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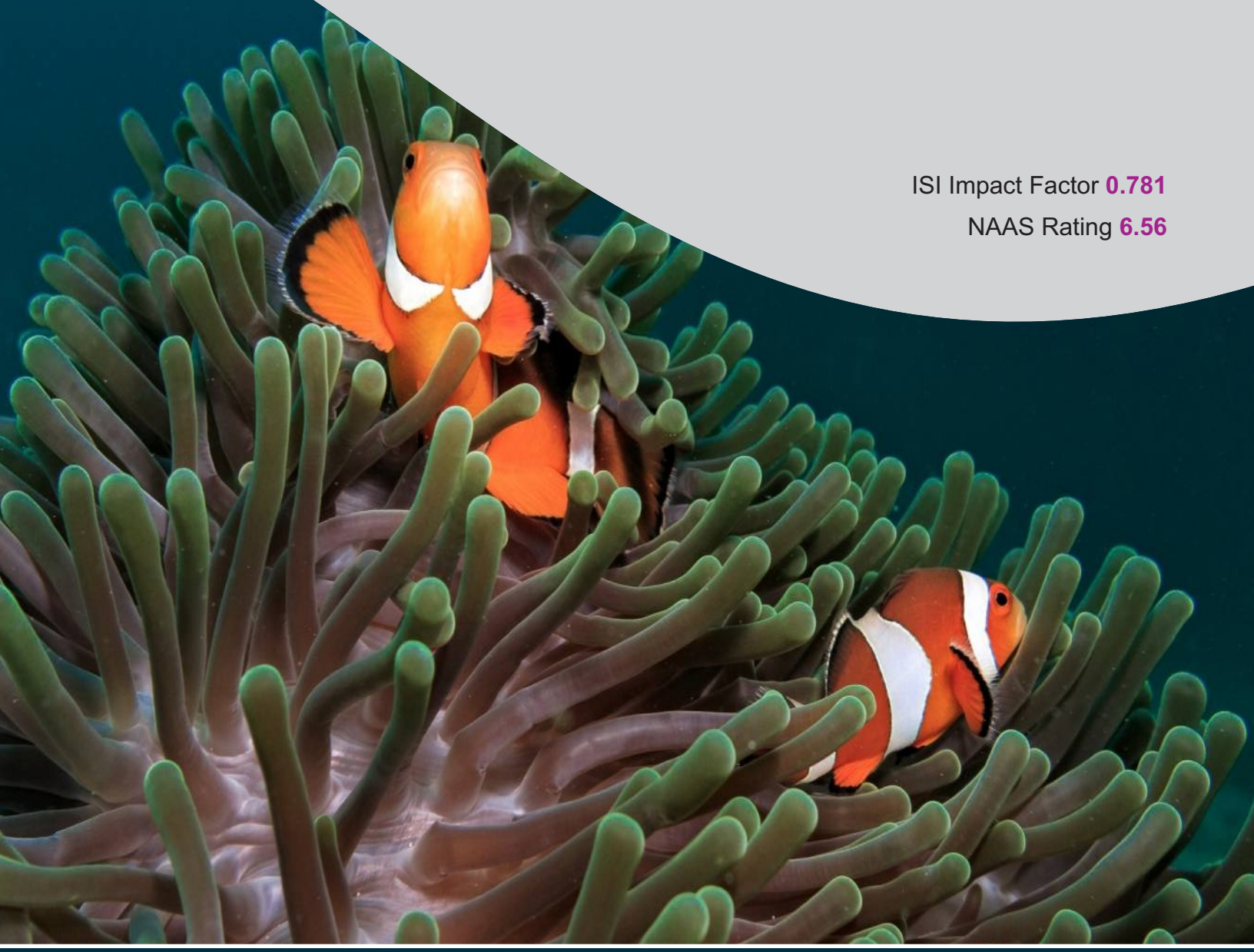

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Studies on delignification in jute (*Corchorus* spp L.) fibre with promising lignin degrading bacterial isolates

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Abstract

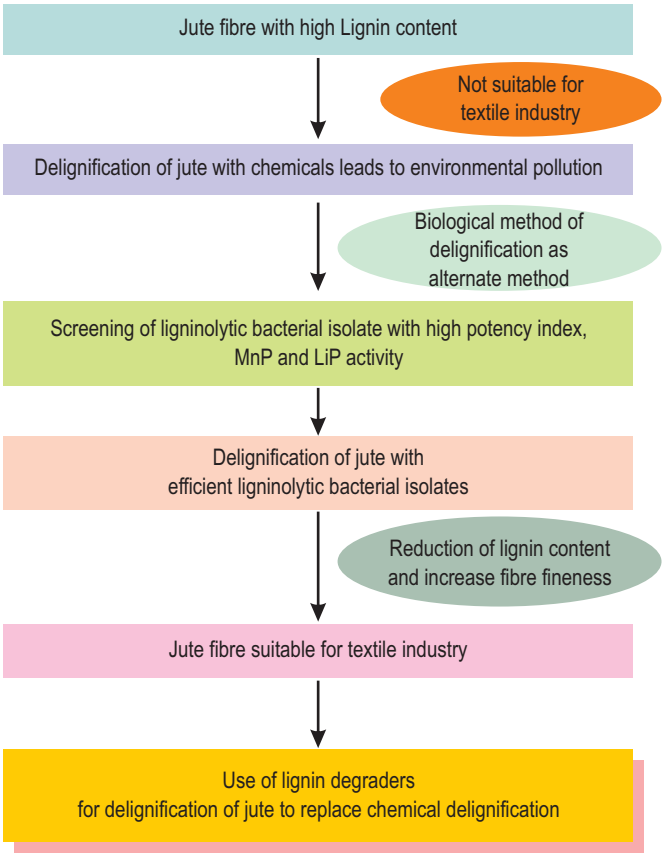
Aim: To investigate the reduction of lignin content in jute (*Corchorus spp.* L.) with promising lignin degrading bacterial isolates.

