

## Proteomic analysis of 2,4,6-trinitrotoluene degrading yeast *Yarrowia lipolytica*

Khilyas I., Lochnit G., Ilinskaya O.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

---

### Abstract

© 2017 Khilyas, Lochnit and Ilinskaya. 2,4,6-trinitrotoluene (TNT) is a common component of many explosives. The overproduction and extensive usage of TNT significantly contaminates the environment. TNT accumulates in soils and aquatic ecosystems and can primarily be destroyed by microorganisms. Current work is devoted to investigation of *Yarrowia lipolytica* proteins responsible for TNT transformation through the pathway leading to protonated Meisenheimer complexes and nitrite release. Here, we identified a unique set of upregulated membrane and cytosolic proteins of *Y. lipolytica*, which biosynthesis increased during TNT transformation through TNT-monohydride-Meisenheimer complexes in the first step of TNT degradation, through TNT-dihydride-Meisenheimer complexes in the second step, and the aromatic ring denitration and degradation in the last step. We established that the production of oxidoreductases, namely, NADH flavin oxidoreductases and NAD(P)<sup>+</sup>-dependent aldehyde dehydrogenases, as well as transferases was enhanced at all stages of the TNT transformation by *Y. lipolytica*. The up-regulation of several stress response proteins (superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase) was also detected. The involvement of intracellular nitric oxide dioxygenase in NO formation during nitrite oxidation was shown. Our results present at the first time the full proteome analysis of *Y. lipolytica* yeast, destructor of TNT.

<http://dx.doi.org/10.3389/fmicb.2017.02600>

---

### Keywords

2,4,6-trinitrotoluene, Biodegradation, Old yellow enzymes, Proteomic assay, TNT, *Yarrowia lipolytica*

### References

- [1] Adamia, G., Ghogoberidze, M., Graves, D., Khatishashvili, G., Kvesitadze, G., Lomidze, E., et al. (2006). Absorption, distribution, and transformation of TNT in higher plants. *Ecotoxicol. Environ. Saf.* 64, 136-145. doi: 10.1016/j.ecoenv.2005.05.001
- [2] Barreto, L., Garcera, A., Jansson, K., Sunnerhagen, P., and Herrero, E. (2006). A peroxisomal glutathione transferase of *Saccharomyces cerevisiae* is functionally related to sulfur amino acid metabolism. *Eukaryot. Cell* 5, 1748-1759. doi: 10.1128/ec.00216-06
- [3] Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., and Freeman, B. A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U.S.A.* 87, 1620-1624. doi: 10.1073/pnas.87.4.1620

