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Phenol remediation by peroxidase from an invasive mesquite: Turning an environmental wound into wisdom

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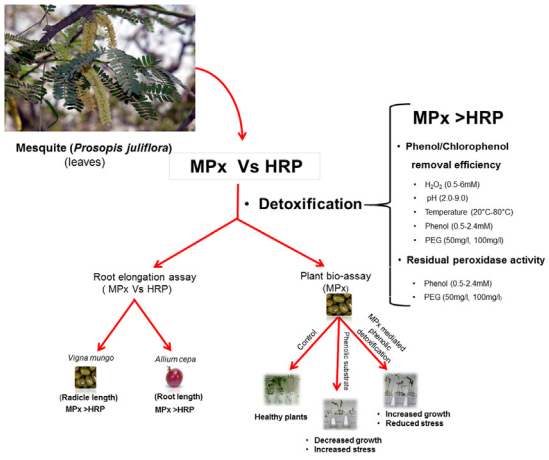
HIGHLIGHTS

- Peroxidase from invasive mesquite (MPx), for phenolic removal is reported.
- 0.3U/ml MPx removes >92% of phenol, 2,4-dichlorophenol & 4-chlorphenol within 30 min.
- MPx removes phenolics more efficiently than HRP at wide pH and temperature range.
- MPx removes phenols & retains residual activity, at high phenol levels or without PEG.
- MPx reduces phenolic toxicity and enhances plant growth.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study examines mesquite (*Prosopis juliflora*), an invasive species, to yield peroxidase that may reduce hazards of phenolics to living organisms. As low as 0.3U of low-purity mesquite peroxidase (MPx) efficiently remove phenol and chlorophenols (90–92%) compared with Horseradish peroxidase (HRP) (40–60%). MPx shows a very high removal efficiency (40–50%) at a wide range of pH (2–9) and temperature (20–80 °C), as opposed to HRP (15–20%). At a high-level of the substrate (2.4 mM) and without the addition of PEG, MPx maintains a significant phenolic removal (60– ≥ 92%) and residual activity (~25%). It proves the superiority of MPx over HRP, which showed insignificant removal (10–12%) under similar conditions, and no residual activity even with PEG addition. The root elongation and plant growth bioassays confirm phenolic detoxification by MPx. Readily availability of mesquite across the countries and easy preparation of MPx from leaves make this tree as a sustainable source for a low-technological solution for phenol remediation. This study is the first step towards converting a biological wound of invasive species into wisdom and strength for protecting the environment from phenol pollution.

1. Introduction

Phenols are essential for industrial growth but deteriorate the quality of environment [1]. Annual production of phenols is ~10

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million tons a year, and released into the environment through various industries, such as mining, petroleum refining, polymer processing, coal conversion, organic synthesis and fibre processing [2]. Mono and dichloro phenols, the largest groups of phenols, are used in manufacturing pesticides, fungicides, disinfectants, etc. Chlorination of water supplies and agriculture runoff further add to levels of chlorophenols in the environment [3] and deteriorate the quality of water, soil, and air. Phenolics are cytotoxic, mutagenic, genotoxic and potentially carcinogenic, and therefore pose health hazards to the living organisms. USEPA also enlisted phenol as one of the 16 priority pollutants for environmental management [1]. In developing countries, hosting >50% of world's population, poor infrastructure, relaxed environmental policy especially for the small-scale industries coupled with the lack of policy implementation result in the high exposure to environmental toxicants to the living organisms [4]. Therefore, an efficient, low-technology method for environmental management of phenols is needed [5].

Conventional physicochemical methods of wastewater treatment are often challenged with the cost, efficiency, the need for extensive facility and generation of hazardous by-products [6]. Peroxidase-based methods are preferred because of high efficiency and specific catalysis to yield insoluble polymers, which can be easily removed [7]. HRP is most widely studied plant peroxidase, which is efficient over a fairly wide range of pH, temperature, and phenol levels, etc. However, the requirement of high concentration of purified enzyme, its inactivation by reaction by-products, and need of polyethyleneglycol (PEG) adjuvant to minimize inactivation are some of the bottlenecks for its wide application [8,9]. The PEG-induced increase in chemical oxygen demand of treated water poses a further environmental challenge. To find a sustainable alternative, peroxidase from soybean [10,11], turnip [12], bitter gourd [13], etc. have also been investigated. These peroxidases also suffer from inactivation and need an adjuvant. Further, because of diverting crops and agriculture land, these alternatives may not be considered sustainable [14].

