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Isolation and screening of lignin peroxidase producing microorganism from different geographical location of Andaman and Nicobar island

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Abstract

Lignin play an important role in total carbon balance in our earth ecosystem, moreover it is second largest carbon source present in earth environment. There are several ligninolytic enzyme synthesis by microorganism used for degradation of lignin and revert it back in the environment. In the present study lignin peroxidase producing bacterial and fungi were isolated on mineral salts medium supplemented with Lignin.Qualitative determination of lignin peroxidase was done by plate screening method using Guaiacol, Pyrogallol, methylene blue, ramezol brilliant blue and kraft lignin as indicators. Positive cultures were further screened quantitatively in liquid medium using Veratryl alcohol as a substrate. Fungal culture (FSV 3) gave maximum lignin peroxidase production of 131.18 U/ml after 72 hours of incubation.

Keywords:kraft lignin, Lignin, Lignin peroxidase,Veratryl alcohol.

1. Introduction

Lignin peroxidase belong to group of lignolytic enzyme. Lignolytic enzyme includes lignin peroxidase (Lip), Laccases, Manganese peroxidases, Versatile peroxidases, Chloroperoxidases, and Cellobiose dehydrogenase (Hariharan and Nambisan, 2013). Lignin peroxidase (Lip) (E.C 1.11.1.14) work on a principal of oxidation and reduction as it belongs to oxidoreducatase family (Patil, 2014).

Lignin can be degraded by chemical method but comparing with the harsh chemical treatment, lignolytic enzyme play important role to overcome recalcitrant effect of paper and pulp effluent, dye industry and agrowaste.There are several researches carried out for degradation of lignin from paper and pulp industry. According to Karigar and Rao (2011), Lignolytic enzyme is used to remove toxic chemicals substance such as orthodox and paradiphenols, polyamides, cyclic compound, halogenated phenols and may other recalcitrant compound. In future microbial derived lignin peroxidase will play an important to remove phenolic pollutant from environment not only this but Lip have several applications in the field of bioremediation, dermatology as well as in cosmetology field. (Faladee*t al*, 2016)

Moreover, Egwin (2015) reported that lignin peroxidase as one of the useful enzyme for biotechnology as well several other industries. As microbial derived lignin peroxidase being one of the industrial important enzyme and have several useful applications the objectives of present research study is to screen microorganism from soil sample which give high lignin peroxidase activity.

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2. Material and Methods

2.1Collection of soil sample

Soil sample were collected from diverse geographical region of Andaman and Nicobar island. Soil sample were collected in sterile polybag and were brought in laboratory.

2.2Isolation of Lignin peroxidase producing microorganism

Enrichment of soil samples were carried out. 1 gm of Soil was enriched in 50 ml media containing 0.5% kaft lignin supplement with minimal media. Minimal media solution containing following ingredient (per litre): $NaNO_3$ 6g, KCl0.52g, $MgSO_4.7H_2O$ 0.52g and KH_2PO_4 0.82g. Enriched soil samples were incubated for 144 hours in rotatory shaker having Rpm 120 at 30^0C. From Enriched soil samples, bacterial strains were isolated on nutrient agar plate, while fungal culture were isolated on potato dextrose agar. Nutrient agar plate and potato dextrose agar plate were incubated at 37^0 C and 25^0 C respectively.

2.3 Primary screening

Primary screening of isolates was done by qualitative plate method using different lignin model such as Guaiacol, Pyrogallol, methylene blue, ramezol brillent blue and kraft lignin.

2.3.1 Guaiacol Plate assay

Isolated cultures were inoculated on Guaiacolmedia plates containing minimal basal salts supplemented with 3% agar and 0.01% Guaiacol(Anuja*et al*, 2017).Plates were incubated at 30 °C for 5 days.Lignin peroxidase activity was visualized on the plates as dark brown halo of Guaiacoloxidation.

2.3.2 Pyrogallol Plate assay

Isolated cultures were screened using drop test. Isolated cultureswere plated on minimal basal salts supplemented with 3% agar. Plates were incubated at 30°C for 2 days. Oxidation of Pyrogallol was observed by applying few drops of 0.4% Pyrogallol Solution and 0.1% Hydrogen peroxide on to the edge of microbial colony (Ayodeji, 2017). Detection of lignin peroxidase was confirmed around the colony showing yellow-brown color of Pyrogallol oxidation.

2.3.3 Dye decolorization plate assay

Isolated cultures were inoculated on plates containing minimal basal salts supplemented with 3% agar and 0.25 g/l methylene blue (V. Sasikumar, 2014) and 0.5 g/l ramezol brilliant blue (Tito sumandono, 2015). Plates were incubated at 30° C for 5 days. Clear zone of dye decolorization around the microbial colony indicates presence of lignin peroxidase activity.

