STUDY OF LIGNINOLYTIC BACTERIA ISOLATION AND CHARACTERIZATION FROM DHAMDHA AGRO FIELD OF BHILAI-DURG REGION

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Abstract

Lignin is a complex, three-dimensional aromatic polymer consisting of dimethoxylated, monomethoxylated and non-methoxylated phenylpropanoid subunits. It is the most abundant renewable carbon source on Earth. The majority of plant biomass, including stems and leaves, is composed of lignocellulose. In present study isolation, identification and characterization of ligninolytic bacterial flora were done from the agro-field, using a lignin residue. 2 types of soil samples black and mixed soil were selected from agro fields of ***Dhamdha of Bhilai-Durg*** *for isolation of ligninolyitc bacterial colony. Microbes from both mixed and black soil samples were grown in solid media by Hungate method and result shown that three types of bacterial colonies (1-MS, 2-BS), were isolated from Dhandha agro field and used to check the activity of lignolytic capability. Out of 3 types colonies only* ***1****types of colony (j-MS), was shown potential of lignin degradation. The Morphological, gram's reaction and endospores staining reaction, biochemical characteristics of the isolate obtained from this agro-field soil samples identified with reference to Bergey's Manual of Determinative Bacteriology. These identified isolate (j) - Bacillus species was shown presence of Laccase, Manganese peroxidase (MnP) and Lignin peroxidase (LiP), etc. lignin degrading enzymes. This results concluded that Bacillus sps. strain was able to degrade lignin substrate which was second abundant and waste material in the world. It is also concluded that to expand on the range of products which can be obtained from lignocellulosic biomass. In this study, soil bacteria was isolated by enrichment on Kraft lignin and evaluated for their ligninolytic potential as a source of novel enzymes use to generate 2nd generation biofuel from waste streams of pulping.*

Keywords: *Lignocellulosic, 2nd generation biofuel, Laccase, Manganese peroxidase (MnP), and Lignin peroxidase (LiP), Bacillus species*

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1. INTRODUCTION

Lignin is a complex, three-dimensional aromatic polymer consisting of dimethoxylated, monomethoxylated and non-methoxylated phenylpropanoid subunits, (Martinez *et al.*, 2005) [1]. It is found in the secondary cell wall of plants, where it fills the spaces between the cellulose, hemicellulose and pectin components, making the cell wall more rigid and hydrophobic. Lignin provides plants with compressive strength and protection from pathogens, (Kumar, and coworker, 2008; Rubin, 2008) [2,3]. Lignin can be depolymerized by thermochemical methods such as pyrolysis (thermolysis), chemical oxidation, hydrogenolysis, gasification, and hydrolysis under supercritical conditions (Pandey and Kim, 2011) [4]. However, many of these processes are environmentally harsh and occur under severe conditions requiring large amounts of energy (Ward and Singh, 2002) [5]. Enzymes could provide a more specific and effective alternative for lignin depolymerization. Furthermore, biocatalytic processes generally take place under mild conditions, which lowers the energy input and reduces the environmental impact(Perez *et al.*, 2002; Sun and Chen, 2002) [6,7].Thus, the bacterial ligninolytic potential is still largely unexplored and many novel ligninolytic enzymes may await discovery. These bacterial enzymes may be superior to their fungal counterparts with regard to specificity, thermostability and mediator dependency (Kumar, and coworker, 2008; Wenzel *et al.*, 2002, Rodriguez, 2009) [2, 8, 9]. The enzymes reported to be involved in bacterial lignin degradation are laccases, glutathione S-transferases, ring cleaving dioxygenases (Masai *et al.*, 1999; Perestelo *et al.*,1989) [10,11], monooxygenases and phenol oxidases (Canas *et al.*, 2007) [12]. Such enzymes are also involved in degradation of polycyclic aromatic hydrocarbons, which show similar structural properties and resistance to microbial degradation as lignin (Perestelo *et al.*, 1989) [11].

The enzymes are used for the degradation of many compounds, and it's used for biological functions such as textile, food, paper and pulp industries, organic, medical, pharmaceutical, cosmetic and nanotechnology applications, and bioremediation too, having many functions in the microorganism (Cullen, 1997) [13]. They may also have specific advantages for the depolymerization of the modified lignin residues typically encountered in waste streams from the pulping or 2nd generation biofuel/biobased chemicals industry (International Energy Agency, 2012) [14].

