Isolation and Identification of ligninolytic bacteria from fire affected soils

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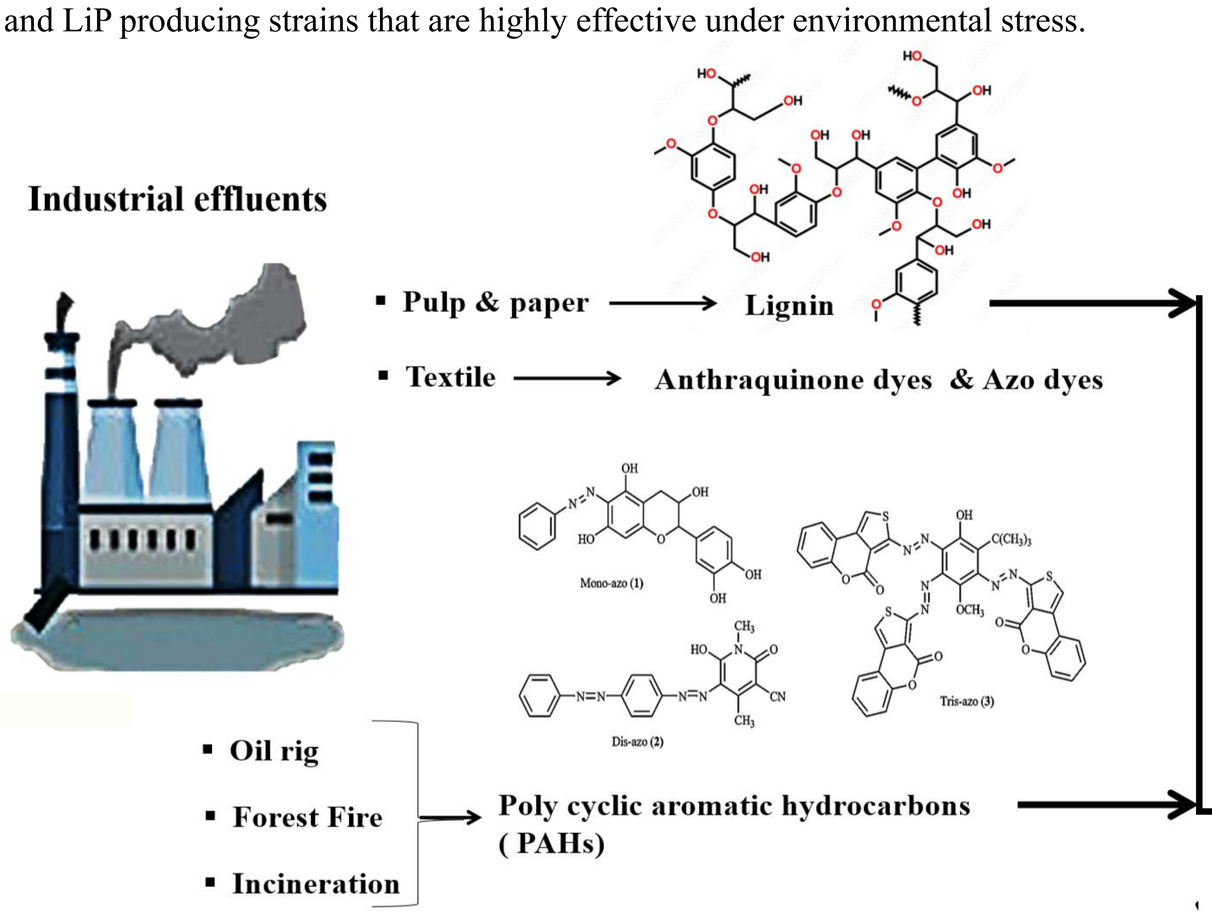
Introduction: Resilient water supply systems are increasingly threatened by anthropogenic pollution and climate variability. Industrial effluents, especially those containing lignin derivatives, synthetic dyes, phenolics, and polycyclic aromatic hydrocarbons (PAHs), frequently enter aquatic environments and challenge conventional treatment approaches (Bugg et al., 2011; Levin et al., 2016). Microbial ligninolytic enzymes, primarily laccase, lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP), offer a promising solution. Their ability to function under mild, eco-friendly conditions reduces reliance on harsh chemicals and minimizes sludge formation, enhancing environmental compatibility (Rodríguez Couto, 2009). Furthermore, these enzymes can be immobilized or integrated into existing infrastructure such as biofilters, membranes, and hybrid bioreactors, enabling scalable and modular deployment (Jarosz-Wilkolazka et al., 2002; Rodríguez-Couto et al., 2003). When synergized with indigenous microbial communities, they contribute to long-term functional stability and ecological sustainability (Pointing, 2001). Fire ecology provides an additional dimension to enzyme sourcing. Fire alters soil physical and chemical properties by increasing pH, releasing ash and aromatic carbon compounds, and generating charred organic matter that acts as a persistent substrate (Certini, 2005; Knicker, 2007). Post-fire soils often harbour fungi and bacteria with enhanced oxidative metabolism, which produce more effective ligninolytic enzymes to break down aromatic residues and polyaromatic hydrocarbons left by combustion (de Boer et al., 2005; Baldrian, 2006). Such microbial populations are thus valuable reservoirs for isolating robust laccase, MnP, and LiP producing strains that are highly effective under environmental stress.



