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Kraft lignin degradation through bacterial strain isolated from soils of timber areas

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ABSTRACT: *Kraft lignin (KL) is the major pollutant in pulp and paper mill effluent and main contributor to the toxicity and color of the effluent. Total fifteen bacterial strains were isolated from soil of timber area and screened for ligninolytic enzyme activity in mineral salt media (MSM) amended with KL (200ppm) along with 1% glucose and 0.3% peptone as additional carbon and nitrogen sources. Out of fifteen, thirteen bacterial strains (TSF1-TSF13) were found to have ligninolytic enzyme activity (Manganese peroxidase;MnP, Lignin peroxidase;LiP and Laccase). Positive result of manganese peroxidase activity was shown by seven strains, Lignin peroxidase activity was shown by ten strains, whereas Laccase activity was shown only by ten strains.*

Key words: *Bacteria,Kraft lignin, pulp and paper mill effluent.*

I. Introduction

Industrialization develops the economic importance of a nation but at the same time it leads to the negative impact on environment (Hossain and Rao, 2014; Raj *et al*., 2014). Economic benefits of the pulp and paper is one of the most important industrial sections in the world due to its economical beneficial role. Still now, pulp and paper mills are facing challenges with the energy efficiency mechanisms and management of the consequential pollutants, considering the environmental feedbacks and enduring legal requirements (Kamali and Khodaparast, 2015). Generally, the pulp paper industry is considered as the most polluting industries in the world (Pokhrel and Viraraghavan, 2004). The color and toxicity of the pulp and paper effluent is mainly due to the presence of Kraft lignin (KL). The KL is formed during alkaline sulfide treatment of lignocelluloe in pulp and paper industry. It differs from natural lignin as it undergoes a variety of reactions including aryl–alkyl cleavages, strong modification of side chains, and various illdefined condensation reactions causing the polymer to fragment into smaller water/alkali-soluble fragments (Chakar and Ragauskas, 2004). Because of high molecular weight and presence of various biologically slable β-O-4 ether bonds, β-5 carbon to carbon and ether linkages in lignin it is highly resistance to microbial degradation (Argyropoulos and Menachem 1997). In most cases, this effluent (row or treated) is exposited into the streams, rivers or other water bodies; resulting in harmful environmental and social impacts (Chandra *et al*., 2011). Thus it is necessary to treat the effluent before throwing. A variety of lignin degrading microbial strains could be found in environment, involving bacteria (vicuna, 1988; Zimmermann, 1990), actinomyces (Ramchandra *et al*., 1988), fungi (sanhez, 2009). *Citrobactor*, *serratia*, *klebsiella*, *Paenibacillus*, *Aneurinibacillus* and *Bacillus* were isolated from the sludge of pulp paper mill as lignin degrading bacteria (Chnadra *et al*., 2007, 2008). One bacterial strain *Bacillus* sp. isolated from Egyptian soil in Kafr El-Dawar area. This strain utilized lignin as a sole carbon source and have potential to efficiently degrade synthetic lignin (Abd-Elsalam and El-Hanafy, 2009). Bacteria releases ligninolytic enzymes i.e. Manganese paeroxidase(MnP), Lignin peroxidase(LiP) and Laccase for degradation (Naz, 2014). Hence, the objective of this study was to isolate bacterial culture capable to degrade KL.

II. Materials and Methods

2.1 Chemicals: - The purified KL powder was purchased from Sigma Aldrich.

2.2 Sample Collection:- For isolation of potential bacterial strains, soil sample was collected from timber area of Raipur, Chhattisgarh. The samples were put into polythene bags and immediately preserved at 4°C. Soil samples were separated, air dried at room temperature, crushed, sieved and collected in separate polythene bags. pH of the soil samples were recorded using pH meter.