2.3.4 Kraft lignin degradation plate assay

Isolated cultures were inoculated on plates containing minimal basal salts supplemented with 3% agar and 0.1% kraft lignin (V. Sasikumar, 2014). Plates were incubated at 30 °C for 5 days. Positive lignin peroxidase activity was shown by clear zone of kraft lignin degradation.

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2.4 Secondary screening

2.4.1 Quantitative screening of lignin peroxidase

Bacterial and fungal isolates were inoculated in minimal medium containing 0.1% glucose for 24 hours at 30°c on rotatory shaker having 120 rpm. After 24 hours, 2% of inoculum was inoculated in production medium containing basal salt solution medium supplemented with 0.1% kraft lignin and incubated at 30°C and 120 Rpm for seven days. After interval of 24 hrs, samples were harvested and centrifuged at 10,000 x g for 10 min at 4°C. The supernatant was used for determination of lignin peroxidase enzyme activity.

2.4.2 Lignin peroxidase enzyme assay

Enzyme assay was carried out using 10mMVeratryl alcohol as a substrate (Tien and Kirk, 1988). The reaction mixture contains 1ml of sodium tartrate buffer (pH 3), 0.5 ml substrate, 0.5 ml culture supernatant and reaction was started using 0.5 ml 5mMH_2O_2. The oxidation of Veratryl alcohol (ε= 9300 x 10 6 m-1 cm-1) was monitored by measuring the change in absorption at 310 nm for 1 min. One unit of enzyme activity defined as amount of enzyme that oxidizes 1μm of Veratryl alcohol per min.

3.0 Result & discussion

3.1 Isolation

Collected soil were enriched in lignin containing media for 144 hours (figure 1), during incubation period only those microorganisms will grow which have capability to utilize lignin as a sole carbon source. In the present study from the enriched soil sample 35 bacterial strain and 10 fungal strain were isolated.

Figure 1. Enrichment of soil sample

3.2 Primary screening

Primary screening was done using different lignin model media such as Guaiacol, Pyrogallol, methylene blue, ramezol brillent blue and kraft lignin.

3.2.1 Guaiacol plate assay

Selected isolates were first screened on Guaiacol containing media. Out of 35 bacterial isolates 23 bacterial strain and out of 10 fungal isolates 4 fungal strain have given dark brown halo of oxidation of Guaiacol around colonies which indicates positive result. (figure 2).

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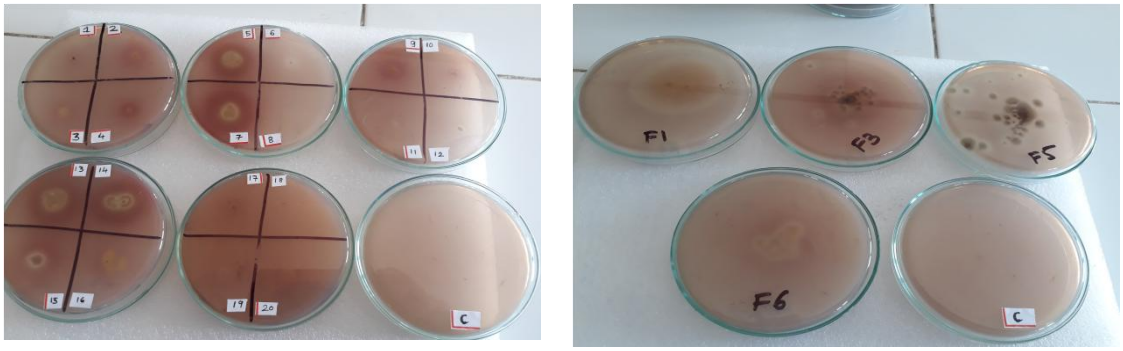
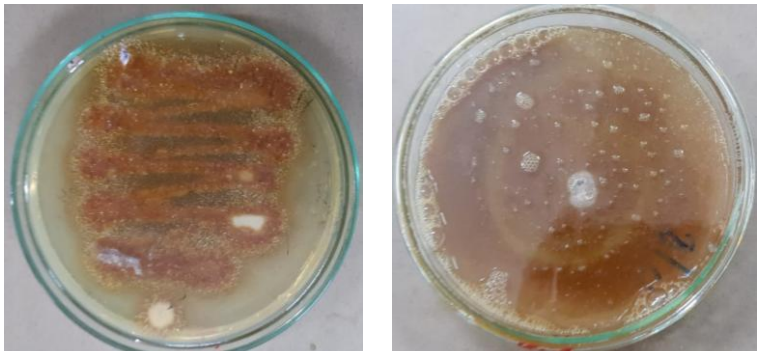


A B

Figure 2. Results of oxidation of Guaiacol (A. Bacterial culture and B. Fungal culture)

3.2.2 Pyrogallol plate assay

Out of 34 bacterial and 10 fungal strains, 3 bacterial and 2 fungal strains gave positive results. After addition of Pyrogallol and h_2o_2, positive cultures had shown highly intense dark brown color around colony of Pyrogallol oxidation (figure 2).



