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Research Article

ISOLATION, ENRICHMENT AND ITS OPTIMIZATION OF LACCASE PRODUCING BACTERIAL STRAIN FROM MANGROVE SOIL COLLECTED FROM VELLAR ESTUARY PARANGIPETTAI

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ABSTRACT

In the current study laccase producing bacterial strain was isolated from mangrove soil at different depth (surface, 0.5, 1, 1.5 and 2). Among the different sample, soil collected from 2 meter depth was black and muddy like nature with temperature 27.9 ˚C soil pH 7.75 showed maximum laccase producing bacterial strain which turn to dark green colour after addition of ABTS. Optimization for laccase enrichment was also carried out in that 0.5 mM copper sulphate with 2mM guaiacol act as a good inducer for enrichment of laccase producers, enrichment broth kept at 40 ˚C for 72 hours is optimized incubation parameters.

Keywords: Laccase, mangrove soil, optimization, enrichment.

INTRODUCTION

Marine organisms in particular marine bacteria were accepted as fruitful and undervalued producers of natural products and chemical (1-3). Mangrove environment are dedicated and wide spread environment along the coastal regions which support biologically different group of organisms including microbes (4). Mangroves have various type of sediment with different levels of contamination which depends on the different human activities (5). Mangrove ecosystem compromises huge numbers of organic matter to the nearby coastal water in the form of detritus; hence it is huge in energy and comprises both attached and free living microbial population (6). Mangroves are coastal wetland forests primarily instigate at the intertidal precincts of estuaries, backwater, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical latitudes (7). It is a connection flanked by terrestrial and marine ecosystem and docks exclusive microbial diversity which are present in coastal areas of tropical countries and maintain rich life over a diet cable that flinches with the trees and the microbiota (8). By backing in numerous stages of the putrefaction and mineralization of litter fall, sediment microorganisms play essential parts in it and create a vital support to the yield of the mangrove ecosystem (9,7). Bacterial counts are usually greater on attached mangrove vegetation than they are on fresh leaf litter owing to attached, undamaged leaves leak amino acids and sugars but do not release much tannin (10).

Overall bioactive compounds from marine source bacteria occupy 70% while other like fungi and other domains takes the rest of it (11). Marine environment is measured as one of the biggest resource for biodiversity and simultaneously it is the minimum established with respect to the task of specific biological function to genes, proteins and enzymes (12). Marine microorganisms has distinctive genetic structures and life habitats because of the environment it lived (13,14). Enzymes from marine microbes are discovered from several marine pedestals comprising mangrove, seagrass, coral feer, open sea, deep sea, coastal regions, estuarine, brackish water, lagoon and hydrothermal vent adittionally archae,bacteria, fungi and viruses also studied to blow the source of the marine world (15). Marine microbes have been accepted as latent source of unique enzymes owing to their enhanced stability, activity and tolerance to risky conditions (16,17). The enzymes secreted by marine microorganisms are differ from terrestrial because of difficulty of the marine environment include high salinity, low temperature and high pressure (18,19). 75% of industrially used enzymes like amylase, protease, lipase, cellulose and laccase are hydrolylic in nature (20).

Laccase enzymes belong to the family of blue multicopper oxidases, which were widely distributed in fungi, plants and insects (21). While in the study of degradation and synthesis of lignin biological part of fungal and plant laccases have shown (22). In recent times laccases have been found to be pervasive in bacteria (23,24). Of the described bacterial laccases, several found to hold sole properties, covering excellent activity and stability under alkaline conditions and high halide tolerance and these may be an additional for specific industrial applications of laccase enzyme (24-26).

Laccase have been revealed in a trivial amount of bacteria comprising *Bacillus subtilis*, *Bordetella campestris*, *Caulobacter crescentus*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Yersinia pestis* and *Stenotrophomonas maltophilia* (24, 27-31). Current work is focussed on difference in the population of laccase producing strain collected from mangrove soil at different depth and optimization of parameters for enrichment.

MATERIAL AND METHODS

Collection of sediment sample:

Sediment samples were collected at different depth (surface, 0.5, 1, 1.5 and 2 meters) from Velar estuary mangrove soil Parangipettai using sterile spatula. 10 gram of sediment from each sample was transferred to sterile polythene bag and kept in ice box. The samples were immediately transferred to laboratory for isolation and screening.