Peroxidases help adapting plants to different stresses. Therefore invasive plants colonizing stressed habitats may serve as a source of novel biomolecules [15–17]. Mesquite (*Prosopis juliflora*) is among world's worst invasive species, which is well adapted to diverse environmental stresses and spread in >129 countries [18]. Thus, the present study on evaluation of mesquite peroxidases (MPx) to serve as low-technology solution for phenol remediation was undertaken with the following objectives: (i) to ascertain the efficiency of the low purity MPx to remove phenol and mono- and dichlorophenols under diverse physicochemical conditions, and its comparison with HRP; (ii) to determine the removal efficiency of MPx at high phenolic levels and analyze the need of PEG to maintain its removal efficiency and residual activity, and its comparison with HRP; and (iii) to determine the potential of MPx to detoxify phenol and chlorophenols using root elongation and plant growth bioassays. The study reports low purity mesquite peroxidase as a low-technology solution that efficiently removes and detoxifies phenols and chlorophenols than HRP. This will pave a way to convert biological wound of invasive species into strength to reduce the environmental hazard of phenols.

2. Materials and methods

2.1. Estimation of peroxidase activity in mesquite leaf extract

2.1.1. Extraction of *mesquite* protein

The leaves of mesquite belonging to Fabaceae, were collected from invasive infested reserve forest of Kamla Nehru Ridge of Delhi (28.6849°N, 77.2161°E). The leaves were washed and stored at −80 °C till further use. The details of chemicals used are given in the Supplementary file. To obtain a low-purity peroxidase, the leaves of *P. juliflora* were homogenized in liquid nitrogen and mixed in 0.1 M chilled sodium-phosphate extraction buffer (pH 7.0) containing 2% polyvinylpolypyrrolidone (PVPP) and 350 mM NaCl [19]. The homogenate was centrifuged at 13,000 × *g* for 30 min at 4 °C, and the supernatant was subjected to partial purification of protein through ammonium sulphate fractionation (fraction I: 0–25% salt saturation; fraction II: 25–80% salt saturation) [20]. The fractions were dialyzed against 0.1 M sodium phosphate buffer (pH 7.0) at 4 °C. Total protein content in crude extract and dialyzed fractions was determined by Bradford's dye-binding assay using BSA as standard [21].

2.1.2. Determination of peroxidase activity

Peroxidase activity in the crude extract and partially purified fractions was determined by estimating guaiacol dehydrogenation product (GDHP) formation (extinction coefficient of $40 Mm^{-1} cm^{-1}$) at OD_{470nm} using H_2O_2 as an oxidizing agent [22]. The 3 ml reaction mixture consists of 10 mM of guaiacol, and 3 μg of protein in 0.1 mM of phosphate buffer, pH 7.0. The reaction was initiated by adding 0.5 mM H_2O_2 and the oxidized product (brownish coloured tetra-guaiacol) was measured at OD_{470nm} over 3 min at RT. The peroxidase activity was expressed as μM guaiacol oxidized/mg protein/min at 25 °C. One peroxidase unit is defined as the amount of protein needed to produce 1 μmol of tetra-guaiacol produced in 1 min at RT [23]. All assays were carried out in triplicates and repeated three times throughout the study. Results are expressed as mean ± standard error.

2.2. Determination of phenolic removal by low-purity mesquite peroxidase (MPx)

2.2.1. Determination of optimum peroxidase units for maximum phenolic removal

The efficiency of MPx to remove phenol, 4-chlorophenol (4-CP) and 2,4-dichlorophenol (2,4-DCP) was determined by estimating the minimum enzyme units required for maximal phenol removal [24] and also compared with HRP. The 30 ml batch reactions were set up with phenolics (0.5 mM) and H_2O_2 in a 1:1 ratio in 0.1 M sodium phosphate buffer pH 7.0 [25]. The optimum enzyme unit was determined by testing various concentrations of MPx or HRP (0.3-3.0U/ml). After 3 h of incubation at RT, the reaction was terminated by adding catalase (125U/ml). Subsequently, alum 0.4% $Al_2(SO_4)_3.14H_2O$ (0.2 ml/ml) was added to increase the flocculation and the pH was adjusted to 6.3 by using 1N HCl/1N NaOH. The reaction mixture was centrifuged at 13000*g* for 20 min and the pellet containing the polymerized phenols was removed. The content of residual phenol in supernatant and percent phenolic removal was determined as described below [26].

2.2.2. Estimation of the residual phenolic content

The content of residual phenol was estimated by standard amino antipyrine colorimetric assay [27]. A 1 ml of post-reaction mixture was diluted to 100 ml with distilled water followed by sequential addition of 2 ml of ammonium chloride/ammonium buffer, 2 ml of 20.8 mM 4-aminoantipyrine in 0.25 M sodium carbonate and 2 ml of 83.4 mM potassium ferricyanide in 0.25 M sodium carbonate. After 15 min of incubation in the dark OD_{510nm} was determined. The residual phenol content in the post-reaction mixtures was estimated by comparing with a respective phenolic substrate taken as standard. Unless mentioned, 0.3U/ml MPx/HRP and 0.5 mM of phenolic substrates were used in all phenolic removal experiments.