2.3 Isolation and purification of KL degrading bacteria

Isolation was done by serial dilution method in which soil sample was serially diluted from 10^{-1} to 10^{-7} and 0.1ml aliquots from each dilution was inoculated in mineral salt medium (MSM) of following composition

(in g/l):Na2HPO4, 2.4; K2HPO4, 2.0; NH4NO3, 0.1; MgSO4,0.01; CaCl2, 0.01; D-glucose, 10.0; peptone, 3.0; Agar,15; at pH 7.6±0.2 and trace element solution (1 ml/l) amended with KL (200 mg/l) (designated hereafter as KL-MSM) The samples were incubated at 30°C for a period of 7 days. When samples exhibited decolorization, an aliquot (0.1 ml) was spread on KL-MSM agar plates and incubated at 30°C. Fifteen phenotypically different colonies were picked and purified by repeated streaking on the same medium. The purified strains were designated as TSF1, TSF2, TSF3, TSF4, TSF5, TSF6, TSF7, TSF8, TSF9, TSF10, TSF11, TSF12, TSF13, TSF14 and TSF15.

2.4 Screening of potential bacterial strains for ligninolytic enzyme activity

The isolated and purified bacterial strains were screened for Manganese peroxidase, Lignin peroxidase and Laccase activity by plate assay method. MSM with different substrate was used for screening. The substrate used for manganese peroxidase was Pnenol red (0.1%), for Lignin peroxidase Azure B (0.002%). While Laccase activity was detected in nutrient agar medium (NAM) containing (in g/l) peptone, 5.0; beef extract, 3.0; NaCl, 5.0; $CuSO_4$ (1mM) and guaiacol as substrate (Chandra and Singh, 2012).

III. Results

Bacterial colonies were isolated from soil sample of timber area by serial dilution method on L-MSM media. Total fifteen bacterial strains were isolated, purified and screened for ligninolytic enzyme activity.

Qualitative screening of bacterial strains for ligninolytic enzyme activity

Out of fifteen, thirteen bacterial strains have capability to produce ligninolytic enzymes. Strains were screened for Manganese peroxidase, Lignin peroxidase and Laccase activity by plate assay method. MSM with different substrate was used for screening. Conversion of dark pink to yellow indicated the presence of MnP activity, disappearance of blue color showed the positive Lip and brown color halos indicated positive laccase activity (**Table 1**).

Table.1. Isolated and screened lignin degrading bacteria

S.No.	Bacterial strains	Ligninolytic enzymes		
		Manganese peroxidase	Lignin peroxidase	Laccase
1	TSF1	-	+	+
2	TSF2	-	+	+
3	TSF3	-	+	+
4	TSF4	-	+	-
5	TSF5	+	+	+
6	TSF6	+	+	+
7	TSF7	+	+	-
8	TSF8	+	-	+
9	TSF9	+	+	+
10	TSF10	+	-	+
11	TSF11	+	-	+
12	TSF12	-	+	+
13	TSF13	+	+	-

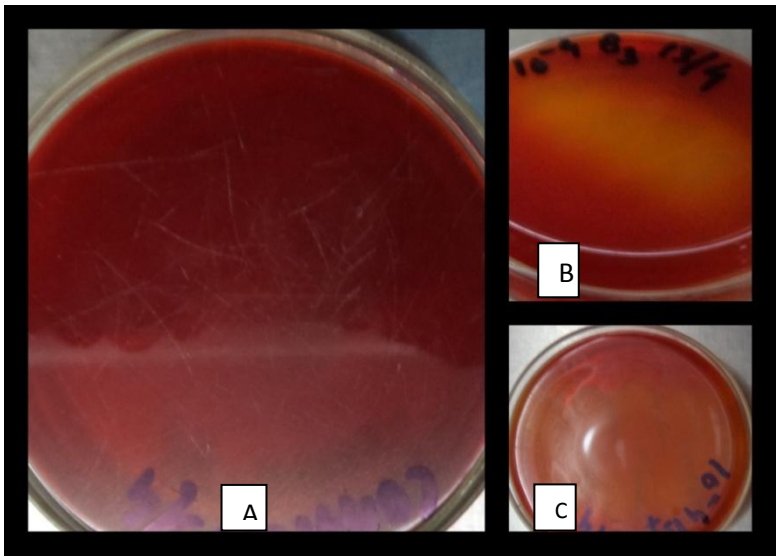

Figure.1: Screening of bacterial strains for the presence of manganese peroxidase activity.(A)control (B)TSF3 (C)TSF7