Matters of soil:

Soil sample were analysed to check the contents of soil at different depth. Visual observation was carried out to check the particles present in the soil.

Enrichment and isolation of bacteria:

Enrichment and screening method was adapted from Coll et al., 1993 (32) with slight modifications. 0.1 gram of sediment from each sample were transferred to nutrient broth supplemented with 0.2mM of $CuSO_4$ and 2 mM of guaiacol and incubate the broth at 37 ˚C for 48 hours for enrichment of laccase producing bacteria

Optimization of enrichment:

Enrichment for laccase production can be optimized by various parameters like incubation period, temperature, pH, copper sulphate concentration, guaiacol concentration and agitation.

Effect of incubation period:

To check the optimum incubation time for enrichment of culture flask containing broth was incubated for different incubation period (12, 24, 48, 72 and 96 hrs) after different incubation period the broth was serially diluted and plated into copper sulphate containing plates.

Effect of temperature:

To check the optimum temperature for enrichment, culture flask containing broth was incubated at different temperature (30, 35, 40, 45 and 50) at optimized incubation period. After incubating at different temperature the broth was serially diluted and plated.

Effect of Copper sulphate:

To check the effect of copper sulphate on enrichment of organism, broth was supplemented with different concentration of $CuSO_4$ (0.5, 1, 1.5, 2 and 2.5mM) was used and incubated with optimized

temperature and incubation period. After incubating at different temperature the broth was serially diluted and plated.

Effect of guaiacol:

To check effect of guaiacol on enrichment of organism, broth was supplemented with different concentration of guaiacol (0.5, 1, 1.5 and 2mM) was used and incubate with optimized parameters. After incubating at different temperature the broth was serially diluted and plated.

Effect of pH on enrichment:

To check the optimum pH for enrichment, culture flask containing broth was adjusted before inoculation to different pH ranges from 4 to 10 and incubated with optimized parameters. Then the broth was serially diluted and plated on copper sulphate containing nutrient agar plate.

Screening of potential strain:

Then the plate with optimized culture was incubated at 37 ˚C for 24 hours. After 24 hours the plates containing bacterial colonies were flooded with 1mM ABTS (2, 20-azino-bis-[3-ethyl benzothiazoline-6-sulphonic acid]). After addition of ABTS, colonies appear green in colour was the positive strain for laccase production.

Counting of colonies:

Plates containing colonies were subjected to counting to differentiate the laccase producing organism from total bacterial population.

RESULTS

Nature of soil:

S.no	SAMPLE(m)	COLOUR	NATURE	CONTENTS	pH	TEMPERATURE (°C)
1	Surface	Greenish brown	Sandy	Dry leaves, Shells, Living organisms and excretes.	7.81	28.7
2	0.5	Greenish brown	Semi sandy	Shells and dead organisms	7.86	28.5
3	1	Black	Muddy	Decayed organisms, mangrove leaves and sticks.	7.79	28.3
4	1.5	Black	Muddy	Some decayed matters	7.76	28.1
5	2	Black	Muddy	Bare soil	7.75	27.9

Isolation of laccase producing strain:

Samples collected from different depth (surface, 0.5, 1, 1.5 and 2 meters) were processed for isolation of laccase producing bacterial strain. From the five different depth sample, soil collected from surface containing excretes shown more bacterial population 4.3×10^5 CFU/mg, 3.8×10^5 CFU/mg, 4.1×10^5 CFU/mg, 3.6×10^5 CFU/mg and 3.9×10^5 CFU/mg in surface, 0.5, 1, 1.5 and 2 respectively. But when coming to laccase producing strain 2 meter shows maximum laccase producing colonies 4.7×10^1 CFU/mg and 4.3×10^1 CFU/mg, 3.1×10^1 CFU/mg, 2×10^2 CFU/mg and 2.2×10^1 CFU/mg for 1.5, 1, 0.5, and surface soil respectively. Laccase producing colonies turn to green colour after addition of ABTS.