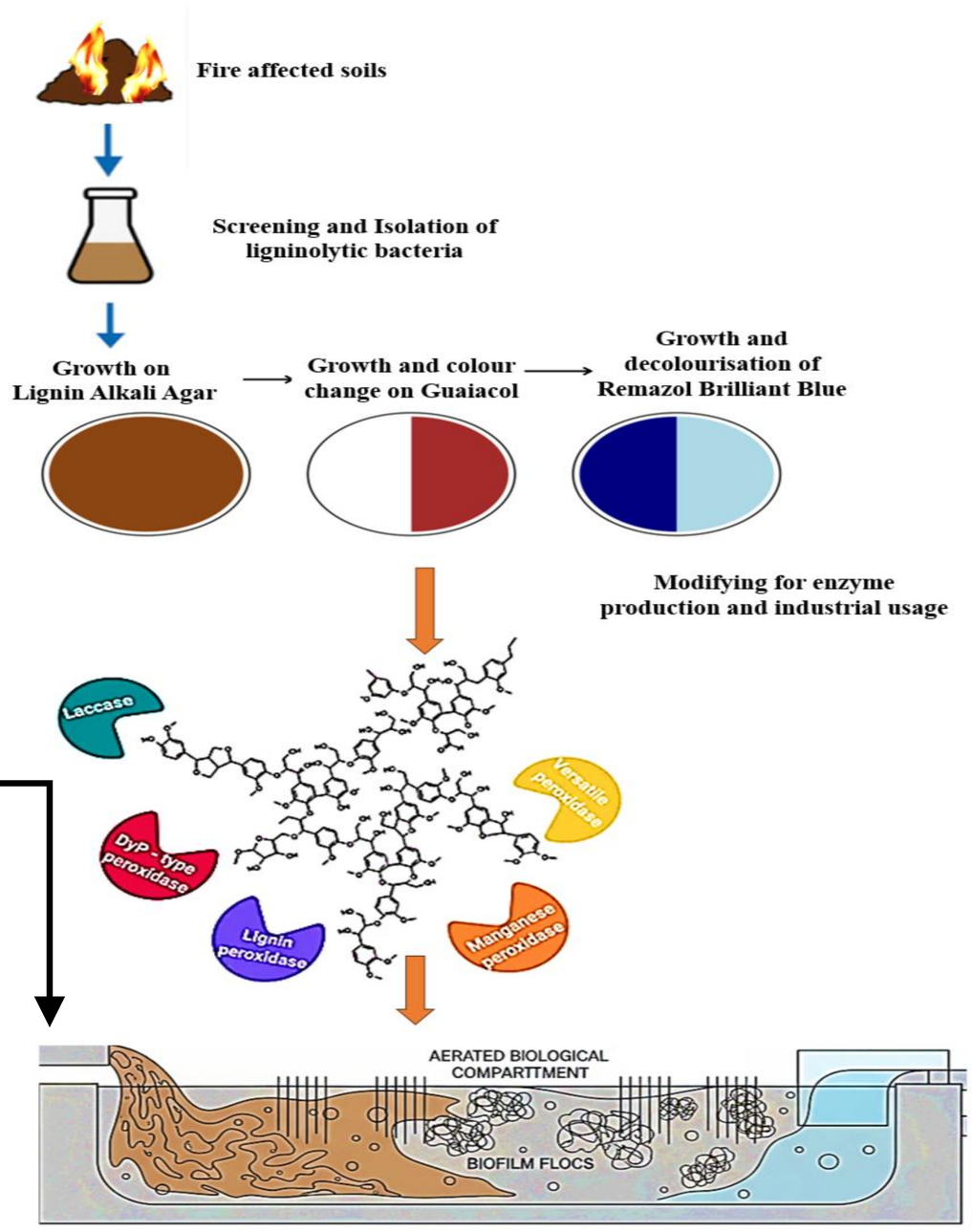
Methods: Soil samples analysed from wet sclerophyll forest in Southeast Queensland subjected to long-term (since 1972) prescribed burning ,also from eucalyptus forest in East Gippsland, Victoria affected by wildfire. All samples had taken 5 years after the last fire.

- The first screening applied by making soil serial dilution and inoculation on the following media and multiple streaking to isolate pure bacteria.
  1- 10-fold diluted Nutrient broth (3.4g/L) + 15g/L Agar
  2- Lignin (1g/L) + M9 (56.5 g) + Yeast extract (0.1 g/L) + Agar
- PCR for the genes involved in lignin degradation (Glycoside hydrolases Family 1, Lignin peroxidase, Laccase, Cellulase, Manganese peroxidase, Catechol 2, 3 dioxygenases and PAH hydratase-aldolase) confirmed the existence of the genes of interest.
- As a prove of ligninolytic activity, pure isolates inoculated on the following media.

