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Bacterial Degradation of Lignin: A Prospective for Lignocellulosic Biofuels

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Abstract:- Lignin, a recalcitrant compound, forms very strong complexes with cellulose and hemicelluloses in plant materials, which serves as substrate for biofuels. Delignification of these substrates to yield pentose and hexose sugars is usually overcome by mechanical or chemical pretreatment methods. These methods generate toxic byproducts which inhibit the further processing of substrates. Bacterial enzymes, gene manipulations, medium formulations and synergistic effects of mixed cultures of bacteria offer better degradation of lignin. This method of biodegradation could serve as a cost effective and ecofriendly method towards production of second generation biofuels.

Keywords:- *Lignin; Biodegradation; Lignocellulose; Biofuel; Bacteria; Actinomycetes.*

I. INTRODUCTION

Lignin is a highly branched, aromatic complex polymer of hydroxycinnamyl alcohols (or monolignols), coniferyl alcohol (G-lignin), sinapyl alcohol (S-lignin) and *p*-coumaryl alcohol (H-lignin) (table I) which is resistant to microbial degradation [14], [47]. The complex three dimensional network of non phenolic phenyl propanoid units linked together by ether and carbon bonds gives lignin its recalcitrance.

Lignocellulosic biomass which contains higher proportions of cellulose could serve the purpose of second generation biofuels. The decomposition of lignin constitutes an important process in the biological deterioration of plant materials for industrial products derived from lignocelluloses. The major lignocellulosic substrates which have high potential for second generation biofuels are listed in the table II.

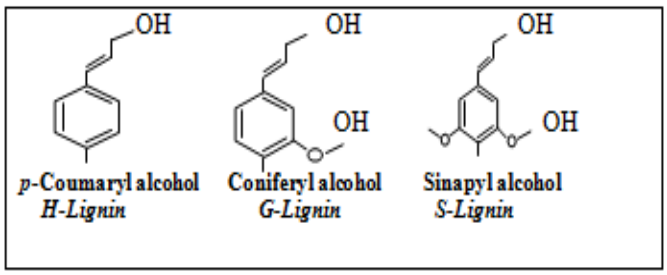

Table 1:- Basic Structural Units of Lignin

Lignocellulosic substrate		Cellulose	Hemicellulose	Lignin
Hardwoods	Eucalyptus	54.1	18.4	21.5
	Oak	40.4	35.9	24.1
	Poplar	50.8-53.3	26.2-28.7	15.5-16.3
Softwoods	Douglas fir	44.0	11.0	27.0
	Pine	42.0-50.0	24.0-27.0	20.0
	Spruce	45.5	22.9	27.9
Straw	Miscanthus	43.1-52.2	24.8-33.9	9.2-12.5
	Wheat	34.2	23.7	13.9
	Barley	33.3	20.4	17.30
	Paddy	45.5	23.0	8.2
	Oat	37.6	23.3	12.8
	Rice	29.2-34.7	23.0-25.9	17.0-19.0
	Sorghum	32-35	24-27	15-21
	Canola	42.4	16.4	14.1
Switch Grass		31-32	35–50	20.4-25.2
Corn cobs		35.9	32.7	18.8
Corn stalks		61.2	19.3	6.9
Cereal		45-55	26-32	16-21
Coconut husk		39.3	16.1	29.8
Sugarcane bagasse		38.2	25.0	24.0

Table 2:- Chemical composition of lignocellulosic materials having potential for Bioethanol [1], [11], [27], [24], [5], [29], [45], [50].

Lignocellulosic biomasses are potential sources for sustainable alternative and more ecofriendly fuels. Effective, economical and ecofriendly degradation methods need to be developed for the processing of lignocellulosic feedstock for future industrial applications.

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II. BACTERIAL STRAINS INVOLVED IN LIGNIN DEGRADATION

The major classess of bacteria found helpful in degrading lignin is categorized into Actinomycetes, Firmicutes, Bacteriodetes and three classes of (α, β and γ) proteobacteria [13]. Based on the morphological aspects of attack bacterial degradation has been categorized into cavity-formation, erosion, and tunneling [10]. The biodegradation by bacteria is achieved through a complex of enzymes including extracellular peroxidases, Dye decolourising Peroxidases (DyPs) and laccases [12]. The phenolic hydroxyl groups linked together by ether bonds in lignin molecule must be broken down by an effective lignin biodegrader.