A B

Figure 3. Results of oxidation of Pyrogallol (A. Bacterial culture and B. Fungal culture)

3.2.3 Dye decolorization plate assay.

Methylene blue and Ramezol brillent blue dyes are used for screening of lignin peroxidaseenzyme producing microorganism as it acts as lignin model. In the present study, out of 34 bacteria strain, 3 bacterial strain were giving clear zone of methylene blue and ramezol brillent blue dye decolorization while 1 fungal stain was giving positive result.

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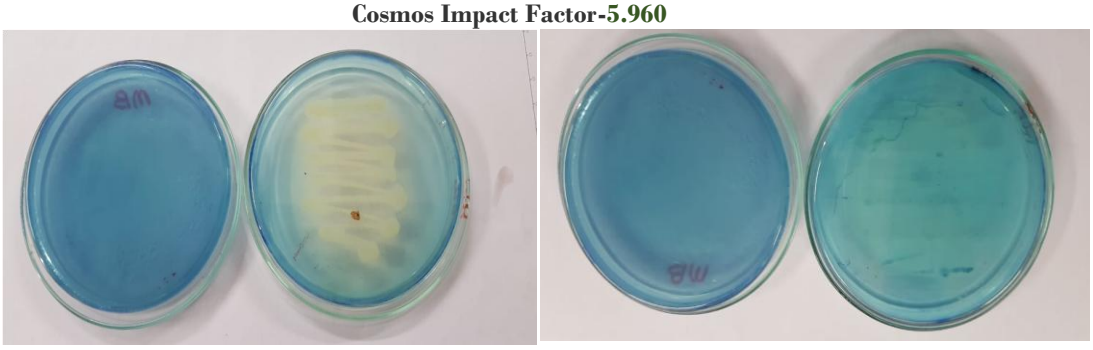
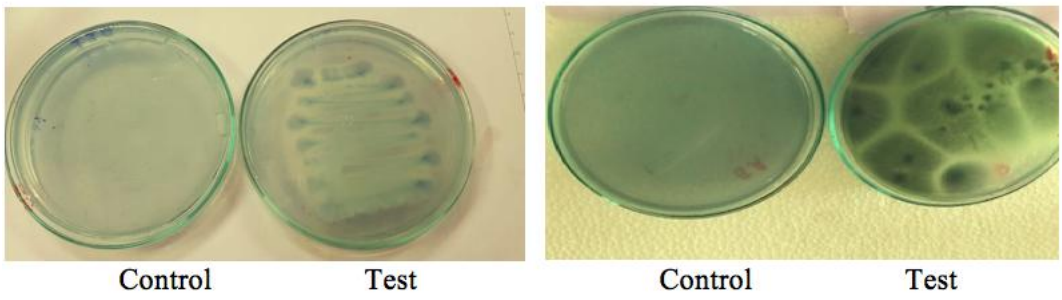


control test control test

A B

Figure 4. Results of Methylene blue decolorization (A. Bacterial culture and B. Fungal culture)

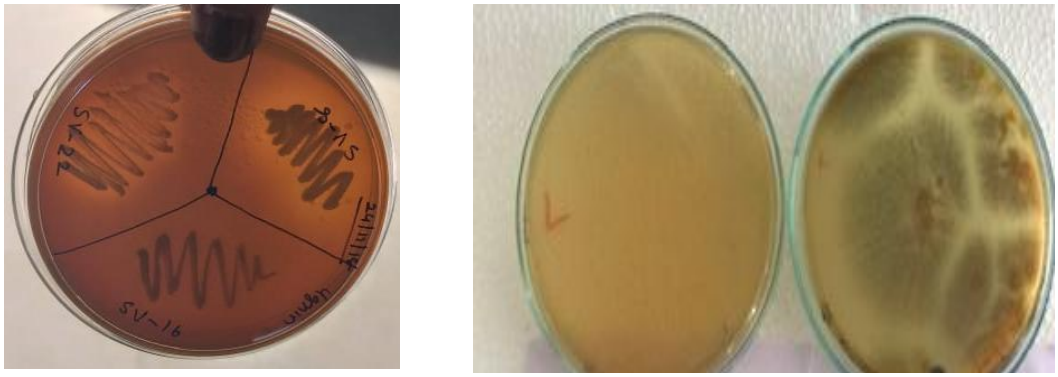


A B

Figure 5. Results of Ramezol Brillant blue decolorization (A. Bacterial culture and B. Fungal culture)

3.2.4 **Kraft lignin degradation plate assay**.