Optimization of enrichment:

In enrichment, incubate the enrichment broth for 72 hours gives maximum number of laccase producing colonies (4.9×10^1 CFU/mg), optimum temperature for enrichment was 40 ˚C shows (5.1×10^1 CFU/mg) maximum positive colonies for laccase production. Optimum concentration of copper sulphate (0.5 mM) and guaiacol (2 mM) shows 9×10^2 CFU/mg and 4.9×10^1 CFU/mg respectively, optimum pH for enrichment was pH 7 shows 4.3×10^1 CFU/mg and enrichment broth which kept in the shaking condition gives maximum laccase producing population 4.6×10^1 CFU/mg, broth which kept without shaking gives less isolates.

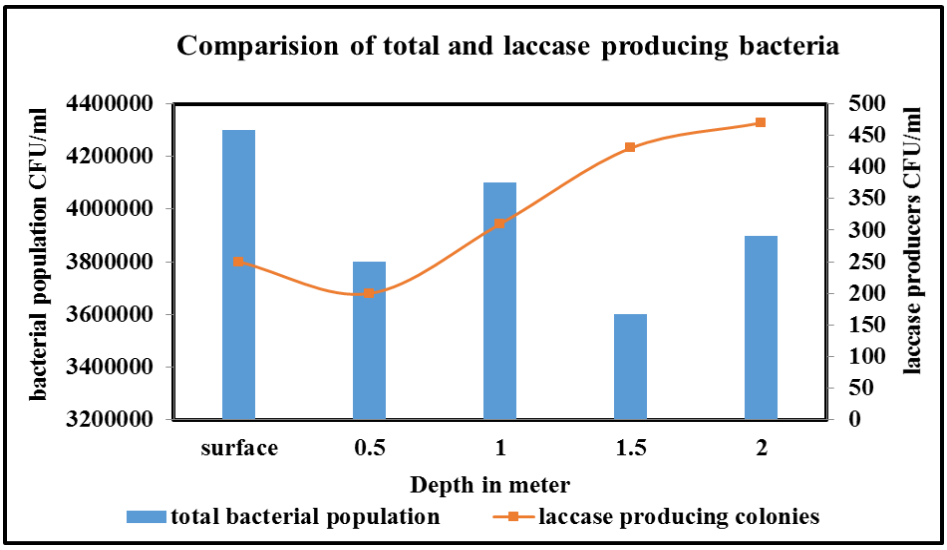

Figure 1: Comparison of total and laccase producing bacterial strain in different depth of mangrove soil. Maximum bacterial population was observed at surface collected soil and maximum laccase producing strain was shown at 2 meter depth.

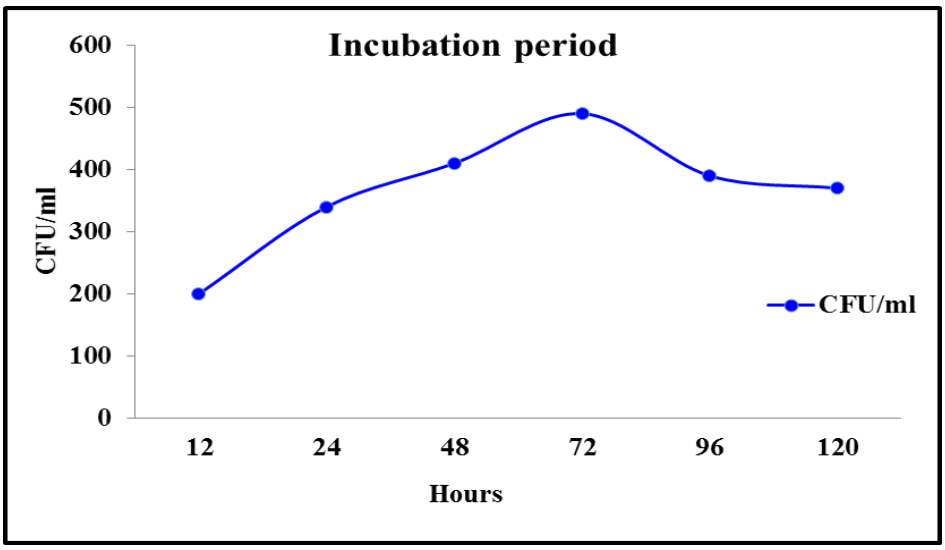

Figure 2: Optimization of enrichment at different incubation time. 72 hours of incubation show maximum laccase producing bacterial population.