Bacterial laccases are different from fungi in terms of high tolerance to temperature, salt and acid/ alkaline conditions [53]. This makes them fit for various industrial applications.

Lignin peroxidase is defined by the ability to oxidize nonphenolic substrates. Extracellular heam peroxidases which have high redox potential generate a protein oxidizer to form a catalytic tryptophan free radical that interacts with and degrades lignin polymer [38].

DyPs are classes of heam containing peroxidases having wide substrate specificity and are divided in four classes. Class 'A-C' is mainly found in bacteria, while class 'D' are extracellular fungal representatives [39].

Earlier reports of lignin degradation by soil microbes on various plant lignocellulosics concluded that the soil microbes were a better degrader of cellulose and pentosans than lignin [34]. But if suitable conditions are provided, the degradation of lignin could be as great as that of cellulose. Soil bacteria such as *Pseudomonas* and *Flavobacterium* were able to degrade 20-30% of the lignin extracted from wood flour [41].

A scheme for the disorganization of lignin using six biodegradative strains of Actinomycetes including different strains of *Streptomyces* on dimeric model lignin compounds has been developed, as a method to increase the accessibility of hemicelluloses and cellulose [21].

Lignin degrading capacity of bacteria is dependent on the type of substrate used [33].

Study on the lignocellulose degrading property of *Streptomyces flavovirens* on douglas fir phloem, stated that the organism was a better degrader of cellulosic components and a very slow lignin degrader [42].

Delignification efficiency of *Streptomyces viridosporus T7A* was proven on native wheat straw lignin and corn lignocellulose which on incubation after8 weeks reduced the lignin and carbohydrate contents by 19.7 and 44.7% respectively by weight [55], [16]. The removed lignin was accumulated as water soluble acid precipitable, polyphenolic polymeric lignin (APPL). Later on this enzyme was characterized to be a heam protein, which was the first report on lignin peroxidase in bacterium [36]. APPL yields appeared to be correlated with biodegradability of lignocellulosics [8].

High depolymerisation of lignin and enrichmement of cellulose (around 2 times more than the uninoculated sample) was observed using *Streptomyces griseorubens*, after 21 days of inoculation on paddy straw [40]. Potential of *Streptomyces griseorubens C-5* for production of laccase and peroxidase was proved on rice straw, in which the Acid detergent lignin was reduced from 7.20 to 2.99% along with reduction in cellulose and hemicelluloses [52].

Mixed cultures of bacteria are more effective in delignification of various substrates. Delignification efficiency of 43.6% was obtained in bark chips using a mixed culture of *Bacillus polymyxa* and *cellulomonas sp,*. 37.8% using *Bacillus polymyxa* and *Bacillus coagulans* and 35.5% using *Bacillus polymyxa* and *Bacillus circulans* [18].

Streptomyces sp. KS1025A shows faster and higher laccase producing capacity when compared to white rot fungi *Pleurotus ostreatus* for lignin degradation in reduced time [7]. *Streptomyces psammoticus* [32] isolated from marine and mangrove areas is a potent lignin degrader with the production of LiP, MnP and laccases. Bacterial strains belonging to the phylum α-Proteobacteria (*Ochrobactrum sp., Ochrobactrum pectoris, Ochrobactrum pituitosum, Agrobacterium sp.),* γ-Proteobacteria (*Enterobacter ludwigii, Enterobacter cloacae,* Actinobacterial strains such as *Microbacterium sp., Mic. oxydans, and* Firmicutes species such as *Paenibacillus sp. Lysinibacillus sphaericus* has been studied for lignin removal using milled pine as substrate [37]. The decrease in lignin content holds a maximum for *Lysinibacillus sphaericus* (24%) followed by *Enterobacter cloacae* (22.7%) after 7 days of pretreatment.

A low cost method of optimization of medium formulation was developed rather than strain improvement using a new bacterial strain *Streptomyces cinnamomensis* to produce a crude laccase-LiP complex for the degradation of species-specific plant lignins [23]. The laccase activity of *S. cinnamomensis* was found to be 5.1 times higher than the initial average activity on optimization of medium formulation.

