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SCREENING, ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF LACCASE PRODUCING BACTERIA FROM RAIGAD DISTRICT SOIL

Microbiology

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ABSTRACT

Screening and isolation of Laccase producing bacteria from Raigad District soil was carried out to assess the diversity of Lignocellulose degrading bacteria. For isolation Laccase producing bacteria nutrient medium containing guaiacol was used. Total ten isolates were isolated from soil samples collected from different regions of Raigad district. Preliminary screening of Laccase producing bacteria was carried out by growing isolated colonies on to the nutrient medium containing guaiacol. Green colour formation by using ABTS confirms the potential laccase producing capability of isolates. Ten bacterial isolates showed positive results. As an outcome predominant isolates were identified as Pseudomonas spp. Bacillus sp. The findings show that these microorganisms have significant potential for use in applications for the treatment of Lignocellulose degradation and lignin related environment pollutants.

KEYWORDS

Laccase, Guaiacol, Nutrient medium, Bacillus spp.

1. INTRODUCTION:

Lignocellulose is the organic compounds which comprises about 95 % of land based biomass produced by plants. Lignin forms tight association with cellulose and hemicelluloses to form lignocelluloses as a rigid recalcitrant material in woody plants (Hannah L. Woo et al, 2014) [2]. Laccases are the copper containing enzymes. These enzymes are capable of oxidation of a variety of aromatic amines as well as phenolic compounds (Hemaraju et al, 2018) [1]. Recently several developments have helped to make bioremediation feasible. Varieties of bacteria are capable of decontaminating the polluted soil and water.

Biodegradation of Lignin is difficult because of the complex structure. Lignin is water insoluble polymer. To undergo fully metabolized like other polymer must be broken down extracellularly into fragments that are small enough to enter the cells.

The goal of this research is to isolate novel lignin degrading bacteria present in Raigad district soil. The Raigad district area is known for coastal area, forest area and its biodiversity. The district forms an important part of the traditional 'Konkan Plain'. The Sahayadri (Western Ghats) in the east send several transverse numbers of subsidiary hills west towards denying the plains of a uniform level and continuous character. On the basis of variation in local relief, the district has been classified into six groups, Sahayadri hills, Konkan forested hills, Sudhagad plateau, Ulhas basin, Kal-Savitri Valley and Raigad coast.

The main types of soils found in Raigad district are, Forest soil, Varkas soil, Rice soil, Khar or Salt soil and coastal soil. The variety of soil types helps in studying diversity of lignin degrading microorganisms.

2. MATERIALS AND METHODS:

Sources of Isolates:

The Laccase producing bacteria were isolated from Raigad district soil. Four types of soil samples were collected from different regions of Raigad district based on type and region of the soil, forest area soil, Rice field soil and coastal area soil. These soil samples were used for screening and isolation of Laccase producing bacteria. Figure 1, Figure 2, Figure 3 and Figure 4 shows different soil sample.

Figure 1: Konkan forest soil

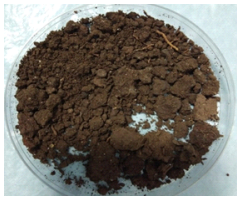

Figure 2: Paddy field soil

Figure 3: Sahayadri plateau soil

Figure 4: Coastal soil

Collection of soil samples:

Soil samples were collected from different locations based on region and soil types of Raigad district in Maharashtra India. These samples were used for screening and isolation of Laccase producing bacteria. The samples were collected from 8-10 cm depth using a sterile spatula. The collected sample was transferred in a pre-sterilized self-sealing polybags. The soil samples were brought to the laboratory and stored under refrigerated condition at 2-8°C temperature.

Enrichment medium for Laccase producing bacteria:

The enrichment medium used for inoculation of collected soil samples was nutrient broth supplemented with 0.2 mM CuSO4 and 2 mM Guaiacol as an inducer. (M. Kuddus et al, 2013) [8].

Enrichment of Laccase producing bacteria:

The enrichment of Laccase producing bacteria from soil was done in 250 ml conical flask. 10 gm of soil sample collected from different locations of Raigad district were inoculated in 100 ml of nutrient broth containing 0.2 mM CuSO4 and 2 mM Guaiacol as an inducer. The inoculated flasks were incubated at 37°C temperature for 48 hours.

