Fungi as a promising tool for bioremediation of soils contaminated with aromatic amines, a major class of pollutants

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In their recent article, (Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Rev. Microbiol.* **9**, 177–192 (2011))¹, Harms *et al.* highlight that the potential use for fungi in bioremediation has not received the attention it deserves. We fully agree with Harms *et al.* and wish to further highlight the promising metabolic capabilities of these organisms in the remediation of a major class of pollutants, the aromatic amines (AA).

In a pilot study, we provided proof-of-concept remediation experiments in which *Podospira anserina*, through its arylamine *N*-acetyltransferase 2 (NAT2) enzyme, detoxifies the highly toxic pesticide residue 3,4-dichloroaniline (3,4-DCA) in experimentally contaminated soil samples². 3,4-DCA is the major breakdown product of the phenylurea herbicides diuron and linuron and of the anilide propanil. It belongs to the class of AA, an important and diversified class of soil pollutants. Many AA are toxic to most living organisms³. In particular, AA account for 12% of the 415 chemicals known or strongly suspected to be carcinogenic in humans⁴. Some aniline derivatives, such as 3,4-DCA or 3,5-DCA, are persistent in soils and waters and exhibit potential toxicity^{5,6}. Residues of sulphonamides, an important class of AA drugs, have been detected in manure at levels of up to 12 mg per kg⁷. The ecotoxicological effects of these drug contaminations remain to be studied more thoroughly. However, toxic effects have already been reported^{8,9}. AA contamination can involve industrial chemicals, some of which, including azo dyes, have been shown to be toxic¹⁰. Soils can also be contaminated by toxic nitroaromatic compounds¹¹.

Exploiting the ability of microorganisms to transform AA pollutants is a promising approach for bioremediation. Most studies have focused on conversion of 3,4-DCA into its acetylated form. First, it has been shown that acetylated 3,4-DCA is less toxic than 3,4-DCA; second, some

soil bacteria and fungal strains acetylate 3,4-DCA^{12,13}. Although aniline derivatives undergo complex transformations in soils, these studies open up new possibilities in bioremediation.

The major detoxification pathway of AA depends on the activity of NATs¹⁴. NATs affect the bioavailability of many AA drugs and carcinogens. Using the potential degradative properties of NATs expressed in soil microorganisms, we explored putative AA bioremediation pathways using soil bacteria or filamentous fungi^{2,13}. We chose to focus our studies on the bioremediation potential of *P. anserina*. This fungus only reproduces by sexual means, it is a non-pathogenic cosmopolitan species and its spread is easy to control. Targeted gene disruption experiments revealed that only one NAT, NAT2, is required for the growth and survival of the fungus in the presence of toxic AA. These findings provided a new basis for the bioremediation of AA-contaminated soils.

Given the detoxifying activity of NATs, the presence of NAT-encoding genes in many other fungi¹⁵ and the fungal biomass in soils, our studies show that fungal bioremediation of AA represents a promising perspective. Further studies are needed for setting up bioremediation protocols in natural conditions and to assess the possible effects of other soil fungi on AA biodegradation.

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Published online 18 April 2011

- Harms, H., Schlosser, D. & Wick, L. Y. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Rev. Microbiol.* **9**, 177–192 (2011).
- Martins, M. *et al.* An acetyltransferase conferring tolerance to toxic aromatic amine chemicals: molecular and functional studies. *J. Biol. Chem.* **284**, 18726–18733 (2009).
- Kim, D. & Guengerich, F. P. Cytochrome P450 activation of arylamines and heterocyclic amines. *Annu. Rev. Pharmacol. Toxicol.* **45**, 27–49 (2005).
- US National Toxicology Program. 11th Report on Carcinogens. (US Department of Health and Human Services, 2005).
- Ito, Y., Matsuda, Y. & Suzuki, T. Effects of 3,4-dichloroaniline on expression of *ahr2* and *cyp1a1* in zebrafish adults and embryos. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **152**, 189–194 (2010).
- Lee, J. B. *et al.* Microbial biodegradation and toxicity of vinclozolin and its toxic metabolite 3,5-dichloroaniline. *J. Microbiol. Biotechnol.* **18**, 343–349 (2008).
- García-Galan, M. J., Díaz-Cruz, M. S. & Barcelo, D. Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC–MS/MS). *Talanta* **81**, 355–366 (2010).
- Jin, C., Chen, Q., Sun, R., Zhou, Q. & Liu, J. Eco-toxic effects of sulfadiazine sodium, sulfamonomethoxine sodium and enrofloxacin on wheat, Chinese cabbage and tomato. *Ecotoxicology* **18**, 878–885 (2009).
- Sartorius, M. *et al.* Sulphadimethoxine inhibits *Phaseolus vulgaris* root growth and development of N-fixing nodules. *Chemosphere* **76**, 306–312 (2009).
- Khalid, A., Arshad, M. & Crowley, D. E. Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains. *Appl. Microbiol. Biotechnol.* **78**, 361–369 (2008).
- Frische, T. Screening for soil toxicity and mutagenicity using luminescent bacteria — a case study of the explosive 2,4,6-trinitrotoluene (TNT). *Ecotoxicol. Environ. Saf.* **51**, 133–144 (2002).
- Tixier, C. *et al.* Biotransformation of phenylurea herbicides by a soil bacterial strain, *Arthrobacter* sp. N2: structure, ecotoxicity and fate of diuron metabolite with soil fungi. *Chemosphere* **46**, 519–526 (2002).
- Rodrigues-Lima, F. *et al.* Cloning, functional expression and characterization of *Mesorhizobium loti* arylamine *N*-acetyltransferases: rhizobial symbiosis supplies leguminous plants with the xenobiotic *N*-acetylation pathway. *Mol. Microbiol.* **60**, 505–512 (2006).
- Dupret, J. M. & Rodrigues-Lima, F. Structure and regulation of the drug-metabolizing enzymes arylamine *N*-acetyltransferases. *Curr. Med. Chem.* **12**, 311–318 (2005).
- Martins, M., Dairou, J., Rodrigues-Lima, F., Dupret, J. M. & Silar, P. Insights into the phylogeny of arylamine *N*-acetyltransferases in fungi. *J. Mol. Evol.* **71**, 141–152 (2010).

Competing interests statement

The authors declare no competing financial interests.