Manganese dependent lignin peroxidase DypB from *Rhodococcus jostii RHA1*, a powerful degrader of PCBs, shows selective degradation of some lignin compounds. The recombinant DypB obtained from *R. jostii* degraded wheat straw lignocellulose over a period of 48 hours in the presence of $MnCl_2$ [2].

Pantoea sp. SD-I, a rice endophytic bacterium, is a proved biodegrader of aromatic compounds, shows lignin degradation activity (about 33.1%) on rice straw. On the bio-refinery point of view, the species has limited application as it degrades 80.1 and 59.6% of cellulose and hemicellulose respectively [51].

The synergistic effect of bacterial consortiums harboring two *Paenibacillus sp*., two *Pseudomonas sp*., one *Mic. pumilum* and one *Acinetobacter* sp. was effective in degrading lignin upto 60.9% in reeds with very negligible cellulose decomposition of 2% in 15 days. This synergism was found to be much more effective than the degrading capacity of *Ps.*, alone which could only degrade 26.7% lignin in *L.oligensis* in a period of 45 days [48], [54]. Pseudomonas sp. PKE117 could degrade the structure of lignin, cellulose and hemicellulose and was a better degrader than white rot fungi in softwoods.

The enzymes extracellular peroxidases and laccases require hydrogen peroxide and dioxygen respectively, as a co-substrate for the degradation of lignin. Bacterial species such as *S. viridosporus* and *N. autotrophica* utilize extracellular peroxidases for degradation whereas *P. putida, Rhodococcus RHA1* and *Rhodococcus sp.* use extracellular laccases for lignin degradation. The observations were based on the two new spectrophotometrical assays (Continuous fluorescent assay and UV-Visible assay) for monitoring the breakdown of lignin component of plant lignocellulosics [3].

Aneurinibacillus aneurinilyticus, a bacterium isolated from pulp and paper sludge, was observed to degrade 43% lignin from kraft lignin. The bacterium did not use lignin as a sole source of carbon but the degradation was done as a co-metabolism on addition of glucose [35]

Bacterial lignin degradation can be explained on various catabolic pathways [31]. The presence of various enzyme systems in *Sphingomonas paucimobilis* SYK-6 serves it as a versatile lignin degrader. The bacterium can utilize and degrade variety of lignin derived compounds such as biphenyl, β-aryl ether, phenylcoumarane, diaryl propane and various lignin derived monoaryls such as vanillin, vanillate, syringaldehyde, syringate.

B. pumilus and *B. atrophaeus* isolated from rainforest soils was identified to have high laccase activity. After 7 days of incubation these strains have degraded model lignin dimer guaiacylglycerol-β -guaiacyl ether by 27.5 and 35.1% respectively, which is the most abundant linkage in lignin compounds (45–50% in softwood and 60% in hardwood) [22].

Two native Egyptian bacterial Strains *B. subtilis* (EU344808) and *Bacillus sp.* (EU344809) were identified which could utilize lignin as sole carbon source. *B. subtilis* degraded 64% of the total lignin whereas *Bacillus* sp. degraded only 59% lignin [19].

Natural lignin from *spartina* lignocellulose, Kraft lignin from *P. elliottii*, and also lignin from *S. alterniflora* was degraded by Arthrobacter sp. [25]. The study reveals the strain as a versatile lignin degrader which could be applied on other lignocellulosic for biodegradation of lignin. It also suggests that the ability of a microbe to degrade natural lignin can be confirmed by the degradation studies using ^{14}C lignin labeled lignocellulosic substrate.

Two bacterial strains *S. viridosporus* and *S. setonii* grown on soft wood (spruce), hard wood (maple) and grasses shows a high lignin degrading capacity. The efficiency of degradation was substrate dependent and maximum degradation was observed in grasses. *S. viridosporus* removed approximately 44% of the lignin component from the grass lignocellulose compared to hardwood (32%) and softwood (30.9%) [6].

Bacterial strains such as *S. viridosporus* and *S. badius* could utilize indulin AT lignin (purified form of lignin, devoid of carbohydrates) as a sole carbon source [20].