In the present study, the characterization and identification of naturally ligninolytic bacterial flora from the agro-fields, using a model industrial lignin residue from the Kraft process.

2. MATERIALS AND METHODS

2.1 Sources of Isolates: The ligninolyitc bacterial colony were isolated from 2 types of soil samples black and mixed soil of agro fields of Dhamdha of Bhilai-Durg..

2.2 Isolation and Selection of Ligninolytic Bacteria: Both mixed and black soil samples used to isolate ligninolyitc bacterial colonies were grown in solid media by Hungate method (Ogimoto and Imai, 1981) [15]. In warm condition, media was divided into 3 tubes. Each selective substrate then dissolved, and then poured 15 ml each into Petri disc. Microbes sources soil sample were serially diluted with 10^{-5} dilution then (100µl) soil sample was inoculated for 7-14 days. The growing colonies then were counted and identified. The lignin degrader bacteria was selected qualitatively based on the diffusion zone diameter that formed around colony (Subbarao, 1993; Samingan, 1998; Martani and Coworkers, 2003) [16, 17, 18]. Each isolate was inoculated by spot method on nutrient agar that contains 1% tannic acid (Subbarao, 1993) [16]. Diffusion and clear zone were measured after 7 days of anaerobic incubation. Diffusion zone with colony size was used to determine the selected isolates.

2.3 Identification of Selected Microbes: The pure cultures of lignin degrading microbes were selected and subjected to various morphological studies, various types of differential staining (Gram's and endospore) and biochemical characterization tests (catalase test, starch hydrolysis, Indole, MR-VP, simmon's citrate agar, fermentation, H_2S production, nitrate reduction, urease, casein hydrolysis, gelatin hydrolysis) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbon, 1974) [19].

2.4 Lignin Degradation Study in Pure Culture: After screened and identification of lignin degrading bacterial strains were used to check the quantitative estimation of lignin degradation capability by Chandara and coworker method (Chandra *et al.*, 2007) [20]. For the measurement of lignin degradation, 1 ml of samples were centrifuged at 15000 rpm for 5 min. Supernatant (250µl) was diluted by adding 2.5 ml phosphate buffer (pH 7.6) and absorbance measured at 280 nm for lignin degradation on a UV-visible spectrophotometer (Perkin Elmer Lambda EZ201 UV/VIS Spectrometer) Lara and coworkers method, 2003 [21].

2.5 Enzyme Assays: Presence of lignin degrading enzymes activity in lignin degrading bacteria were measured by Laccase activity by Machado and Matheus method (2006) [22], Manganese peroxidase (MnP), Glenn and coworkers, method (1986) [23], and Lignin peroxidase (LiP), Tie *et al.*, method (1988) [24].

3. RESULTS AND DISCUSSIONS

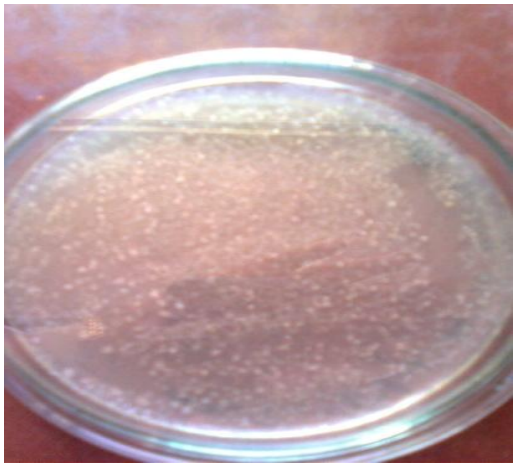
In the present study, the isolation and characterization of naturally ligninolytic bacterial flora from **Dhamdha** agro-fields **of Bhilai-Durg** were done. The bacteria were isolated from 2 types of soil samples black and mixed soil of agro fields.

3.1 Isolated Bacterial Colonies from Dhamdha Agro Fields: Three types (1-MS, 2-BS), of bacterial colonies were isolated from both mixed and black soil samples of Dhamdha agrofield and used to check the activity of lignolytic capability (Table-1, Figure-1).

Table-1: Bacterial Colonies Isolated From Dhamdha Agro-Field.

Sample Site	Dhamdha Agro Field	
Types of Soils	Mixed Soil	Mixed Soil
Types of	1	2
No. of Colonies	j- 10	J-38 K-24

Fig -1: Shown Bacterial Colonies Isolated From Dhamdha Agro-Field.