Methodology: Promising lignin degrading bacterial isolates were screened on the basis of potency index, MnP (manganese peroxidase) and LiP (lignin peroxidase) activities. Very efficient ligninolytic isolates were used for laboratory scale delignification trial and the resultant fibre was tested for lignin content, fibre strength and fineness. The efficient isolates were identified up to species level with Biolog Inc. based on the metabolic fingerprinting of the isolates.

Results: Out of 95 ligninolytic bactetial isolates, twenty isolates having potency index >1.10 on the basis of Azure-B dye degradation test were selected for enzyme assays. Five promising isolates (L3, L9, L10, L26 and L30) were selected for delignification trial on the basis of high MnP (126 – 482 U l^{-1} min^{-1}), and LiP (558.7 – 615.6 U l^{-1} min^{-1}) activities. The isolate L9 performed best among the five isolates and could reduce lignin content from 11.33 to 8.84% i.e. a reduction of 21.97% from the control. All the five isolates were identified as *Bacillus spp.*

Interpretation: Delignification of jute by using lignin degrading bacteria without any environmental hazard may be considered as an alternate method of chemical delignification for minimization of environmental pollution.

Key words: Delignification, High lignin, Jute, Ligninolytic bacterial isolates, Textile industry



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Introduction

Jute (*Corchorus capsularis* L. and *C. olitorius* L.) is a very important cash crop of eastern India, cultivated for fully biodegradable lignocellulosic bast fibres used for diversified jute products, geotextiles and paper pulp and to some extent in textile industries. Jute lignin is a complex polyphenolic polymer composed of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units in 2:32:66 ratio with a S/G ratio of 2.1 (Del Rio *et al*., 2009). The lignin content in jute fibre varies from 13.3 to 15% (Del Rio *et al*., 2009; Chakraborty *et al*., 2015; Sengupta and Palit, 2004), which is high as compared to other important bast fibres like flax and ramie having less than 5% lignin (Sengupta and Palit, 2004) and is extensively used in textile industry. Higher lignin content in jute fibre is restricting its use as a textile fibre. Lignin content in jute fibre has inverse relationship with fibre fineness (Meshram and Palit, 2013) and photo yellowing of jute products also occurs because of reaction of α-carboxyl group of lignin with UV-radiation (Chakraborty *et al*., 2015) making jute fibre unsuitable for fine quality fabrics and apparel production.

So, there is an urgent need for low-lignin containing jute fibre in jute based industries to enable them to produce high quality textile products. There are two possible ways for getting low lignin jute fibre. Development of low lignin jute variety by specialized breeding and genetic engineering techniques is one of the ways to get low lignin jute fibre, and the second way is delignification of jute fibre either by chemical or enzymatic action using lignin degrading microbes.

Bleaching of wood pulp to remove lignin, xylan by the paper industry, discharge of large amount of toxic chlorinated and aromatic compounds in the environment are the major source of environmental pollution (Shoham *et al*., 1992). Use of laccase and mediator system (LMS) for delignification of jute lignin showed that the molecular weight of lignin from LMS treated jute fibre was lower than the lignin from the control jute fibre which could guide the enzymatic process of ligno-cellulose materials in textile and allied sector (Zhang *et al*., 2014). Jute fibre is chemically modified for various diversified textile use, where jute lignin is either modified or partially degraded by hydrolysis with sulphuric acid leading to sulphonated lignin in the waste solution creating environmental hazard (Hussain *et al.,* 2002). In the traditional process, the lignin in jute fibre is eliminated mainly by degumming using some chemicals, which often causes severe environmental pollution (Kumar *et al.,* 2014*;* Kamali and Khodaparasat, 2015). In order to overcome the disadvantages of chemical degumming, biological method of delignification may serve as an alternative option with very few limitations (Huang *et al.,* 2013). It involves the use of microorganisms or immobilized microbial sub molecules such as enzymes, which is cheap and environmental friendly.

