reesei* et *T. virens*. A) *N*-acetylation of 2-AF by fungal extracts. Activities were measured after lysis of fungal biomass with 2-AF (1mM) and AcCoA (1mM). Data are presented as mean ± SD of three independent experiments. **B)** *T. reesei* or *T. virens* were grown in potato dextrose liquid medium in the presence of 250 µM 3,4-DCA. At different time points, 3,4-DCA and acetyl 3,4-DCA were detected in the growth medium and quantified by HPLC. Data were normalized with control to account for spontaneous degradation of 3,4-DCA. Data are presented as mean ± SD of three independent experiments.$^{a,b}p < 0.05$. **C)** *T. reesei* or *T. virens* were used to inoculate soils contaminated with 3,4-DCA (20 mg.kg^{-1}). At various time points, 3,4-DCA and acetyl 3,4-DCA were extracted from soil samples and analysed by HPLC. Data are presented as mean ± SD of five independent experiments. $^{a,b}p < 0.05$.

Functional characterization of recombinant enzymes : (HYPVI)NAT1 and (HYPJE)NAT1

- These two NAT enzymes present the same substrate specificity.
- (HYPVI)NAT1 efficiency is higher than (HYPJE)NAT1 for 7 of 10 substrates tested.

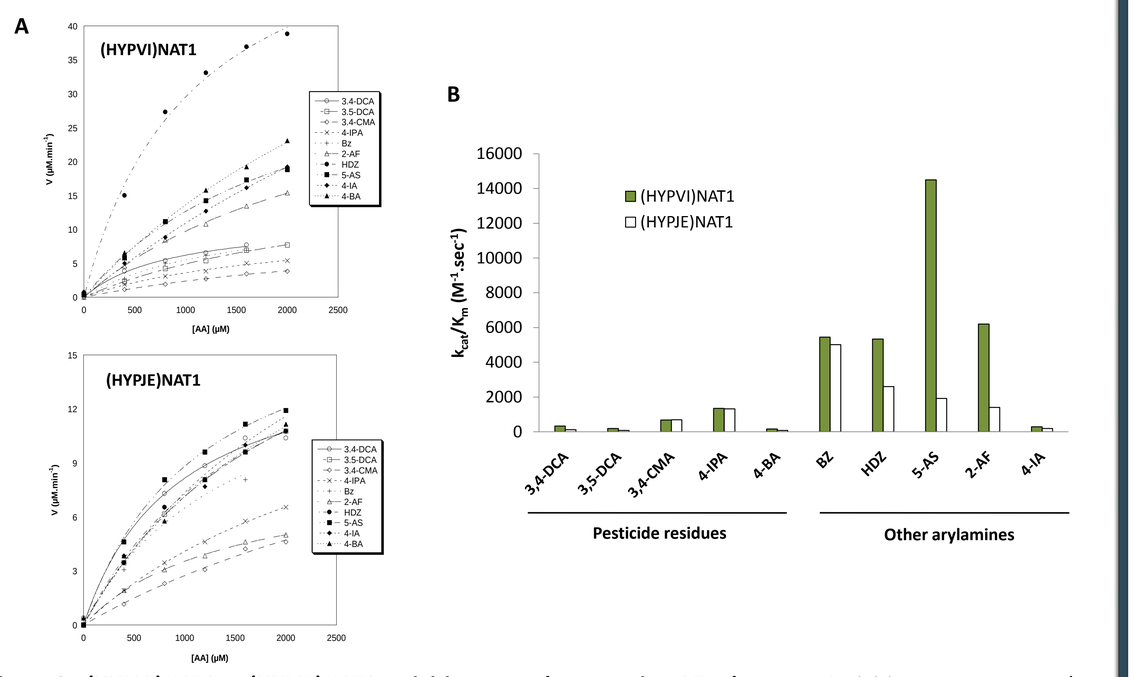

Figure 2 : (HYPVI)NAT1 et (HYPJE)NAT1 activities towards aromatic NAT substrates. Activities were measured by the DTNB test. This test quantifies the hydrolysis rate of AcCoA (400µM) in presence of AA. Apparent V_{max} and K_m were determined by direct curve-fitting to Michaelis-Menten equation. **A)** Michaelis-Menten kinetic characterization of (HYPVI)NAT1 and (HYPJE)NAT1. **B)** Comparaison of catalytic efficiencies (k_{cat}/K_m app). Error for triplicate values were at maximum of ± 5%. 3,5-DCA : 3,5-(dichloroaniline); 3,4-CMA : 3-chloro-4-methylaniline; 4-IPA : 4-isopropylaniline; 4-BA : 4-bromoaniline; BZ : benzidine; HDZ : hydralazine; 5-AS : 5-aminosalicylate; 2-AF : 2-aminofluorene; 4-IA : 4-iodoaniline

Complementation of *P. anserina ΔNAT1/2* with (HYPJE)NAT1

- In presence of 3,4-DCA : **(Fig. 4A)**
  - Growth *of P. anserina WT* is few impaired,
  - Growth *of P. anserina Δ(PODAS)NAT1/2* is completly inhibited,
  - Growth *of P. anserina Δ(PODAS)NAT1/2–(HYPJE)NAT1* increases compare to *Δ(PODAS)NATs.*
- Introduction of (HYPJE)NAT1 in *P. anserina Δ(PODAS)NAT1/2* increased resistance to AA. It's confirmed by NAT activities in fungal extracts. **(Fig. 4B)**
- (HYPJE)NAT1 promotes acetylation and tolerance to 3,4-DCA.

Strain	NAT activity (pmol.min^{-1}.mg^{-1})
WT	808 ± 151
Δ(PODAS)NAT1/2	37 ± 0,5
(HYPJE)NAT1	512 ± 19

Figure 4 *:* Contribution of (HYPJE)NAT1 enzyme to tolerance to 3,4-DCA. A) *P. anserina WT*, *P. anserina Δ(PODAS)NAT1/2* and *P. anserina Δ(PODAS)NAT1/2–(HYPJE)NAT1* tolerance to 3,4-DCA. Indicated strains grown on M2 agar with 3,4-DCA (0 or 200 µM). **B)** *N*-acetylation of 2-AF by fungal extracts. Activities were measured after lysis of fungal biomass with 2-AF (1mM) and AcCoA (1mM). Data are presented as mean ± SD of three independent experiments.

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. (HYPJE)NAT1 and (HYPVI)NAT1 showed the same substrate specificity and similar activities.

In vivo, both species metabolize efficiently 3,4-DCA, but only *T. reesei* use the *N*-acetylation pathway to detoxify AA. These results were confirmed by complementation assays : (HYPJE)NAT1 expressed in *P. anserina Δ(PODAS)NAT1/2* increases acetylation and tolerance to 3,4-DCA.

This study show that *T. reesei* and *T. virens* may be good candidates for use in bioremediation.

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