Efficient lignin decomposing microbial consortium to hasten rice-straw composting with moderate GHGs fluxes

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Abstract

We hypothesised that lignin decomposition microbial consortium would make rice-straw decomposition faster as straw contain around 15–24% lignin. In this study, we isolated lignin degrading microbes from four natural sources and based on their ability towards lignin degradation four microbial strains and their combination (2 bacteria (LB 8, LB 18) and 2 fungi (LF 3, LF 9) were selected for rice straw decomposition. During straw decomposition greenhouse gases emission, enzymatic activities (β -glucosidase, cellulase, laccase), reduction in lignin content, weight loss and carbon nitrogen ratio (C:N) were quantified. The β -glucosidase, cellulase and laccase activities were higher in LB 18 + LF 3 consortium as compared to others. The lignin content was also decreased (8.9–9.5 to 6.6–7.9%) continuously from initial to 28th days of composting under LB 18 + LF 3. We found the microbial consortium LB 18 + LF 3 decomposed the rice straw faster as indicated by reduction of C:N ratio and reduction of lignin, hemicellulose and cellulose contents of 60, 19.2, 41.5 and 10.3%, respectively at 28th day from initial compare to other strains/consortium. However, higher, CH_4 and CO_2 fluxes were observed at 28th days after composting (1.36 and $200.7 \text{ mg m}^{-2} \text{ h}^{-1}$) with no significant trend in N_2O flux. Further, the consortium identified could be tested for *in-situ* straw decomposition with proper moisture management to evaluate its potential in field condition. Therefore, we conclude that use of lignin decomposing microbial consortium has the potential to hasten the composting of rice straw in large scale, so viable option to reduce the menace of straw burning.

Introduction

India is the global prime producer of rice (*Oryza sativa*) accounting for about 20% of world rice production. In India 43.2 m ha of area is under rice cultivation. The main byproducts of rice are straw, rice husk and rice bran. Approximately, 760 million tons of rice straw produced per year globally which is the 1.5 times greater than per ton of rice-grain production (FAO 2017; Satlewal et al. 2018). The disposal of this surplus straw creates a major concern now a day in all the rice growing areas (Borah et al. 2016). Moreover, since last two decades farmers prefer to burn this straw to clear the field for the timely sowing of wheat in northern and north-western parts of India. Open field-burning of straw release a large amount of pollutants including methane and fine/ inhalable particles, toxic gases such as carbon monoxide (CO), carcinogenic polycyclic aromatic hydrocarbons and volatile organic compounds (VOCs) (Tipayarom and Oanh, 2007) which are responsible for various environmental pollution and human health hazards. Burning of rice straw emits 0.7–4.1g of CH_4 and 0.019–0.057g of N_2O per kg of dry rice straw and other gaseous pollutants such as SO_2 , NO_x , HCl, and to some extent, dioxins and furans (Oanh et al. 2011).

Rice straw is carbohydrate polymers with heterogeneous complexes. It is a lignocellulosic material which consists of three major biochemical compounds like, cellulose, hemicellulose and lignin. Cellulose is composed of glucose units, which are linked together by β -1, 4-glycosidic bonds in linear and crystalline forms. Hemicellulose is a hetero-polymer consisting of xylose, mannose, arabinose, galactose and glucose in short and highly branched chain. Lignin is made of the structurally complex-carbohydrate, consisting of various bio-chemically stable linkages having high molecular weight (Gupta et al. 2016). After cellulose, lignin is the second most abundant renewable biopolymer in nature. And it is also the most abundant aromatic polymer in the biosphere (Rahman et al. 2013).

Ex-situ rice straw composting is a good option in India to tackle the issues like straw-burning and soil health management simultaneously. The main bottle-neck of rice straw decomposition both in in-situ and ex-situ condition is higher lignin content (5–24%) takes more time for straw composting (Garrote et al. 2002). Therefore, microbes originally from natural sources and with higher lignin decomposing potential are an environment friendly and economic option for large-scale straw decomposition. Further, it is well known that the microbes produce higher ligninolytic enzymes that hasten the rice-straw composting. However, enzymes which degrading lignin are extra-cellular in nature and lignin having structurally heterogeneous and chemically more complex that cannot enter the cell for intracellular action (Rahman et al. 2013). Ligno-cellulolytic enzymes have substantial applications in various industries including chemicals, brewery and wine, food, fuel, textile and laundry, animal feed, paper-pulp and also in agriculture (Howard et al. 2003).

Decomposition of rice straw brings much needed organic manure to the soils that improves soil fertility as well as productivity. Incorporation of rice straw directly to the soil is certainly associated with immobilization of plant nutrients. High C: N ratio of straw and having considerable amount of silica, lignin make it difficult to decompose. Therefore, farmers favour *in-situ* burning of rice straw in the field causes great nutrient and economic losses. Rice straw burning also causes serious environmental problems which emits aerosols and greenhouse gases (Chang et al. 2013).

In our study we focused on the isolation of lignin degrading microbial isolates from natural lignin rich sources, such as elephant dung, dead plant-bark, vermicomposting pits and compost pits. After that most efficient bacterial and fungal strains as well as their

respective consortium were evaluated for rice straw composting. Therefore, objective of our study was to use the efficient lignin degrading microbial strains for rice straw decomposition in shorter time in eco-friendly manner.