Fungal enzymes have limited industrial utility in relation to its protein expression, genetic manipulations and lack of stability under high pH, oxygen limitation and higher lignin concentrations (Crawford and Muralidhara. 2004)., whereas bacterial enzymes are relatively easy to produce. The studies on lignin degradation by using bacterial isolates and increase in production of ligninolytic enzymes of bacterial origin for delignification have been observed in recent years (Renugadevi *et al*., 2011). A pectinolytic bacterial consortium capable of secreting pectinase and xylanase enzymes was used as talc based microbial formulation (Das *et al.,* 2015; Das *et al*., 2018) and as endospores (Chattopadhyay *et al*., 2019) for jute retting was found to improve jute fibre quality but impact on delignification was not studied. Further, xylanase production by immobilized cells of *Bacillus pumilus* was 40.5 and 132.6 % higher over its free cells respectively after 48 and 72 hr of the incubation (Kundu and Majumdar, 2018). The ability to degrade lignin by bacteria is more advantageous compared to fungi such as the ability to adapt better in anaerobic conditions (Huang *et al*., 2013) and higher potential due to its environmental adaptability and biochemical versatility (Abd-Elsalam and El-Hanafy, 2009).

Ligninolytic heme-peroxidases including manganese peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP) of either fungal or bacterial origin play central role in delignification (Ruiz-Duenas and Martinez, 2009). The MnP and LiP oxidizes respectively the phenolic and non-phenolic component of lignin (Falade *et al*., 2016). Besides the oxidation of non-phenolic substrates, guaiacol, acetosyringone, catechol, vanillyl alcohol and syringic acid like phenolics are susceptible to the oxidative potential of LiP (Wong, 2009).

With the advantages of immense environmental adaptability and biochemical versatility, bacteria deserve to be studied for their ligninolytic potential. Therefore, this study aimed to screen, isolate and identify novel bacteria producing lignin degrading enzymes that have potential for delignification of ligno-cellulosic biomass of jute.

Materials and Methods

Collection of water samples and isolation of ligninolytic bacterial strains: The jute retting water samples were collected from various jute growing districts of West Bengal. Decayed material of fallen trees and soil samples were collected from the ICAR-CRIJAF campus and sawmills of North 24 parganas district. Enumerations of lignin degrading bacterial strains from the above collected samples were done on lignin agar media (lignin sulfonic acid Na salt- 1%; NaCl- 0.5%; $MgSO_4$- 0.02%; peptone-0.5%; KH_2PO_4- 0.05%; and agar- 1.8% at pH-7.5) following serial dilution technique and pour plate method (Parmer and Schmidt, 1966). The plates were then incubated at 37°C for 48 hr. The efficient ligninolytic isolates were selected and were separately streaked on fresh lignin-agar plates to obtain single bacterial colonies.

Screening of efficient ligninolytic bacterial isolates: Ligninolytic isolates were then grown on LBM medium (lignin modifying enzyme basal medium) composed of (KH_2PO_4- 1gl^{-1}; $C_4H_{12}N_2O_6$- 0.5 g l^{-1}; MgSO4.7H_2O- 0.5 g l^{-1}; $CaCl_2$.2H_2O- 0.01 gl^{-1}; yeast extract- 0.01 gl^{-1}; $CuSO_4$.5H_2O- 0.001 g l^{-1}; $Fe(SO_4)_3$- 0.001 gl^{-1}; $MnSO_4$.H_2O-0.001 g l^{-1}; 20% glucose solution-10 ml l^{-1} and 1.6% agar powder) containing 0.01% Azure-B to check their azo dyes degradation ability. Glucose solution was separately autoclaved and mixed with the medium just before plating. Inoculation was done by spot plate method, following the qualitative method of Pointing (1999). A clear hydrolysed zone around the colonies indicates the lignin degrading ability of the bacterial strains. The potency index of each isolate was calculated as the ratio of zone diameter to the colony diameter. The isolates showing higher potency index (≥1.10) were selected for further screening on the basis of their laccase activity. These bacterial isolates were again plated on LBM agar plates supplemented with 0.1% ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) to check their production capacity of extracellular laccase. The colourless ABTS agar medium turns green due to oxidation of ABTS to ABTS-azine in the presence of laccase, which is considered as a positive test for laccase activity (Niku-Paavola *et al*., 1990).

Enzyme activity assays: Bacterial isolates screened on the basis of their dye degrading ability were selected for enzyme assay. All the isolates were freshly inoculated in 10 ml of 0.5% lignin broth and grown for 18 hr at 130 rpm at 34°C. The starter cultures were then used as inoculum (5% V/v) into 25 ml of 0.5% lignin broth and incubated at 34°C with shaking at 130 rpm for 6 days. Aliquots were collected for 24-144 hr. After centrifugation of cultures at 10,000 rpm for 5 min, supernatants were used as the sole source of enzyme for lignin peroxidase (LiP) and manganese peroxidase (MnP) assays (Magalhaes *et al.,* 1996; Pease *et al*., 1991).