- [4] Beckman, J. S., and Koppenol, W. H. (1996). Nitric oxide, superoxide, and peroxyxynitrite: the good, the bad, and ugly. *Am. J. Physiol.* 271(5 Pt 1), C1424-C1437
- [5] Boelsterli, U. A., Ho, H. K., Zhou, S., and Leow, K. Y. (2006). Bioactivation and hepatotoxicity of nitroaromatic drugs. *Curr. Drug Metab.* 7, 715-727. doi: 10.2174/138920006778520606
- [6] Borch, T., and Gerlach, R. (2004). Use of reversed-phase high-performance liquid chromatography-diode array detection for complete separation of 2, 4, 6-trinitrotoluene metabolites and EPA Method 8330 explosives: influence of temperature and an ion-pair reagent. *J. Chromatogr. A* 1022, 83-94. doi: 10.1016/j.chroma.2003.09.067
- [7] Caballero, A., Lazaro, J. J., Ramos, J. L., and Esteve-Nunez, A. (2005). PnrA, a new nitroreductase-family enzyme in the TNT-degrading strain *Pseudomonas putida* JLR11. *Environ. Microbiol.* 7, 1211-1219. doi: 10.1111/j.1462-2920.2005.00801.x
- [8] Cho, Y. S., Lee, B. U., Kahng, H. Y., and Oh, K. H. (2009). Comparative analysis of 2, 4, 6-trinitrotoluene (TNT)-induced cellular responses and proteomes in *Pseudomonas* sp. HK-6 in two types of media. *J. Microbiol.* 47, 220-224. doi: 10.1007/s12275-008-0108-0
- [9] Claus, H. (2014). "Microbial degradation of 2, 4, 6-Trinitrotoluene In Vitro and in natural environments," in *Biological Remediation of Explosive Residues*, ed. S. N. Singh (Cham: Springer International Publishing), 15-38
- [10] Esteve-Nunez, A., Caballero, A., and Ramos, J. L. (2001). Biological degradation of 2, 4, 6-trinitrotoluene. *Microbiol. Mol. Biol. Rev.* 65, 335-352. doi: 10.1128/membr.65.3.335-352.2001
- [11] Farah, M. E., and Amberg, D. C. (2007). Conserved actin cysteine residues are oxidative stress sensors that can regulate cell death in yeast. *Mol. Biol. Cell* 18, 1359-1365. doi: 10.1091/mbc.E06-08-0718
- [12] Fitzpatrick, T. B., Amrhein, N., and Macheroux, P. (2003). Characterization of YqjM, an Old Yellow Enzyme homolog from *Bacillus subtilis* involved in the oxidative stress response. *J. Biol. Chem.* 278, 19891-19897. doi: 10.1074/jbc.M211778200
- [13] French, C. E., Nicklin, S., and Bruce, N. C. (1998). Aerobic degradation of 2, 4, 6-trinitrotoluene by *Enterobacter cloacae* PB2 and by pentaerythritol tetranitrate reductase. *Appl. Environ. Microbiol.* 64, 2864-2868
- [14] Ho, E. M., Chang, H. W., Kim, S. I., Kahng, H. Y., and Oh, K. H. (2004). Analysis of TNT (2, 4, 6-trinitrotoluene)-inducible cellular responses and stress shock proteome in *Stenotrophomonas* sp. OK-5. *Curr. Microbiol.* 49, 346-352. doi: 10.1007/s00284-004-4322-7
- [15] Ignarro, L. J., Napoli, C., and Loscalzo, J. (2002). Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: an overview. *Circ. Res.* 90, 21-28. doi: 10.1161/hh0102.102330