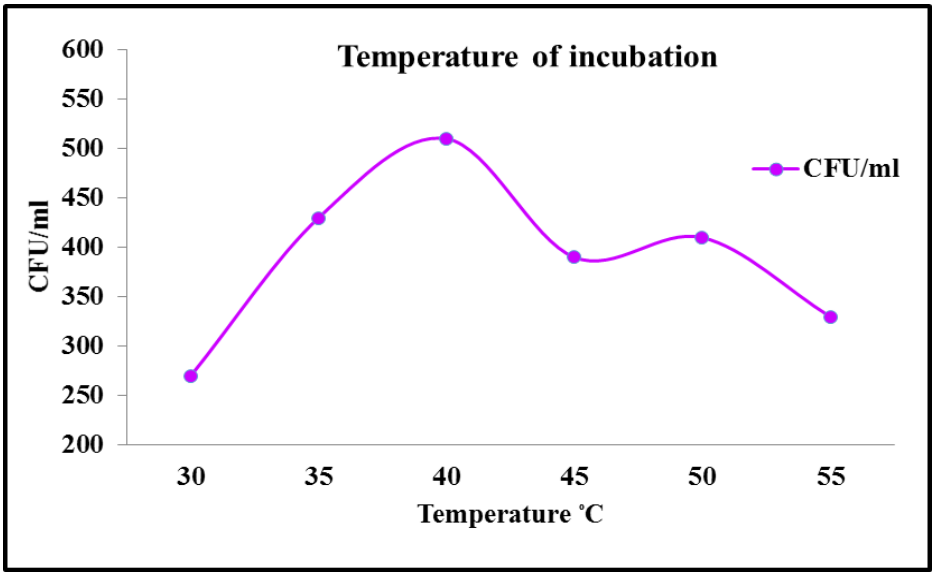

Figure 3: Optimization of enrichment at different temperature. 40 °C of incubation show maximum laccase producing bacterial population.

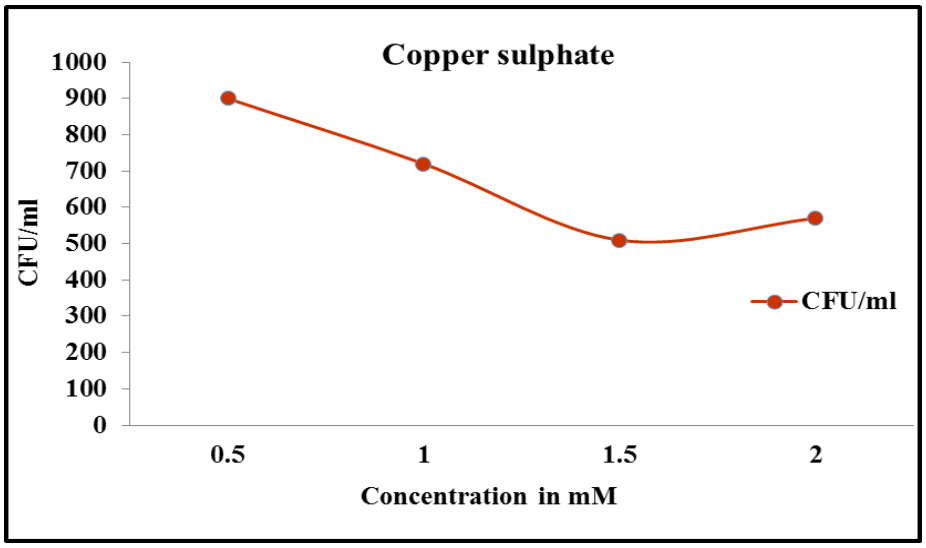

Figure 4: Optimization of enrichment at different concentration of copper sulphate. 0.5 mM copper sulphate shows maximum laccase producing bacterial population.