Lignin peroxidase activity: Lignin peroxidase activity was determined based on demethylation of methylene blue dye, where methylene blue acts as a substrate and enzyme LiP demethylates in the presence of H_2O_2 (inducer). The reaction mixture contains 0.1 ml of 0.1mM H_2O_2, 32 µM methylene blue as substrate, 10 µl of culture supernatant as enzyme and 1 ml of 50 mM sodium potassium tartrate (pH-4) buffer. The solution was incubated at room temperature for 1 hr. The absorbance was measured at 650 nm using an UV-visible spectrophotometer (Mecasys, Optizen). A test control (without culture supernatant) was run simultaneously. The amount of decolourization of methylene blue dye was calculated as decrease in absorbance at 650 nm l^{-1} min^{-1}.

Manganese peroxidase activity: A reaction mixture containing 25 mM lactate; 0.1 mM $MnSO_4$; 1 mg ml^{-1} bovine serum albumin (BSA); and 0.1 mg ml^{-1} of phenol red in 20 mM sodium succinate (pH 4.5) buffer in a total volume of 1 ml in which 0.5 ml culture supernatant was added. The reaction was initiated by adding 0.1 ml of 0.1 mM H_2O_2 at 30°C and was stopped after 1 min with 50 µl of 10% NaOH and the absorbance was measured with an UV-visible spectrophotometer (Mecasys, Optizen) at 610 nm. The control assays of phenol red oxidation in the absence of Mn^{2+} were carried out by omitting $MnSO_4$ from the reaction mixture for every isolate. The MnP activity was calculated and expressed as increase in absorbance at 610 nm l^{-1} min^{-1}.

Delignification trial with selected lignifying bacterial isolates: The bacterial strains selected on the basis of their potency index and enzyme producing ability (LiP and MnP), were used in a laboratory scale delignification trial with retted jute fibre to check delignification ability. These isolates were freshly inoculated in 100 ml of 0.5% lignin broth and grown for 18 hr separately. In each 2 l round bottom flasks, approximately 200 g of retted jute fibre was immersed in 1l of minimal media (peptone 5 gl^{-1}; glucose 10 gl^{-1}; KH_2PO_4 2 gl^{-1}; $MgSO_4$ 1 gl^{-1}; and NaCl 1 gl^{-1} at pH-7.5) and autoclaved. The flasks were then inoculated with the broth culture of selected bacterial strains (5% v/v) separately and incubated at 34°C for seven days. After incubation, fibre samples were washed and used for lignin and fibre quality estimation.

Estimation of lignin in jute fibre samples: Lignin content of retted fibres was estimated as acid detergent lignin (ADL) using FibreCap™ 2021 of the Fibertec™ 2021 system (FOSS Analytical A/S, Hilleroed, Denmark) as described by Kundu *et al*. (2012). Lignin content was estimated in jute fibre samples used for delignification study before and after delignification trial. A control was also run for each of the three replicates with untreated jute fibre to compare the amount of lignin reduction in treated ones.

Fibre quality analysis: Tensile strength of treated and untreated fibre samples was measured by the method of Roy *et al*. (2009) using an electronic fibre bundle strength tester and fibre fineness of samples were estimated by using the airflow method of Bandyopadhyay and Sinha (1968).

Identification of bacterial strains: The efficient ligninolytic bacterial strains were identified up to species level by using the Biolog Inc. (Hayward, U.S.A), an advanced tool for characterization and identification of microorganisms based on the metabolic fingerprinting pattern of the isolates using the software ML_51_01_ml3. Freshly grown microbial colonies were suspended in inoculating fluid and cell density was adjusted as per recommendation. A 100 µl bacterial suspension was then inoculated into GEN III micro plates of Biolog and incubated up to 22 hr at 33 ºC. The micro plates were read after 8, 16 and 22 hr of incubation by using the Biolog Inc, for identification.

Statistical analysis: The data were analysed for standard deviation by using Microsoft excel 2010 version.

Results and Discussion

The jute retting water samples, decayed plant materials and soil samples were used to isolate the ligninolytic bacterial isolates on lignin agar media. Ninety-five ligninolytic bacterial isolates were isolated initially from the collected samples. After that, the pure strains of these isolates were used to check for their lignocellulolytic enzyme activity through qualitative methods *i.e.* dye-degradation ability. Decolourization of dye Azure-B has been positively correlated with production of lignin peroxidase and Mn dependent peroxidase. Among the isolated strains, some isolates could degrade Azure-B dye by creating colourless or halo (hydrolized) zone around the bacterial growth on the Azure B agar plate (blue in colour). The area of dye decolourization was then recorded. None of the 95 isolates showed any green spot on colourless ABTS agar plate, thus indicating negative result which signifies no laccase production. On the basis of Azure B dye degradation test, twenty bacterial isolates showing potency index ≥ 1.10 were selected for enzymatic assay. Among these twenty isolates, four isolates had potency index more than 2 (Fig.1). The ligninolytic isolates L3, L9, L26 and L30 had potency index of 2.60, 2.49, 2.42 and 2.58 respectively. The potency indexes of these four isolates (L3, L9, L26 and L30) were much higher compared with the remaining ligninolytic isolates selected for enzyme assay. Screening of bacterial isolates on the basis of potency index for pectinolytic activities has also been reported by Das *et al*. (2015).

