Discovery and mutation of a novel enzyme for the removal of environmental pollutants

Environmental pollution is one of the biggest challenges in the world today, as society faces the consequences of intense industrial development. The widespread use of pesticides in agriculture leads to high quality products, while minimizing losses in order to meet the huge demand due to the growing planet population. However, the excessive use of pesticides and fertilizers in modern agricultural practices has led to environmental pollution, with negative consequences for humans.

The remediation or treatment of contaminants by conventional methods (both physical and chemical) is a costly, time-consuming, invasive approach that causes environmental deterioration. According to an estimate, the cleaning/restoring of all contaminated sites in the USA requires a capital investment of approximately US $1.7 trillion. Bioremediation has emerged as a safe, reliable, effective, low-cost and environmentally friendly alternative technology to achieve sustainable remediation of hazardous and recalcitrant pollutants. US Environmental Protection Agency (USEPA) defined bioremediation as a treatment process in which microorganisms (or their products) are employed to degrade or modify toxic pollutants to less harmful forms, thus diminishing environmental pollutants generated by various anthropogenic activities.

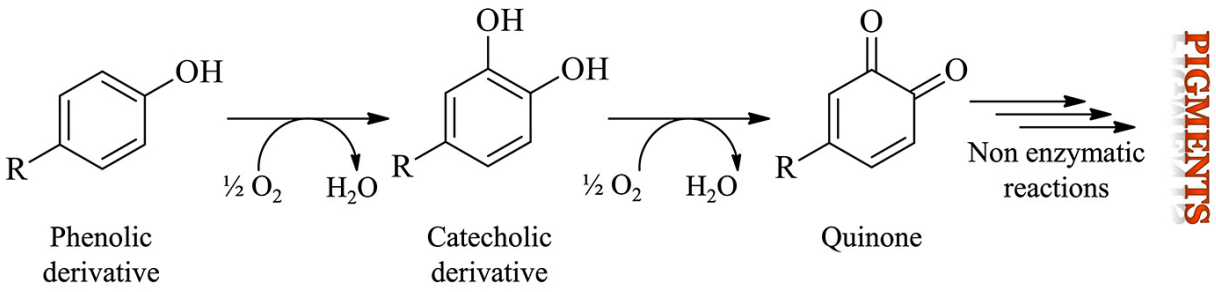

Fig. 1.

Chlorophenols (CPs) are common organic pollutants introduced in the environment by the activities of various industries (mostly paper and textile) and are mainly associated with the production, use, and degradation of several pesticides. CPs may also be produced when wastewater or drinking water is disinfected with chlorine. Some CPs have been listed by the USEPA as priority contaminants, since they impose many health risks for living organisms, like DNA damage, oxidative stress, toxicity, and carcinogenicity. Enzymatic bioremediation of these pollutants is often superior to microbial bioremediation, due to the higher tolerance of enzymes for concentrated CPs.

Polyphenol oxidases (PPOs) are enzymes that catalyze the oxidation of phenolic compounds into quinones, which are reactive molecules that undergo non-enzymatic reactions to form pigments (Fig. 1). PPOs are found in all domains of life, distributed from bacteria to humans, and their main role is the formation of melanins and other phenolic polymers, mainly for protective purposes. In different animal phyla, melanins are formed by various types of precursors (mainly tyrosine) and are located in the skin, hair, and eyes of mammals; bird feathers; skin in reptiles, amphibians, and fish; and in the exoskeleton of insects. In plants and mushrooms, PPOs have been well studied, due to the undesirable postharvest browning they cause, which downgrades the value of these products.

Mechanistic and structural studies of such enzymes are important for developing potent inhibitors for use in hyperpigmentation-associated diseases in humans (like melanoma). Other than that, PPOs have been used as biocatalysts in reactions with applications in food, pharmaceutical, and cosmetic industries and also as biosensors for the detection of small amounts of phenolics. However, the use of this family of enzymes in the bioremediation field has been very limited.

The aim of our study was to discover a PPO that would act on CPs and remove them (Fig. 2). The bioinformatics analysis performed led to a PPO sequence in the genome of the fungus *Thermothelomyces thermophila*, which had low homology to known ones. The recombinant enzyme presented some unique features such as optimum reaction conditions (65 °C and pH 7.5-8), high thermostability and a very wide substrate spectrum. Additionally, the enzyme could remove some of the CPs tested.

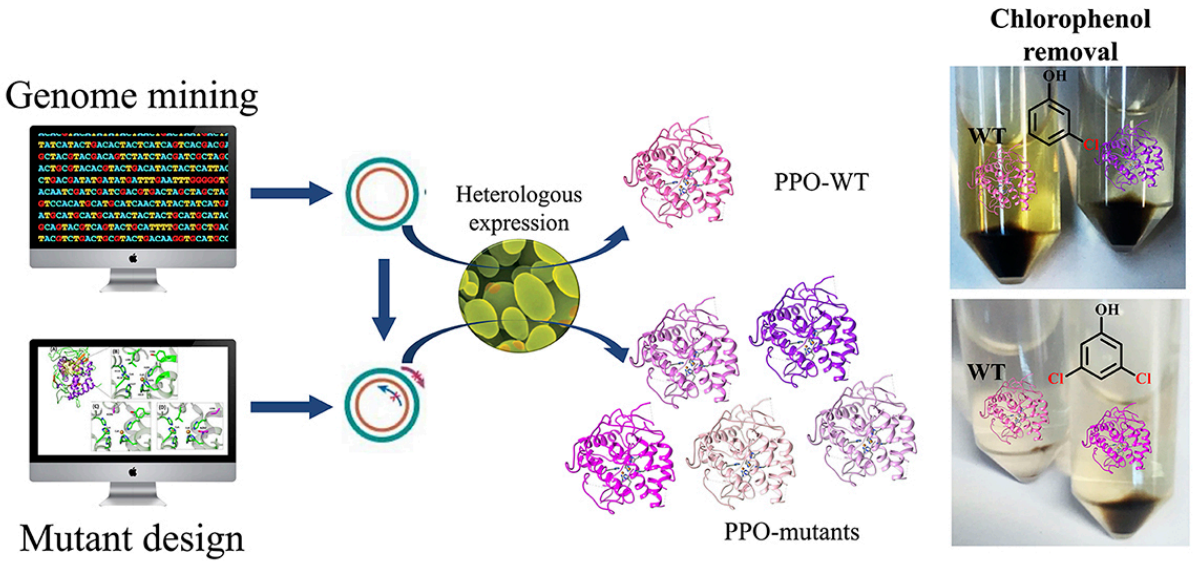

Fig. 2.

In order to increase this activity in CPs, point mutations were performed on the sequence of PPO, using protein engineering tools. The five mutated enzymes constructed, presented altered specificity and activity compared to the original enzyme (wild-type – WT). In fact, one of them increased its activity on one of the CPs by 5.3 times (Fig. 2).

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