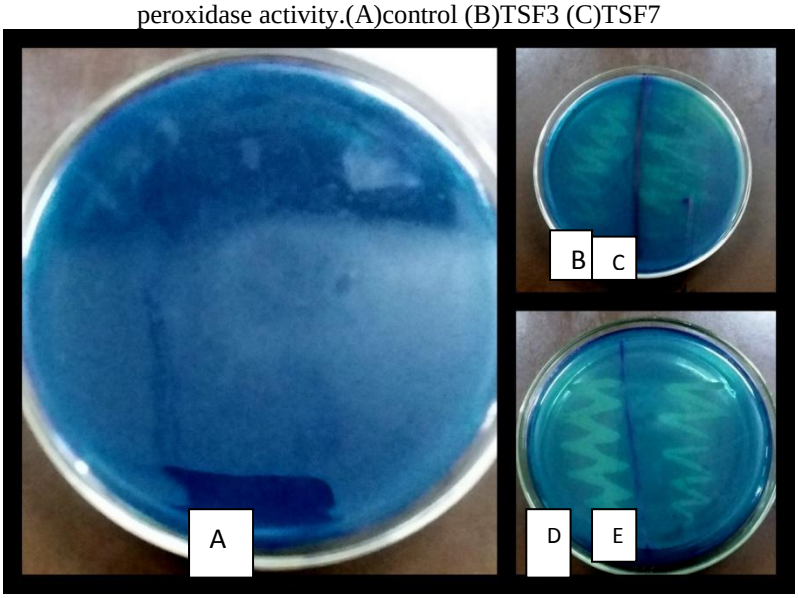

Figure.2: Screening of bacterial strains for the presence of Lignin peroxidase activity.(A) control (B)TSF3 (C)TSF7 (D)TSF12 (E) TSF13

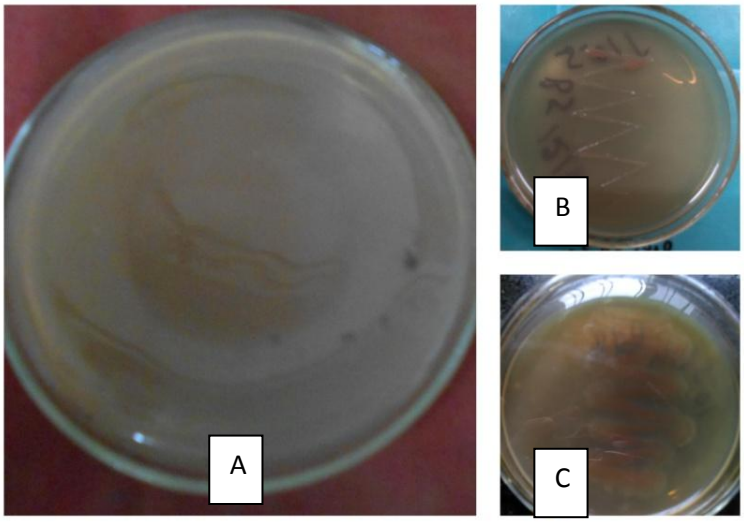

Figure.4: Screening of bacterial strains for the presence of Laccase activity.
(A) Control (B) TSF11 (C)TSF12

IV. Conclusions

It is concluded from this study that soils of timber area contains KL degrading bacteria. Bacteria strains were screened for their ligninolytic enzyme activity. This study concluded that the only two bacterial strains TSF5 and TSF6 release all the three enzymes i.e. MnP, LiP and Laccase.

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