Twenty Azure B positive strains having higher potency index were then used for enzymatic assay. These isolates were grown in lignin broth media and the presence of manganese dependent peroxidase and lignin peroxidase enzyme was checked spectrophotometrically, starting from 24th hr of incubation till 144th hr. The enzyme production assay was carried out in triplicate and the mean value was taken for further interpretation.

The data presented in Table 1 indicates the manganese peroxidase activity by twenty ligninolytic isolates under study in different time intervals ranging from 24 to 144 hr of incubation. After 24 hr of incubation, only four isolates (L3, L10, L23 and L26) showed very low activities (1 to 3 U l^{-1}min^{-1}), but the remaining 16 isolates did not show any activity. All the isolates, except isolate no L75, showed manganese peroxidase activity after 48 hr of incubation, but isolate L75 did not show any activity even after 144 hr of incubation. The Mn peroxidase activities of isolates L3, L9, L10, L13, L15, L23, L26, L30 and L42 were increased with increase in incubation time up to 96 hr and thereafter the activities reduced. After 96 hr of incubation, the highest Mn peroxidase was recorded by the isolate L10 (482 U l^{-1} min^{-1}) followed by L26 (294 U l^{-1} min^{-1}), L9 (275 U l^{-1} min^{-1}), L3 (147 U l^{-1} min^{-1}) and L30 (126 U l^{-1} min^{-1}). Interestingly, the isolate L44 showed increasing Mn peroxidase activity up to 120 hr of incubation (102 U l^{-1} min^{-1}) and then maintained the same activity even after 144 hr of incubation. The remaining ligninolytic isolates (L47, L53, L62, L67, L71, L74, L75, L82, L87 and L89) did not show any definite trend of increase in activity and they did not show any activity after 120 and 144 hr of incubation. From the Mn peroxidase activity data, it was visible that the isolates L3, L9, L10, L26 and L30 recorded higher enzymatic activity besides recording higher potency index among the isolates under study. These promising ligninolytic isolates (L3, L9, L10, L26 and L30) with higher MnP activity were identified as *Bacillus spp*. In earlier studies, *Bacillus spp* with higher delignification activity were also reported by Huang *et al*. (2013) and Woo *et al*. (2014).

The lignin peroxidase activity of isolates L3, L9, L10, L13, L15, L23, L26, L30, L44, L62, L67 and L71 increased with time of incubation up to 96 hr, thereafter the enzyme activity of these isolates reduced at 120 and 144 hr of incubation (Table 2). Among these isolates, the highest lignin peroxidase activity of 615.6 U l^{-1} min^{-1} after 96 hr of incubation was recorded by the isolate L9 followed by L3 (571.2 U l^{-1} min^{-1}), L26 (566.2 U l^{-1} min^{-1}), L30 (560.7 U l^{-1} min^{-1}) and L10 (558.7 U l^{-1} min^{-1}). The lignin peroxidase activity of the isolate L9 after 96 hr of incubation was higher by 7.8 to 24.8% over all the isolates under study. The remaining ligninolytic isolates (L42, L47, L53, L74, L75, L82, L87 and L89) showed increase in lignin peroxidase activity up to 72 hr of incubation, thereafter the enzyme activity reduced with increase in incubation time up to 144 hr. Among these isolates, the highest lignin peroxidase activity after 72 hr of incubation was recorded by the isolate L75 (557.5 U l^{-1} min^{-1}) followed by L74 (552.0 U l^{-1} min^{-1}) and L42 (551.7 U l^{-1} min^{-1}). Five isolates viz. L3, L9, L10, L26 and L30 recorded higher lignin peroxidase activity after 96 hr of incubation, which also recorded higher manganese peroxidase activity and potency index among the 20 isolates under study. Jute lignin is a complex polyphenolic polymer which has major components like guaiacyl and syringyl. Phenolics like guaiacyl and syringyl are highly susceptible to oxidative potential of LiP (Wong, 2000), hence the isolates L3, L9, L10, L26 and L30 having higher LiP could play a crucial role in delignification of jute fibre. Further, these five promising isolates were identified as *Bacillus spp.* This is in agreement with the findings of Huang *et al*. (2013) and Woo *et al*. (2014), who had reported *Bacillus* spp. with higher delignification capacity.