- [16] Iman, M., Sobati, T., Panahi, Y., and Mobasheri, M. (2017). Systems biology approach to bioremediation of nitroaromatics: constraint-based analysis of 2, 4, 6-trinitrotoluene biotransformation by *Escherichia coli*. *Molecules* 22:E1242. doi: 10.3390/molecules22081242
- [17] Khan, H., Harris, R. J., Barna, T., Craig, D. H., Bruce, N. C., Munro, A. W., et al. (2002). Kinetic and structural basis of reactivity of pentaerythritol tetranitrate reductase with NADPH, 2-cyclohexenone, nitroesters, and nitroaromatic explosives. *J. Biol. Chem.* 277, 21906-21912. doi: 10.1074/jbc.M200637200
- [18] Khilyas, I. V., Ziganshin, A. M., Pannier, A. J., and Gerlach, R. (2013). Effect of ferrihydrite on 2, 4, 6-trinitrotoluene biotransformation by an aerobic yeast. *Biodegradation* 24, 631-644. doi: 10.1007/s10532-01-9611-4
- [19] Kumagai, Y., Kikushima, M., Nakai, Y., Shimojo, N., and Kunimoto, M. (2004). Neuronal nitric oxide synthase (nNOS) catalyzes one-electron reduction of 2, 4, 6-trinitrotoluene, resulting in decreased nitric oxide production and increased nNOS gene expression: implication for oxidative stress. *Free Radic. Biol. Med.* 37, 350-357. doi: 10.1016/j.freeradbiomed.2004.04.023
- [20] Lee, B. U., Cho, Y. S., Park, S. C., and Oh, K. H. (2009). Enhanced degradation of TNT by genome-shuffled *Stenotrophomonas maltophilia* OK-5. *Curr. Microbiol.* 59, 346-351. doi: 10.1007/s00284-009-9443-6
- [21] Lee, B. U., Park, S. C., Cho, Y. S., and Oh, K. H. (2008). Exopolymer biosynthesis and proteomic changes of *Pseudomonas* sp. HK-6 under stress of TNT (2, 4, 6-trinitrotoluene). *Curr. Microbiol.* 57, 477-483. doi: 10.1007/s00284-008-9272-z
- [22] Letzel, S., Göen, T., Bader, M., Angerer, J., and Kraus, T. (2003). Exposure to nitroaromatic explosives and health effects during disposal of military waste. *Occup. Environ. Med.* 60, 483-488. doi: 10.1136/oem.60.7.483
- [23] Mulla, S. I., Talwar, M. P., and Ninnekar, H. Z. (2014). "Bioremediation of 2, 4, 6-trinitrotoluene explosive residues," in *Biological Remediation of Explosive Residues*, ed. S. N. Singh (Cham: Springer International Publishing), 201-233
- [24] Naumenko, E. A., Ahlemeyer, B., and Baumgart-Vogt, E. (2016). Species-specific differences in peroxisome proliferation, catalase, and SOD2 upregulation as well as toxicity in human, mouse, and rat hepatoma cells induced by the explosive and environmental pollutant 2, 4, 6-trinitrotoluene. *Environ. Toxicol.* 32, 989-1006. doi: 10.1002/tox.22299
- [25] Nizam, S., Verma, S., Borah, N. N., Gazara, R. K., and Verma, P. K. (2014). Comprehensive genome-wide analysis reveals different classes of enigmatic old yellow enzyme in fungi. *Sci. Rep.* 4:4013. doi: 10.1038/srep04013

