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Synergistic Effect of Bacterial Consortium for Enhanced Laccase Production by Submerged Fermentation

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Abstract

Industrialisation is rapidly changing the pace of economy by leaps and bounds. At the same time, the effects of pollution are evident in terms of infiltration and accumulation of hazardous substances in environment at large. Rajasthan has witnessed tremendous growth in small scale industries, one of them being the handmade paper industry. Globally, the finished product notably known as “Sanganeri handmade paper” is being appreciated for its ethnic hues and multi-usage. The current practices of paper manufacturing rely on intensive mechanical pulping process utilizing an array of raw materials finally leading to enormous volumes of effluent. A combination of mechanical and chemical pulping process has certain identifiable gaps in the form of high production cost, high energy consumption and generation of large volumes of solid waste and effluents rich in high Biological Oxygen demand, Chemical Oxygen Demand, synthetic dyes, heavy metals, bleaching agents, lignin and diversified range of xenobiotic compounds; thereby posing an environmental threat. Considering this fact, we proposed a pilot study aimed at cleaner and greener production of handmade paper by bioprospecting of indigenous micro flora. For this study, soil samples were collected in accordance with standard procedures from local handmade paper industry located at Sanganer, Jaipur. Preliminarily, the samples were screened for bacterial isolates capable of producing laccase, an important enzyme responsible for delignification). Laccases (EC 1.10.3.2) are copper-containing oxidase enzymes found in many plants, fungi, and microorganisms. Furthermore, synergistic effect of bacterial consortium was explored for enhanced laccase production through submerged fermentation. Laccase activity as monitored in Cell Free Extract (CFE), was found to be maximum of 60.9 U/ml for bacterial consortium and was highly significant ($p<0.05$) with respect to abiotic control. This pilot study suggested the role of autochthonous micro flora in delignification of raw materials thereby obliterating the energy and cost intensive chemico-mechanical pulping procedure.

Pulp and paper mills are categorized as a core sector industry and 5th largest contributor of industrial water pollution. This industry utilise large volumes of ligno cellulosic components and water during different unit operations and release intensely coloured black liquors chlorinated lingo sulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated compounds including chlorolignins, chlorophenols, chloroguiacols and chloroaliphatics in the effluent . These compounds are acutely toxic being mutagenic and genotoxic in nature . As per the Ministry of Environment and Forest (MoEF), Government of India, the pulp and paper sector has been placed in the “Red Category” which indicates severe polluting potential of the industry. The in-house unit operations of a paper mill is schematically represented in Figure 1 indicating different pollutants being released in a sequential manner . The conventional pulping process involves removal of lignin either by chemical or mechanical means, which otherwise neither cost effective, nor ecofriendly, low yield, toxic by-products are produced . Microbial biodegradation is carried out by different organisms like bacteria, fungus, and algae . Effective Microorganism (EM) is the consortia of valuable and naturally occurring microorganisms which extra cellularly release organic acids and enzymes for utilization and degradation of anthropogenic compounds . Ligninolytic enzymes have found immense usage in many industrial and biotechnological applications including delignification of lingo cellulosic biomass for fuel (ethanol) production; food, brewery, and wine; animal feed; denim stone washing; laundry detergents; paper and pulp industries; and bioremediation of chemical pollutants . Owing to the wellestablished fact of bioefficacy of lignolytic enzymes, the search for efficient production systems has been explored in recent past utilizing mushroom Stereum ostea marine fungi Trichoderma sp. The production system should be cost effective which can be accomplished by using cheaper raw materials and optimizing the fermentation process for scale up is need of an hour . Because of the diverse applications of ligninolytic enzymes in industrial processes, there is a wide interest in the induction, enhancement, and stabilization of these enzymes. The production of ligninolytic enzymes can be stimulated by the presence of a wide variety of inducing substrates mainly aromatic or phenolic compounds related to lignin or lignin derivatives such as ferulic acid, 2,5-xylidine, and veratryl alcohol . Copper as a micronutrient has a key role as a metal activator, induces both laccase transcription, and plays an important role in laccase production . Surfactants can stimulate the growth of spores and increase the bioavailability of less soluble substrates for the fungus. Laccases are multicopper enzymes belonging to the blue oxidases group of enzymes which widely exist in nature and are defined as nomenclature

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wise oxido reductases type according to the Enzyme Commission (EC) which oxidize diphenols and allied substances . The higher plants and fungi predominantly contain laccases .

Screening of potential microbes, indigenous to specific habitats are key to bio prospecting. This pilot study led to characterization of microflora capable of producing laccases in monoculture as well as consortium. Synergistic effect of screened isolates exhibited highly significant laccase activity. Laccases are ubiquitous in nature belongs to multicopper oxidase which catalyze oxidation reaction coupled to water formation on four electron reduction of molecular oxygen. They are presumed to be potential tool of bio pulping thereby reflecting their enormous potential not only in paper and pulp industries but also textile, fertilizers and other allied industrial sectors. The devised study which is selectively and specifically based at bio prospective strategy would play a pivotal role in generation of an eco-friendly process which would lead to development of a cleaner production technology thereby obliterating mechanical and chemical processes which are energy and cost intensive. Further insights may be based on metagenomics based analysis to screen for otherwise unexplored microbes which may culminate into potential microbial –factories.

Keywords: Bacterial consortium; Cleaner production; Handmade paper industry; Laccase

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