On the basis of potency index and enzymatic activity (MnP and LiP), five promising ligninolytic bacterial isolates (L3, L9, L10, L26 and L30) were used for delignification study of jute fibre. After delignification study, the resultant jute fibre samples were processed and analysed for lignin content (Fig. 2). The lignin content data indicates that all the five ligninolytic isolates used for delignification study were found to decrease the lignin content in jute fibre compared with the control. This could be due to higher LiP and MnP activities of these five bacterial isolates, which are primarily responsible to carry out the delignification process (Ruiz-Duenas and Martinez, 2009). The delignification

Table 1 : Changes in manganese peroxidase activity of ligninolytic isolates at different time intervals

Ligninolytic isolates	Manganese peroxidase activity (U l^{-1} min^{-1})					
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr
L3	2 ± 0.5	7 ± 2	276 ± 16	147 ± 32	90 ± 2	69 ± 5
L9	0	5 ± 1	125 ± 13	275 ± 9	254 ± 14	57 ± 5.9
L10	3 ± 0.8	90 ± 0.8	13 ± 3.4	482 ± 14.4	10 ± 8	0
L13	0	3 ± 1	9 ± 4	77 ± 4	13 ± 2	2 ± 0.1
L15	0	3 ± 1	10 ± 2	33 ± 11	30 ± 3	25 ± 1.4
L23	1 ± 0.9	5 ± 0.8	9 ± 0.16	106 ± 17.4	18 ± 2.5	5 ± 0.4
L26	1 ± 0.5	8 ± 0.5	90 ± 13.9	294 ± 17.6	108 ± 11.8	97 ± 17.2
L30	0	6 ± 1	82 ± 11	143 ± 37	126 ± 0.011	113 ± 6
L42	0	4 ± 0.8	6 ± 1.2	7 ± 0.8	6 ± 1.2	5 ± 1.2
L44	0	3 ± 0.9	90 ± 4.9	94 ± 6.7	102 ± 12	102 ± 6.9
L47	0	2.3 ± 0.5	13 ± 1.2	1 ± 1.2	0	0
L53	0	2 ± 0.5	7 ± 0.9	2 ± 0.5	0	0
L62	0	4 ± 0.8	7 ± 0.8	0	0	0
L67	0	5 ± 0.1	8 ± 0.9	0	0	0
L71	0	0.3 ± 0.5	1.7 ± 0.5	0	0	0
L74	0	2 ± 0.5	4 ± 0.5	1 ± 0.8	0	0
L75	0	0	0	0	0	0
L82	0	2 ± 0.8	5 ± 1.2	0	0	0
L87	0	4 ± 0.8	7 ± 1.2	1 ± 0.9	0	0
L89	0	1 ± 0.8	3 ± 0.5	6 ± 0.8	0	0

Values (mean ± S.D.) in each row for a particular set

Table 2 : Changes in lignin peroxidase activity of ligninolytic isolates at different time intervals

Ligninolytic isolates	Lignin peroxidase activity (U l^{-1} min^{-1})					
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr
L3	485.5 ± 6.5	507.2 ± 6.3	539.8 ± 2.0	571.2 ± 2.2	516.2 ± 5.9	504.4 ± 7.5
L9	498.9 ± 8.9	522.3 ± 3.5	561.6 ± 6.6	615.6 ± 2.6	525.7 ± 8.8	522.9 ± 6.2
L10	500.3 ± 9.1	511.8 ± 12.8	527.8 ± 6.8	558.7 ± 2.1	534.7 ± 2.3	522.9 ± 2.6
L13	481.6 ± 5.3	495.5 ± 7.0	513.5 ± 1.2	516.6 ± 3.8	498.6 ± 2.8	491.2 ± 4.8
L15	490.4 ± 7.3	493.7 ± 2.9	527.6 ± 4.5	539.3 ± 1.9	526.0 ± 4.0	511.9 ± 3.0
L23	481.2 ± 3.9	500.5 ± 4.3	508.9 ± 5.3	521.8 ± 1.6	513.4± 5.1	502.8 ± 3.2
L26	478.9 ± 12.2	492.6 ± 9.5	506.1 ± 7.5	566.2 ± 5.5	533.9 ± 7.3	494.2 ± 5.6
L30	490.9 ± 8.4	499.8 ± 4.6	506.4 ± 7.8	560.7 ± 2.9	535.4 ± 10.0	509.5 ± 8.1
L42	492.5 ± 10.4	539.1 ± 10.6	551.7 ± 10.2	512.3 ± 11.2	504.8 ± 9.8	483.0 ± 11.2
L44	495.2 ± 13.1	501.7 ± 11.4	515.9 ± 10.4	525.4 ± 10.8	509.1 ± 9.2	504.9 ± 8.4
L47	484.4 ± 7.7	520.7 ± 8.1	547.9 ± 11.3	519.3 ± 11.7	510.8 ± 8.0	498.2 ± 9.0
L53	487.6 ± 13.3	503.7 ± 9.0	526.7 ± 12.4	493.2 ± 9.9	487.5 ± 7.6	476.2 ± 10.5
L62	496.0 ± 9.2	502.1 ± 9.6	521.7 ± 8.4	549.0 ± 9.2	523.0 ± 10.8	500.7 ± 15.0
L67	482.3 ± 8.3	509.6 ± 9.7	522.0 ± 7.1	529.8 ± 8.2	517.6 ± 8.0	491.9 ± 10.4
L71	510.0 ± 12.5	515.3 ± 7.4	521.9 ± 10.3	545.3 ± 18.0	526.4 ± 9.4	514.4 ± 10.6
L74	489.5 ± 10.5	513.4 ± 12.4	552.0 ± 10.5	508.4 ± 12.6	507.8 ± 14.0	497.0 ± 12.7
L75	493.7 ± 8.7	543.2 ± 10.7	557.5 ± 5.8	542.1 ± 7.3	529.5 ± 6.2	508.4 ± 8.9
L82	493.1 ± 14.1	512.9 ± 11.1	545.7 ± 11.1	528.3 ± 11.4	512.1 ± 13.8	503.0 ± 15.9
L87	479.9 ± 5.5	512.8 ± 9.9	545.1 ± 10.5	525.4 ± 10.0	521.3 ± 6.8	503.2 ± 11.6
L89	481.4 ± 8.5	515.0 ± 7.71	548.0 ± 11.7	529.7 ± 11.1	503.6 ± 9.3	507.4 ± 9.8