The strain which were giving Guaiacol, Pyrogallol and dye decolorization test positive result were further screened using Kraft lignin. Out of 4 bacterial cultures, 2 bacterial and 1 fungal culture have given clear zone of lignin degradation.



control test

A B

Figure 6. Results of Kraft Lignin degradation (A. Bacterial culture and B. Fungal culture)

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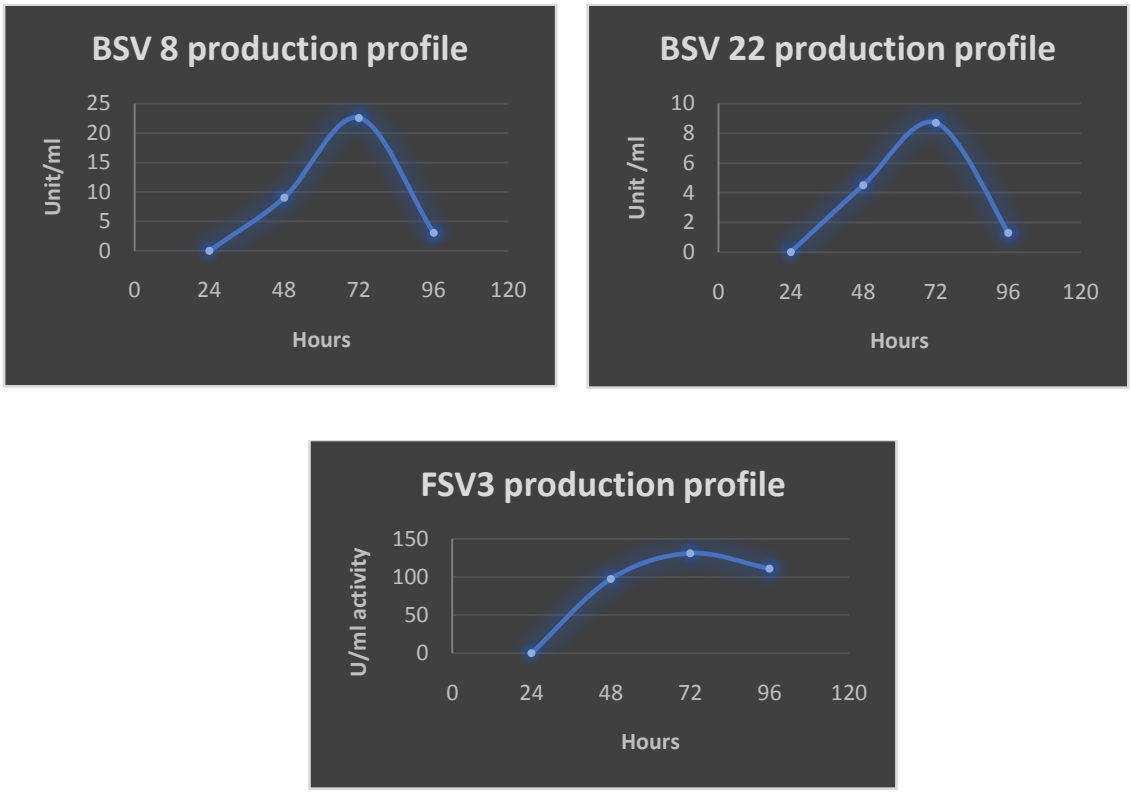
4.0 Secondary screening

4.1Quantitative screening of lignin peroxidase production

Two bacteria strain and one fungal strain were selected after primary screening and were further screened using Veratryl alcohol as substrate for lignin peroxidase assay. Submerged cultivation of cultures was carried out for production of lignin peroxidise. Enzyme activity was monitored after every 24 hrs of incubation. The maximum enzyme activity was observed after 72 hours of lignin peroxidase production. Fungal isolate SVF 3 gave maximum production of 131.18 u/ml, while bacterial stain BSV 8 and BSV 22 have given 22.58 u/ml and 8.70 u/ml lignin peroxidase production respectively. (graph 1).



Graph 1. Production profile of BSV8, BSV 22 and FSV 3 for lignin peroxidase production

5.0 Conclusions

Present research work aimed to screen microorganism producing lignin peroxidase from soil samples collected from Andaman and Nicobar island. For screening of lignin peroxidase production different indicators like Guaiacol, Pyrogallol and 0.1% kraft lignin were used. After screening on different lignin model stepwise, out of 35 bacterial isolate and 10 fungal isolate, 2 bacterial and1 fungal isolates were selected for further quantitative studies. Fungal isolate FSV 3 showed highest lignin peroxidase activities (131.18 U/ml) on 72 hours of incubation using submerged cultivation.

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