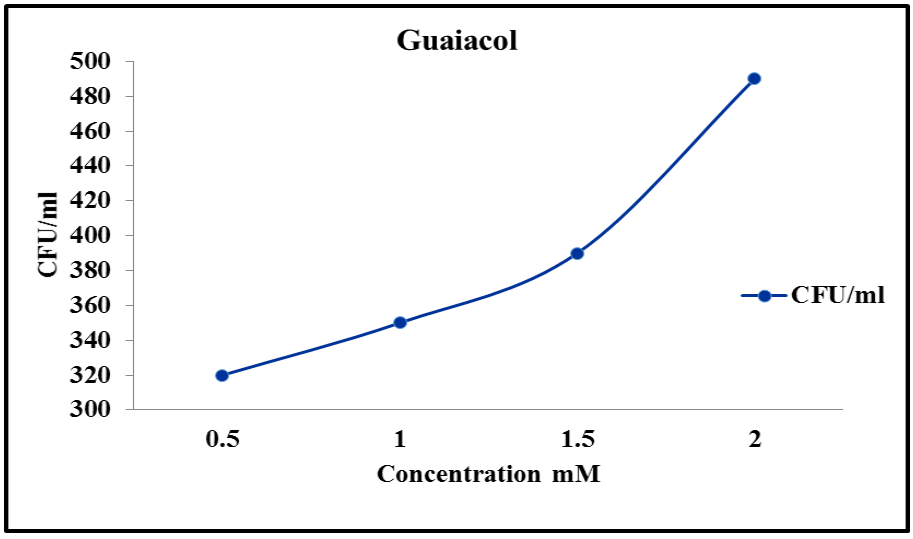

Figure 5: Optimization of enrichment at different concentration of guaiacol. 2 mM guaiacol shows maximum laccase producing bacterial population.

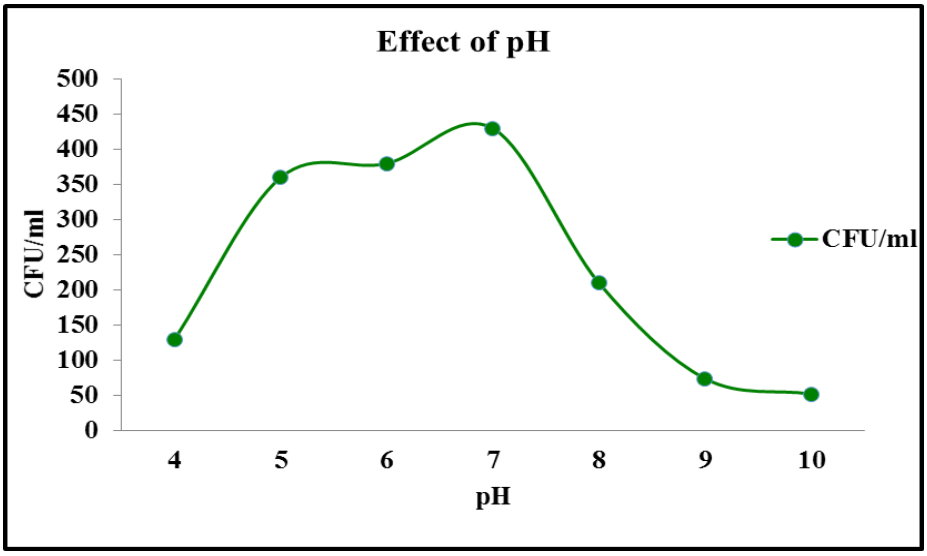

Figure 6: Optimization of enrichment at different range of pH. 7 pH shows maximum laccase producing bacterial population.

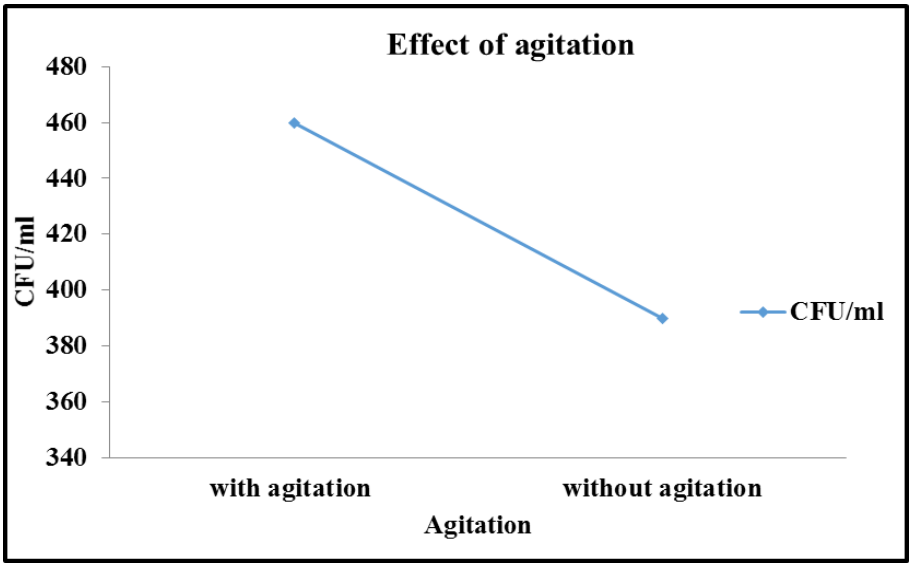

Figure 7: Effect of agitation on enrichment of broth culture incubated at shaking incubator showed maximum laccase producers.