Values (mean ± S.D.) in each row for a particular set

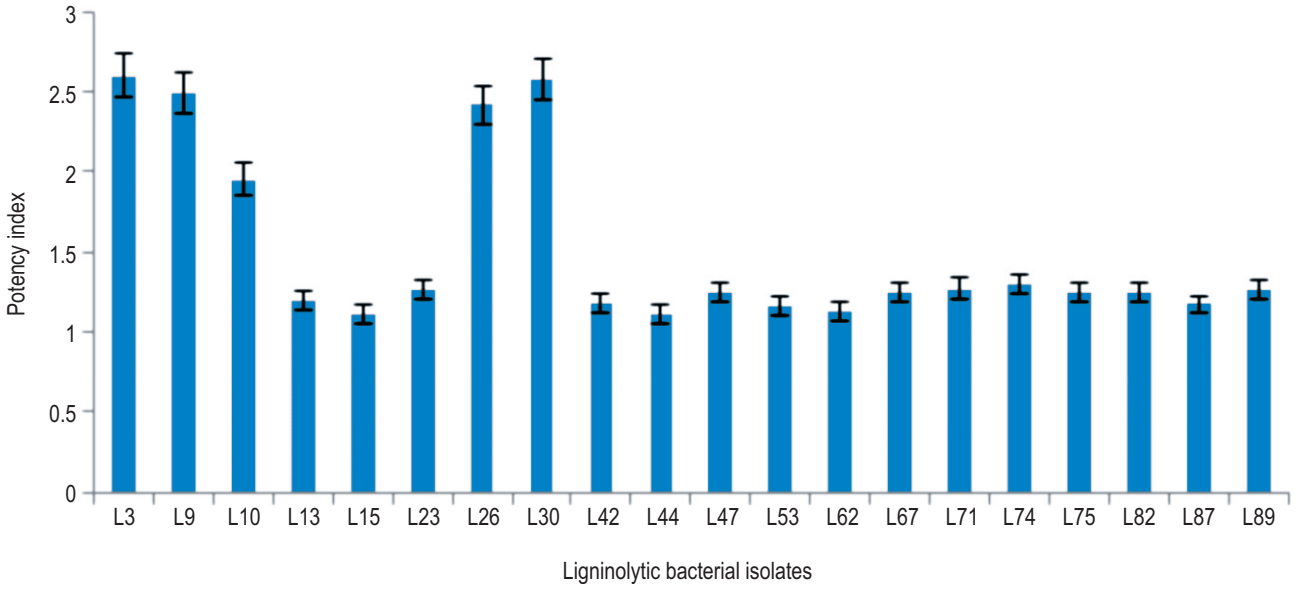

Fig. 1 : Potency Index of different ligninolytic isolates in Azure-B dye test.

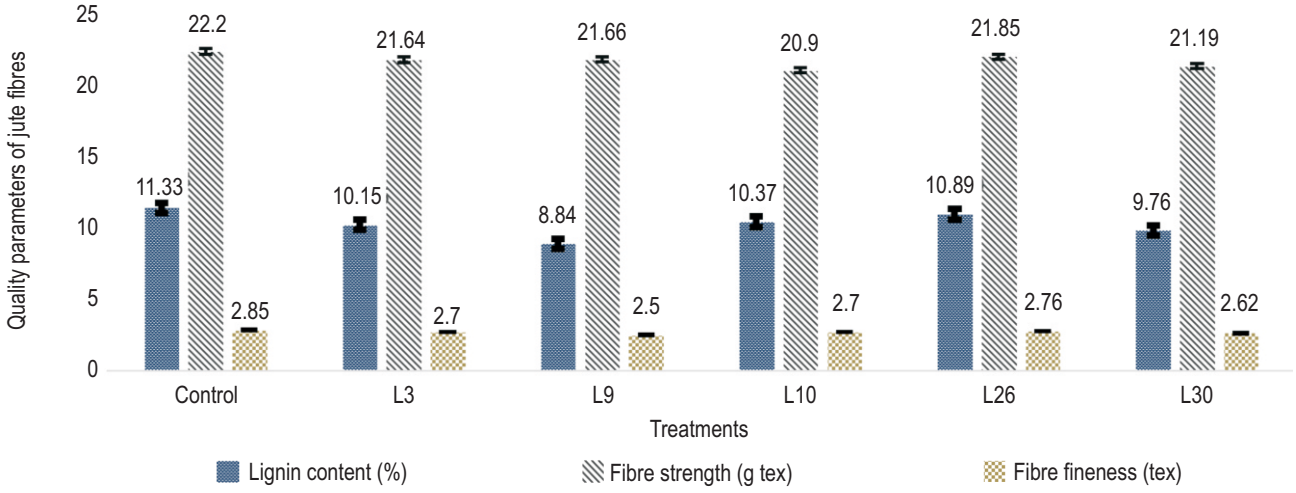

Fig. 2 : Effect of delignification with promising ligninolytic isolates on jute fibre quality.