3.3 Identification of Selected Ligninolytic Bacterial Colony: In present studies Dhamdha agro field **1** bacterial colony (j-MS), was shown potential of lignin degradation.

The Morphological characteristics of the obtained from the water samples on Nutrient Agar (NA) and Eosin Methylene blue (EMB) agar is shown in (Table-3, Fig-1). The gram's reaction and endspores staining reaction for the characterization of isolates obtained are also shown (Table-4). The Biochemical characteristics of the isolates obtained from this agro-field soil samples is shown in Table-4. The isolated bacterial colony was identified with reference to Bergey's Manual of Determinative Bacteriology [5]. This identified isolate was (j)- *Bacillus* species (Table 3).

Table-3: Shown Morphological Characteristic of Isolated Microbes from Dhamdha Agro-Field.

Isolate	Morphological Characteristics	Organism
j	**Spore forming, Gram positive rods, creamy white colony on Nutrient Agar, entire margin**	***Bacillus* sp.**

Table-4: Shown Biochemical Test for Identification of Isolated Bacteria Dhamdha Agro-Fields

S. No.	Biochemical test	Dhamdha Agro Field
		j Bacterial colony
1.	**Motility test**	**+**
2.	**Catalase test**	**+**
3.	**6.5% NaCl**	**-**
4.	**Glucose fermentation Test**	**A/G**
5.	**Lactose fermentation Test**	**A**
6.	**Sucrose fermentation Test**	**A/G**
7.	**Starch Hydrolysis Test**	**-**
8.	**Indole Test**	**-**
9.	**MR Test**	**-**
10.	**VP Test**	**+**
11.	**Citrate Test**	**-**
12.	**Urease Test**	**-**
13.	**Gelatin Hydrolysis Test**	**+**
14.	**H_2S Production Test**	**+**
15.	**Nitrate Utilization Test**	**+**
16.	**Lipid Hydrolysis Test**	+
17.	**Oxidase Test**	**+**

Note: A -Acid, A/G-Acid/Gas, + =Positive, - =Negative,

3.4 Detection of Lignin Degrading Enzyme: Present studies of lignin degrading bacteria (j) - *Bacillus* species shown that all three lignin degrading enzymes Laccase, Manganese peroxidase (MnP) and Lignin peroxidase (LiP) were presence (Table- 5)**.**

Table-5: Shown Lignin Degradation Study of Isolated Bacteria from Dhamdha Agro-Field.

S. No.	Lignin Degrading Bacterial colony	Presence of Lignin Degrading Enzymes		
		Laccase	**Manganese peroxidase (MnP)**	**Lignin peroxidase (LiP)**
1.	***Bacillus* sp.**	+	++	+++

Previous studies related to isolation and charachterization of ligninolytic bacteria and their enzymatic activity by Bandounas, *et al.,* (2011), Rodriguez *et al.,* (2011), Gong *et al.,* (2013) and Rahman *et al.,* (2013) were revealed *Bacillus* sp. have highest potential to degrade lignin due to presence of ligninolytic enzymes [Laccase, Manganese peroxidase (MnP) and Lignin peroxidase (LiP) [25,26,27,28]. Previous study shown similarity with present research work. The bacterial isolate in this study appear to have an alternative type of ligninolytic system. Thus, a new and presumably vast source may be tapped for novel ligninolytic enzyme activities. A few considerations, however, must be taken into account when hunting for novel ligninolytic activities for lignin valorization.

4. CONCLUSION

In the present study, the characterization and identification of naturally ligninolytic bacterial flora from the **Dhamdha** agro-fields **of Bhilai-Durg**, using an industrial lignin residue. The bacteria were isolated from 2 types of soil samples black and mixed soil sample of agro fields. In present investigation found that from the both soil samples of agro-field *Baccillus* sp. was isolated from mixed soil sample and given positive results for various ligninolytic enzymes (Laccase, Magnese Peroxidase and Lignin Peroxidase). This result concluded that *Baccillus* sp. was able to degrade lignin substrate which was second abundant and waste material in the world. It is also concluded that to expand on the range of products which can be obtained from lignocellulosic biomass, the lignin component should be utilized as feedstock for value-added chemicals such as substituted aromatics, instead of being incinerated for heat and energy.

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