DISCUSSION

In our study maximum laccase producing bacterial strain was attained at 72 hrs of incubation which was supported by Afreen et al., 2016 (33) in which higher laccase production was attained at 4th day of incubation. The time taken for laccase enzyme production largely depends on the lag phase and primary metabolism that varies with the media composition (34). This was supported by Singh et al., 2014 in which maximum laccase activity from bacillus sp. was shown on 72 hrs of incubation, 48 hrs in trametes versicolor (35). Muthukumarasamy et al., 2015 (36) in that maximum enzyme activity was attained at 96 hrs of incubation by using two substrate rice bran (267 ± 2.64 U/mL) and wheat bran (238 ± 3.26U/mL) and the production was declined at higher incubation time of 144 h.

Maximum population was observed at incubating culture at 40 ˚C and was correlated to Jetley et al., 2004 (37) report that optimum temperature for spirulina growth and enzyme production was 35 to 40 ˚C temperature above to that will affect both growth and enzyme production. In *Pleurotus, Dichomitus squalens* and *Bjerkendera adusta* optimum temperature for laccase production was 30 ˚C (38, 34). Farnet et al. 2000 (39) and Pointing et al. 2000 (40) reported that 25˚C to 50˚C found as optimal temperature in *Marasmius quercophilus strain and Pycnoporus sanguineus.*

Nature of the inducer for laccase production varies widely from species to species but in general aromatic and phenolic compound have been widely used to produce enhanced laccase production (41, 22). In present work 2 mM guaiacol shows maximum laccase producers which is supported by Hou et al., 2004 (42) Koroljova-Skorobogt'ko et al., 1998 (43) Patel et al., 2014 (44) enhanced laccase production was attained by supplementing 1mM guaiacol as inducer in *Phlebia spp., Pleurotus ostreatus, Coriolus hirsutus*. In other work reported by Mongkolthanaruk et al. 2012 (45) 8mM guaiacol acts as a good inducer in bacteria viz *Rhodococcus sp., Enterobacter sp., and Staphylococcus saprophyticus.*

Laccase are multicopper oxidases therefore containing, copper as micronutrients in media. The concentration of copper may increase or decrease the growth and production of organism, high copper concentration may decrease the growth of organism and suppression of enzyme (46). Copper is reported as a strong inducer for laccase production in fungus species *Trametes versicolor and P. chrysosporium* (47,48). In our study 0.5 mM copper sulphate shows maximum laccase producers as reported by Hao et al. 2007 (49) also found 2.0 mM of CuSO4 as optimal concentration of copper in *Pestalotiopsis sp*. but Palmieri et al. 2000 (50) found 150 µM of copper sulfate as the best concentration for laccase activity.

Thurston, 1994 (51) concluded that the effect of pH is limited in case of laccase enzyme production and also supported by Kuddus et al., 2013 (29) in that *Pseudomonas putida* showed maximum laccase activity at pH 8. Cordi et al., 2007 (52) reported that pH range of 3.0 to 8 will be the range for laccase when syringaldazine was used as substrate, in majority of work it is indicated that pH level were set at initial level but after inoculation most cultivation the level are not maintained (53). Current study reported that bacterial population was maximum in the broth with pH 7, our result supported by the Nyanhongo et al., 2002 (54) in

which laccase from *T. modesta* showed maximum activity in the range of 7 pH.

CONCLUSION

From this study it is concluded that laccase producing isolates present more number in muddy black colour soil which contain only decayed matter at the depth of 2 meters. Optimization of enrichment is same as that of optimization of production in that culture stored at 40 ˚C for 72 hours showed maximum laccase producing bacterial strain and enrichment broth with 0.5 mM copper sulphate, 20 mM guaiacol with pH 7. Broth kept in rotary shaker showed good enrichment compared with flask kept without agitation.

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