

# Exploiting marine fungal biodiversity for the bioremediation of chlorinated aromatic pollutants and discovery of novel enzymes

E. Nikolaivits<sup>1</sup>, A. Agrafiotis<sup>1</sup>, R. Siaperas<sup>1</sup>, A. Termentzi<sup>2</sup>, N. Fokialakis<sup>3</sup>, J. Ouazzani<sup>4</sup>, E. Topakas<sup>1</sup>

<sup>1</sup>School of Chemical Engineering, National Technical University of Athens, Athens, Zografou Campus, 15780, Greece

<sup>2</sup>Department of Pesticides Control and Phytopharmacy, Benaki Phytopathological Institute, Athens, Kifisia, 14561, Greece

<sup>3</sup>Department of Pharmacy, University of Athens, Athens, Zografou Campus, 15771, Greece

<sup>4</sup>Institut de Chimie des Substances Naturelles, ICSN, CNRS, Gif-sur-Yvette, 91198, France

Keywords: marine fungi, bioremediation, 2,4-dichlorophenol, PCBs.

Presenting author email: [snikolai@central.ntua.gr](mailto:snikolai@central.ntua.gr)

In everyday life, we come across over 60,000 chemicals in the types of consumer products, drugs, pesticides, food additives, fuels and industrial solvents (Das and Dash, 2014). Chlorophenols (CPs) are introduced in the environment as metabolites of herbicides and other chlorinated xenobiotics or through anthropogenic activities as effluent discharge of industrial processes; for instance pulp bleaching, dye manufacturing, water disinfection with chlorine, waste burning and wood waste incineration (El-Naas et al., 2017; Honda and Kannan, 2018). In particular 2,4-dichlorophenol (2,4-DCP) has been widely used as a fungicide, pesticide and wood preservative (Huang et al., 2015), being released in high amounts into the environment (ca 10,000 kg in the US during 2014) according to the United States Environmental Protection Agency (US EPA), which listed it as a priority pollutant among other chlorophenols (Honda and Kannan, 2018; Patel and Kumar, 2017). Polychlorinated biphenyls (PCBs) are produced through the fusion of two benzene rings in the presence of chlorine gas. There are 209 different PCB congeners which are considered persistent organic pollutants (POPs) (Nikolaivits et al., 2017). PCBs in wastewaters derive from leaching runoff, leaching from landfills, and improper disposal of chemical waste. Previously disposed capacitors, transformers, inks, lubricants, adhesives, and other PCB-containing products are present in unsecured landfills and currently leach toxic PCB waste into water systems during rain events (Fusi et al., 2017).

Bioremediation is the use of living organisms in order to remove pollutants from soil and water; a method that is considered more cost-effective and environmentally friendly than the conventional techniques (Patel and Kumar, 2017; Ren et al., 2016). Microorganisms – mainly bacteria and fungi – indigenous to the contaminated regions are potential candidates for the task, benefiting from their acquired enzymatic arsenal, aiming to use the contaminants as food, ideally towards their complete mineralization (Srivastava, 2015). Fungi are robust organisms and most of them are usually more tolerant to high concentrations of pollutants compared to bacteria (Srivastava, 2015).

The marine environment is an untapped source of microbial diversity, showing various characteristics valuable for biotechnological applications, including bioremediation (Nikolaivits et al., 2017), especially considering that a great part of the earth's pollution appears in the oceans. An exceptionally under-investigated source of marine biodiversity is the fungal symbionts of invertebrates (e.g. ascidians, cnidarians, and sponges) (Nikolaivits et al., 2017). The mesophotic zone, in particular, is an underexplored marine habitat, probably due to the fact that it is below depths (30-100 m) reached with traditional SCUBA diving techniques. The biodiversity of mesophotic coral systems is considered a potential source of novel symbiotic microorganisms, which can contribute to the enzymatic arsenal of Biocatalysis and specifically biodegradation of recalcitrant pollutants (Nikolaivits et al., 2017).

In the present work, we study the potential of 107 marine-derived symbiotic fungi that were isolated through TASC MAR H2020 project to bioconvert the aromatic chlorinated pollutants 2,4-DCP and PCB29. The metabolites of 2,4-DCP detoxification by the most efficient fungi were elucidated using mass spectrometry and activities of ring-cleaving dioxygenases were measured. Regarding PCB29 bioconversion, enzymatic activities implicated in this process were measured. In both cases, novel enzymes (catechol dioxygenase and laccase) were isolated and biochemically characterized.

## Acknowledgments

Authors would like to acknowledge the financial support of TASC MAR, which is a collaborative European research project funded by the European Union's Horizon 2020 research and innovation programme, under grant agreement N° 634674.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of

the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

## References

- Das, S., and Dash, H. R. (2014). “Microbial bioremediation: A potential tool for restoration of contaminated areas,” in *Microbial Biodegradation and Bioremediation*, ed. Surajit Das (Elsevier), 1–21. doi:<http://dx.doi.org/10.1016/B978-0-12-800021-2.00001-7>.
- El-Naas, M. H., Mousa, H. A., and Gamal, M. El (2017). “Microbial degradation of chlorophenols,” in *Microbe-Induced Degradation of Pesticides*, ed. S. N. Singh (Springer), 23–58. doi:10.1007/978-3-319-45156-5\_2.
- Fusi, S. C., Chan, A. Y., and Kjellerup, B. V. (2017). “Processes of microbial transformation and physical removal of polychlorinated biphenyls (PCBs) in wastewater treatment,” in *Optimization and Applicability of Bioprocesses*, ed. K. A. Purohit H., Kalia V., Vaidya A. (Singapore: Springer Singapore), 101–113. doi:10.1007/978-981-10-6863-8\_5.
- Honda, M., and Kannan, K. (2018). Biomonitoring of chlorophenols in human urine from several Asian countries, Greece and the United States. *Environ. Pollut.* 232, 487–493. doi:10.1016/j.envpol.2017.09.073.
- Huang, Z., Chen, G., Zeng, G., Chen, A., Zuo, Y., Guo, Z., et al. (2015). Polyvinyl alcohol-immobilized *Phanerochaete chrysosporium* and its application in the bioremediation of composite-polluted wastewater. *J. Hazard. Mater.* 289, 174–183. doi:10.1016/j.jhazmat.2015.02.043.
- Nikolaivits, E., Dimarogona, M., Fokialakis, N., and Topakas, E. (2017). Marine-derived biocatalysts: importance, accessing and application in aromatic pollutant bioremediation. *Front. Microbiol.* 8, 265. Available at: <http://journal.frontiersin.org/article/10.3389/fmicb.2017.00265/full> [Accessed February 8, 2017].
- Patel, B. P., and Kumar, A. (2017). Biodegradation and co-metabolism of monochlorophenols and 2,4-dichlorophenol by microbial consortium. *CLEAN - Soil, Air, Water* 45, 1700329. doi:10.1002/clen.201700329.
- Ren, H., Li, Q., Zhan, Y., Fang, X., and Yu, D. (2016). 2,4-Dichlorophenol hydroxylase for chlorophenol removal: Substrate specificity and catalytic activity. *Enzyme Microb. Technol.* 82, 74–81. doi:10.1016/j.enzmictec.2015.08.008.
- Srivastava, S. (2015). “Bioremediation Technology: A greener and sustainable approach for restoration of environmental pollution,” in *Applied Environmental Biotechnology: Present Scenario and Future Trends*, ed. Garima Kaushik (New Delhi: Springer India), 1–18. doi:10.1007/978-81-322-2123-4\_1.