Guaiacol (1g/L) +20-fold diluted Nutrient broth (1.7 g/L) + Agar

Remazol Brilliant Blue R (0.5g/L) + 20-fold diluted Nutrient broth (1.7 g/L) + Agar

- MALDITOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry) used to identify ligninolytic isolates , however, most of the isolates needed sanger sequencing as no match found for them in the MALDI TOF data base.

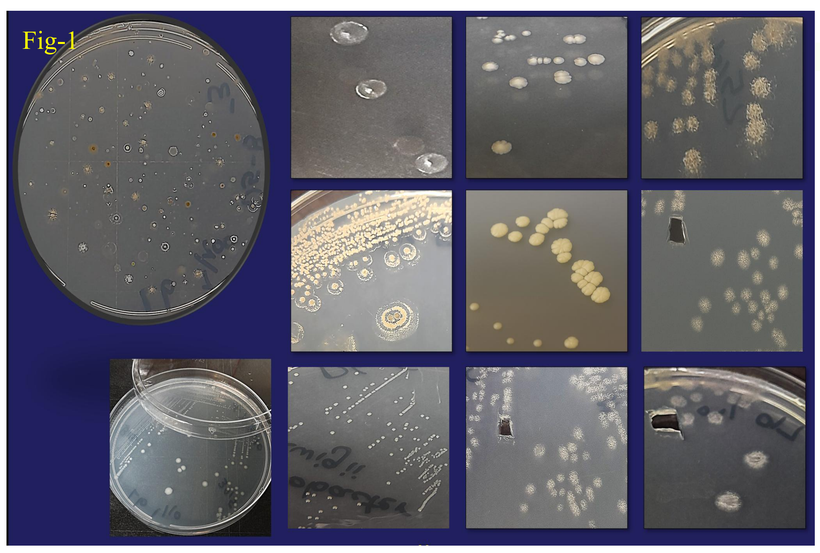


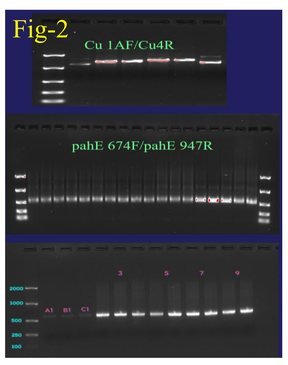
Results:

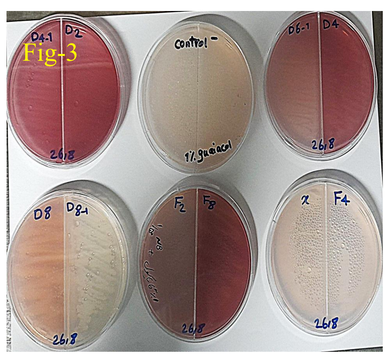
Fig-1: some of the pure isolates , Fig-2 : PCR results, Fig-3 : results on Guaiacol media and Fig-4 : results on RBBR

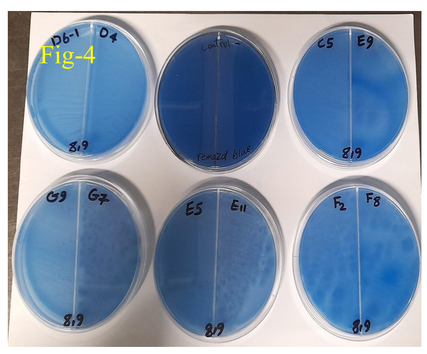
ligninolytic isolates identified by MALDI-TOF:

- *Bacillus cereus*
- *Bacillus thuringiensis*
- *Burkholderia pyrrocinia*
- *Enterobacter lignolyticus*
- *Klebsiella aerogenes*
- *Ochrobactrum anthropi*
- *Ochrobactrum tritici*
- *Penicillium namyslowskii*
- *Purpureocillium lilacinum*
- *Raoultella ornithinolytica*
- *Serratia marcescens*
- *Streptomyces rubiginosus*
- *Yokenella regensburgei*









Conclusion:

Fire reduces overall microbial biomass (notably fungal populations), but surviving and recolonizing microbes often display increased oxidative enzyme activity per cell. This leads to a community more capable of degrading lignin despite a lower total number of microbes (Pold et al., 2015). Persistent or frequent heat events can elevate the long-term functional potential of soil microbial communities for lignin decomposition by altering both species diversity and metabolic strategies (Dove et al., 2022). Harnessing post-fire adapted microbes can support the development of sustainable, enzyme-based technologies for treating polluted water.

In this research, burned soils were a good source of oxidative enzymes produced by a highly divers taxonomic groups of microorganisms. Ligninolytic enzymes are capable of degrading PAH's and Azo dyes as well, and future work will assess the biotechnological potential of these isolates.

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