Isolation of Laccase producing bacteria:
Enriched sample of 1ml was transferred to 99 ml of sterile 0.9% NaCl. The solution were stirred vigorously and allowed to settle down. By using 1 ml of liquid mixture serial dilution were prepared in 0.9% NaCl. About 100 µl of diluted samples were spread on nutrient agar medium containing 0.2 mM CuSO4 and 2 mM Guaiacol as an inducer. For confirmation of laccase producing bacteria 2-3 drops of 1 mm ABTS laccase substrate was added on to the bacterial colonies. Laccase positive colonies were identified by the appearance of green color formation.

Following bacterial colonies were isolated on nutrient agar medium containing 0.2 mM CuSO4 and 2 mM Guaiacol.

Table 1: Colony characters

Sr. No.	Name of Isolate	Colony characters						Gram's nature
		Size	Shape	opacity	color	margin	elevation	
1	LPB-01	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve long rod
2	LPB-02	2 mm	round	opaque	Off white	entire	raised	Gram +ve short rod
3	LPB-03	1-2 mm	round	opaque	Off white	entire	raised	Gram -ve short rod
4	LPB-04	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve long rod
5	LPB-05	1 mm	round	opaque	Off white	entire	raised	Gram +ve short rod
6	LPB-06	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve short rod
7	LPB-07	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve rod
8	LPB-08	1-2 mm	round	opaque	Off white	entire	raised	Gram -ve rod
9	LPB-09	1-2 mm	round	opaque	Off white	entire	raised	Gram +ve cocci
10	LPB-10	1 mm	round	opaque	Off white	entire	raised	Gram -ve short rod

3. RESULTS AND DISCUSSION:

To identify the selected strains biochemical characterization has been done. Different tests are performed they include Indole test, citrate test, Methyl red and Voges-Proskauer test of the Laccase producing bacteria were performed and following observations were drawn,

Table 2: Biochemical characterization of Laccase producing bacteria isolated from Raigad District soil

Sr. No.	Name of Isolate	Formation of Endospore	Motility	Biochemical Tests						Congo red decolorization Test
				Catalase	Oxidase	IMViC Tests				
						Indole	MR	VP	Citrate	
1	LPB-01	+	+	+	-	-	-	+	-	+
2	LPB-02	-	-	-	-	-	-	-	-	-
3	LPB-03	-	-	+	-	-	-	-	-	-
4	LPB-04	+	+	+	-	-	-	+	-	+
5	LPB-05	+	-	+	-	-	-	+	-	+
6	LPB-06	+	-	+	-	-	-	+	-	+
7	LPB-07	+	+	+	-	-	-	-	-	+
8	LPB-08	-	+	+	+	-	-	+	+	+
9	LPB-09	-	-	+	-	-	-	-	-	-
10	LPB-10	-	-	+	-	-	-	-	-	-

Confirmation of Laccase producing activity:
Confirmation of Laccase producing ability of bacterial isolates was performed by adding 2-3 drops of 1 mm ABTS laccase substrate was added on to the bacterial colonies. Laccase positive colonies were identified by the appearance of green color formation. Only these potential laccase producing bacteria based on maximum laccase activity were taken for further study.

DISCUSSION:

The enrichment of Laccase producing bacteria was achieved by using nutrient agar medium containing 0.2 mM CuSO4 and 2 mM Guaiacol as an inducer. Broth medium was enriched for 48 hours. Further isolation of bacterial colonies on agar medium containing 0.2 mM CuSO4 and 2 mM Guaiacol as an inducer. The Laccase producing ability was confirmed by adding 2-3 drops of 1 mM ABTS laccase substrate was added on to the bacterial colonies and confirmed by appearance of green color formation.

Laccase producing bacteria were also isolated from forest by Hemaraju S. and Narasegowda P. N. [1]. Laccase producing bacteria was studied by M. Kuddus et al [8]

Different types of Laccase producing bacterial isolates were isolated from Raigad district soil, which is showing diversity and capability to utilize various biochemicals during study of biochemical characterization.

The Laccase producing capability and activity of bacterial isolates were accessed by using agar medium containing 0.2 mM CuSO4 and 2 mM Guaiacol.

4. CONCLUSION:

Total 10 Laccase producing bacterial isolates were obtained from Raigad district soil with the use of agar medium containing 0.2 mM CuSO4 and 2 mM Guaiacol and their Laccase producing activity was checked. The Laccase producing bacterial colonies showed green color appearance when 2-3 drops of ABTS were place on isolated bacterial colonies. The predominant isolates were Bacillus spp. and Enterobacter spp. The isolates were then maintained on nutrient agar plates for further studies.

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