trial of jute fibre was carried out at slightly alkaline pH (7.5) which might have helped in higher delignification as high S-lignin content in jute fibre is advantageous for delignification due to higher reactivity of S-lignin in alkaline system (Chang and Sarkanen, 1973; Tsutsumi *et al*., 1995). Among the five isolates, isolate L9 was the best performer in delignifying the jute fibre, which had reduced the lignin content from 11.33 to 8.84%, *i.e.* a reduction of 21.97% from the control. The isolate L3 also performed very well, which had reduced the lignin content in jute fibre from 11.33 to 9.76%, *i.e.* a reduction of 13.8% from the control followed by the isolates L3, L10 and L26. The isolate L9 was later identified as *Bacillus subtilis*. The higher delignification capacity of L9 might be due to high LiP (615.6 U l^{-1} min^{-1}) and MnP (275 U l^{-1} min^{-1}) activity which helped in the degradation of phenols like guaiacyl and syringyl present in jute lignin as these phenols are sensitive for degradation to the oxidative potential of LiP (Wong, 2009). Further, the combined action of MnP and LiP helped in oxidation of the phenolic and non-phenolic component of jute lignin respectively (Falade *et al*., 2016) resulting higher delignification of jute fibre. Hence, a reduction of 21.97% in lignin content of jute fibre was observed where fibre was treated for delignification with highly active ligninolytic isolate L9.

The fibre strength data presented in the Fig. 2 revealed that after delignification trial with five promising ligninolytic isolates, the fibre strength reduced marginally with all the isolates compared with the control. Among the isolates, the highest fibre strength (21.85 g tex^{-1}) was recorded with the isolate L9 followed by the isolate L30 (21.66 g tex^{-1}) and L3 (21.64 g tex^{-1}) which were lower than the fibre strength (22.20 g tex^{-1}) recorded in control. The fibre strength recorded in control was higher by only 1.6 % over the best isolate L9.

Interestingly, the fibre fineness increased (decrease tex value) with all the five ligninolytic isolates under study compared with the control (Fig. 2). The maximum increase in fibre fineness (2.50 tex) was recorded with the isolate L9 followed by isolate L30 (2.62 tex) compared with the control (2.85 tex). The lower fibre fineness value (tex value) indicates that the fibre fineness is higher; hence, the higher fibre fineness was recorded with all the ligninolytic isolates compared with the control after delignification study. Higher lignin value in jute fibre reduces the quality and commercial value of the fibre because of higher rigidity and roughness (Sharma, 1986), hence the reduction in lignin value in jute fibre with the ligninolytic bacterial isolates might have increased the fibre quality and spinnability of the fibre as indicated by higher fineness of the resultant fibre (Fig. 2). This higher fibre fineness recorded with all the five ligninolytic isolates correspond to decrease in lignin content in fibre because of delignification. Lowering the lignin content in jute fibre in the present study helped in increase in fibre fineness of jute fibre which is supported by the findings of Meshram and Palit (2013), where they have shown an inverse relationship between lignin content and fibre fineness in jute fibre. The reduction in lignin content of jute fibre by using ligninolytic bacterial isolates helped in increase in fibre fineness and the resultant fibre can also be utilized for textile purposes, due to higher spinnability with increase in fibre fineness (Grishanov *et al*., 2006).

The five promising ligninolytic bacterial strains used for delignification study were identified up to the species level by BIOLOG microbial identification system after 8, 16 and 22 hr of incubation. The isolates L3 and L9 were identified as two different strains of *Bacillus subtilis*, whereas the isolate L10 and L26 were identified as two different strains of *Bacillus amyloliquefaciens* and the isolate L30 was identified as *Bacillus licheniformis*. The bacterial isolates like *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus circulans* were also isolated and reported to help in biodegradation of lignin in compost environment (Tuomela *et al*., 2000) and 87% of the bacterial genera belonged to *Bacillus spp.* during thermophilic phase of composting (Strom, 1985). Hence the ligninolytic bacterial isolates of the present study can play an important role in delignification of jute fibre in near future.

It can be concluded from the present study that, by using efficient lignin degrading bacterial isolates delignification of jute fibre is possible to a great extent making the jute fibre suitable for textile purposes. Further, the environmental pollution from chemical process of delignification can be overcome by using the biological method of delignification using lignin degrading bacterial isolates in place of chemical delignification.

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