- [26] Oh, B. T., Sarath, G., and Shea, P. J. (2001). TNT nitroreductase from a *Pseudomonas aeruginosa* strain isolated from TNT-contaminated soil. *Soil Biol. Biochem.* 33, 875-881. doi: 10.1016/S0038-0717(00)00233-9
- [27] Pak, J. W., Knoke, K. L., Noguera, D. R., Fox, B. G., and Chambliss, G. H. (2000). Transformation of 2, 4, 6-trinitrotoluene by purified xenobiotic reductase B from *Pseudomonas fluorescens* I-C. *Appl. Environ. Microbiol.* 66, 4742-4750. doi: 10.1128/AEM.66.11.4742-4750.2000
- [28] Rylott, E. L., Lorenz, A., and Bruce, N. C. (2011). Biodegradation and biotransformation of explosives. *Curr. Opin. Biotechnol.* 22, 434-440. doi: 10.1016/j.copbio.2010.10.014
- [29] Sens, C., Scheidemann, P., and Werner, D. (1999). The distribution of 14 C-TNT in different biochemical compartments of the monocotyledonous *Triticum aestivum*. *Environ. Pollut.* 104, 113-119. doi: 10.1016/S0269-7491(98)00142-0
- [30] Singh, S. N. (2014). *Biological Remediation of Explosive Residues*. Cham: Springer International Publishing
- [31] Smets, B. F., Yin, H., and Esteve-Nunez, A. (2007). TNT biotransformation: when chemistry confronts mineralization. *Appl. Microbiol. Biotechnol.* 76, 267-277. doi: 10.1007/s00253-007-1008-7
- [32] Spain, J. C. (1995). Biodegradation of nitroaromatic compounds. *Annu. Rev. Microbiol.* 49, 523-555. doi: 10.1146/annurev.mi.49.100195.002515
- [33] Spanggord, R. J., Stewart, K. R., and Riccio, E. S. (1995). Mutagenicity of tetranitroazoxytoluenes: a preliminary screening in *Salmonella typhimurium* strains TA100 and TA100NR. *Mutat. Res.* 335, 207-211. doi: 10.1016/0165-1161(95)00022-4
- [34] Stenuit, B. A., and Agathos, S. N. (2010). Microbial 2, 4, 6-trinitrotoluene degradation: could we learn from (bio)chemistry for bioremediation and vice versa? *Appl. Microbiol. Biotechnol.* 88, 1043-1064. doi: 10.1007/s00253-010-2830-x
- [35] Strassner, J., Furholz, A., Macheroux, P., Amrhein, N., and Schaller, A. (1999). A homolog of old yellow enzyme in tomato. Spectral properties and substrate specificity of the recombinant protein. *J. Biol. Chem.* 274, 35067-35073. doi: 10.1074/jbc.274.49.35067
- [36] Symons, Z. C., and Bruce, N. C. (2006). Bacterial pathways for degradation of nitroaromatics. *Nat. Prod. Rep.* 23, 845-850. doi: 10.1039/b502796a
- [37] Vasquez-Vivar, J., Denicola, A., Radi, R., and Augusto, O. (1997). Peroxynitrite-mediated decarboxylation of pyruvate to both carbon dioxide and carbon dioxide radical anion. *Chem. Res. Toxicol.* 10, 786-794. doi: 10.1021/tx970031g
- [38] Wenge, B., Bönisch, H., Grabitzki, J., Lochnit, G., Schmitz, B., and Ahrend, M. H. (2008). Separation of membrane proteins by two-dimensional electrophoresis using cationic rehydrated strips. *Electrophoresis* 29, 1511-1517. doi: 10.1002/elps.200700546
- [39] Wessel, D., and Flügge, U. I. (1984). A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. *Anal. Biochem.* 138, 141-143. doi: 10.1016/0003-2697(84)90782-6
- [40] Williams, R. E., and Bruce, N. C. (2002). 'New uses for an old enzyme'-the old yellow enzyme family of flavoenzymes. *Microbiology* 148(Pt 6), 1607-1614. doi: 10.1099/00221287-148-6-1607
- [41] Williams, R. E., Rathbone, D. A., Scrutton, N. S., and Bruce, N. C. (2004). Biotransformation of explosives by the old yellow enzyme family of flavoproteins. *Appl. Environ. Microbiol.* 70, 3566-3574. doi: 10.1128/AEM.70.6.3566-3574.2004
- [42] Wittich, R. M., Haidour, A., Van Dillewijn, P., and Ramos, J. L. (2008). OYE flavoprotein reductases initiate the condensation of TNT-derived intermediates to secondary diarylamines and nitrite. *Environ. Sci. Technol.* 42, 734-739. doi: 10.1021/es071449w
- [43] Xia, Y., Cardounel, A. J., Vanin, A. F., and Zweier, J. L. (2000). Electron paramagnetic resonance spectroscopy with N-methyl-D-glucamine dithiocarbamate iron complexes distinguishes nitric oxide and nitroxyl anion in a redox-dependent manner: applications in identifying nitrogen monoxide products from nitric oxide synthase. *Free Radic. Biol. Med.* 29, 793-797. doi: 10.1016/S0891-5849(00)00427-5
- [44] Yao, D., Vlessidis, A. G., and Evmiridis, N. P. (2004). Determination of nitric oxide in biological samples. *Microchim. Acta* 147, 1-20. doi: 10.1007/s00604-004-0212-8
- [45] Zaripov, S. A., Naumov, A. V., Suvorova, E. S., Garusov, A. V., and Naumova, R. P. (2004). [Initial stages of 2, 4, 6-trinitrotoluene transformation by microorganisms]. *Mikrobiologiya* 73, 472-478. doi: 10.1023/B:MICI.0000036983.04480.19
- [46] Ziganshin, A. M., and Gerlach, R. (2014). "Pathways of 2, 4, 6-trinitrotoluene transformation by aerobic yeasts," in *Biological Remediation of Explosive Residues*, ed. S. N. Singh (Cham: Springer International Publishing), 301-311
- [47] Ziganshin, A. M., Gerlach, R., Borch, T., Naumov, A. V., and Naumova, R. P. (2007). Production of eight different hydride complexes and nitrite release from 2, 4, 6-trinitrotoluene by *Yarrowia lipolytica*. *Appl. Environ. Microbiol.* 73, 7898-7905. doi: 10.1128/aem.01296-07
- [48] Ziganshin, A. M., Naumova, R. P., Pannier, A. J., and Gerlach, R. (2010). Influence of pH on 2, 4, 6-trinitrotoluene degradation by *Yarrowia lipolytica*. *Chemosphere* 79, 426-433. doi: 10.1016/j.chemosphere.2010.01.051