39 bacterial strains belonging to *Nocardia* sp. and *Streptomyces* sp. have been studied for dye decolourising activity and the production of laccases and peroxidases. Thirteen of the 39 strains were found to utilize indulin AT as a sole source of carbon [28]. The study revealed that the identified laccase producers were closely related to *S. cyaneus CECT 3335, S. griseorubens C-5, S. ipomoea CECT 3341 and S. psammoticus MTCC 7334* where the role of the laccases has been related to lignin degradation.

Noc. sp., a Gram-positive bacterium which was isolated from a Finnish soil, was identified to decompose lignin and to assimilate lignin degradation products as a carbon source [44]. This serves the purpose of removal of toxic degradation products which would increase the cellulose accessibility.

Studies suggest structural transformation could be induced by *Clostridium thermocellum* in *Populus trichocarpa* lignin [4]. It was also reported that the enzymes responsible for this transformation are entirely different from fungus. He also suggested that Microbial β-etherases which are a class of enzymes responsible for breakage of β-O-4 bonds in lignin may be responsible for these structural changes. These changes increase the accessibility of cellulosic components for Bioethanol production.

Soil bacterium *Enterobacter lignolyticus* SCF1, a facultative anaerobe, was able to degrade 56% of lignin within 48 hours under anaerobic conditions [17]. Earlier reports on E. *lignolyticus* SCF1 suggests that the species is ionic tolerant and can be used for pretreatment of lignocellulosics [26]. The pretreatment of lignocellulosic biomass using ionic solutions have many advantages over conventional methods but is toxic for the microbes used for saccharification and fermentation. This toxicity can be overcome by utilizing *E. lignolyticus* species.

Two acid tolerant dye decolourising peroxidases have been isolated and characterized from bacterial strains *B. subtilis* and *Ps. putida MET94* which could utilize anthraquinonic or azo dyes, phenolics and methoxylated aromatics as substrates [39].

Thermobifida fusca, a thermophilic actinomycete, a major degrader of plant cell walls, shows high potential in biotechnological applications as it can produce a DyP-type peroxidase (TfuDyP) that shows dye decolourising activity

and accepts phenolic and aromatic compounds. This peroxidases show high similarity to lignin peroxidases of fungal family and that they might play a significant role in lignin biodegradation [46], [30].

Four Actinobacterial isolates (four *Mic.*, two *Micrococcus* and *Rhodococcus erythropolis* and two *α*-proteobacteria (*Ochrobactrum*) have been isolated from woodland soil for their lignin degrading capacity on composted wheat straw [43]. *Thermobida fusca*, *Rhizobiales* and *Sphingobacterium* sp were also isolated in which *Sphingobacterium* showed a tenfold increased activity than all other isolates.

A unicellular bacterial strain *Comamonas sp.* B-9 as reported, could utilize kraft lignin as a sole carbon source with high activity for production of Laccases and Mangnese peroxidases [15].

Bacterial species belonging to phylum Firmicutes and Bacteroidetes shows high lignin depolymerisation activity, increasing the accessibility of cellulosic and hemicellulosic components for biofuel production [49]. This lignin depolymerisation was coupled with methane production using *Methanomethylovorans* sp. and *Methanoculleus* sp. which shows the application of delignification of lignocellulosics towards biofuels.

Lignin mineralization and depolymerisation are two entirely different processes [9]. The study was based on the potential of three lignolytic bacterial *strains Pandoraea norimbergensis LD001*, *Pseudomonas sp LD002* and *Bacillus sp LD003*. The ligninolytic potential was assessed based on the growth on lignin fractions and dye decolourising capability. *Bacillus sp. LD003*, showed least efficient growth on lignin fractions but has a high dye-decolourizing capacity on lignin like dyes. The work also opens the potential of various bacterial strains for lignin depolymerisation outside fungal kingdom.

III. CONCLUSION

Even though fungal enzymes are more established in delignification, the study suggests that bacteria are also potential degraders of lignin. The distribution and function of lignin degrading enzymes in different bacteria needs to be explored for their industrial applications. Methods from gene manipulations to optimization of medium formulations will easily cleave the pathway for utilizing bacteria for lignocellulosic degradation. Development of such biodegradation methods will assist in the formulation of feasible and ecofriendly methods for production of biofuels from lignocellulosics.

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