Materials And Methods

2.1 Isolation and selection of efficient lignin decomposing microbial consortium

2.1.1 Sample collection

The samples were collected from four natural lignocellulosic sources like, elephant dung, dead plant-bark, vermicomposting pits and compost pits. The samples were collected in sterilized polythene bags and transported to the laboratory and immediately stored in refrigerator at 4°C.

2.1.2 Alkali lignin extraction

The lignin was extracted from the bark of the woody plant as described by Howard et al. (2003). The bark used for extraction of lignin was dried and grinded to powder and 10 g of powdered bark was taken in a beaker and 5 mL of 1% sulfuric acid was added to it. Then, it was heated in hot air oven at 80°C for 20 minutes. After heating, 100 mL of 4% sodium hydroxide was added and the suspension was boiled for 30 minutes. The dark brown colored alkali lignin was thus obtained, then it was filtered and autoclaved at 15 lbs for 10 min (Sasikumar et al. 2014). After autoclave, this soluble lignin was used as the selective media for isolation of lignin degrading bacteria.

2.1.3 Isolation of lignin degrading bacteria and fungus

The lignin degrading bacteria and fungus were isolated by using nutrient agar medium (NA) and potato dextrose agar (PDA), respectively in which lignin provided the sole carbon and energy source. In the medium, 1% alkaline lignin was added before autoclaving. Serial diluted collected samples were spreaded in respective media for lignin degrading bacteria and fungus from different sources. The bacterial plates were incubated at 37°C for 24 hrs, whereas the fungal plates at 30°C for 48hrs until colonies were developed. After incubation the colony forming units (CFU) were counted. These isolated bacteria and fungus of different morphology and characters were plated onto fresh nutrient agar (NA) and potato dextrose agar (PDA), respectively to obtain pure cultures (Rahman et al. 2013).

2.1.4 Lignolytic activity of isolated microbes

The microbial isolates were transferred to plats having methylene blue dye as an indicator for further screening. The microbes possess lignolytic enzymes undergoes oxidation of indicator dye. The isolated bacteria and fungus were streaked on methylene blue indicator dye (0.25 g L⁻¹) containing NA and PDA plates. The plates were incubated and placed under observation daily for growth and decolorization zone (Bondounas et al., 2011). The decolorized microbial colonies were processed for identification.

2.1.5 Efficiency test of the isolates

The efficiency test of the bacterial and fungal isolates to utilize lignin-substrate was performed in minimal salt media containing lignin (MSM-L). The MSM-L consisted of 1% alkaline-lignin minimum salt medium solution (K₂HPO₄ - 4.55g; KH₂PO₄ - 0.53g; MgSO₄-0.5g; NH₄NO₃ - 5ml in 1 litre deionized water) (Chandra et al. 2007). The isolates were inoculated in 50 mL broth and placed in shaker. Dinitrosalicylic acid solution (Ingredients, g L⁻¹; Di-nitrosalicylic Acid- 10 ml; Phenol- 2.0 ml; Sodium Sulphite- 0.5g; Rochelle salt (Potassium sodium tartarate) - 200g) was used for estimating reducing sugar. The glucose standard solution was prepared for this. The 2mL of culture solution was taken from each conical flask and was transferred to test tubes and then 3 ml of DNS solution and distilled water was added to each test tube and made up the volume to 10 ml. After that test tubes were heated 5 minutes in water bath and cooled down then the absorbance was taken at 540nm.

2.1.6 Identification of lignin degrading bacteria

Molecular identification of the efficient lignin degrading bacterial and fungal isolates were done by polymerase chain reaction (PCR) amplification of 16S rDNA and 18S rDNA, respectively. Amplified DNA were purified using a DNA purification kit (Promega, Madison, WI), and di-deoxy chain termination method was used for individual nucleotide sequences. The forward and reverse DNA sequences of

each were confirmed and compared by using database Basic Local Alignment Search Tool (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>).

2.1.7 Preparation of consortium followed by compatibility test

The most efficient two bacterial (LB 8, LB 18 (MN784664 & MN784667)) and two fungal (LF 3, LF 9 (MK855473 & MK855476)) strains were selected for rice straw decomposition. For preparing the consortium, at first compatibility test of selected microorganisms were done, because incompatibility of the co-isolates may also inhibit the growth of each other. After compatibility test, the bacterial and fungal isolates were inoculated into autoclaved nutrient broth (NB) and potato dextrose broth (PDB) separately. Then bacterial culture was incubated at 37°C for 24 hrs whereas, fungal culture at 30°C for 48 hrs. After incubation, consortiums were prepared by addition of both bacterial and fungal broth. The bacteria and fungus strains alone and their respective consortium were taken for composting of rice straw.

2.2 Evaluation of straw decomposition capabilities of the microbial consortium

An experiment was conducted to study the efficacy of the isolated strains of bacteria, fungus and their consortium for decomposition of rice straw. In this experiment, 27 numbers of 10 kg capacity pots (9 treatments × 3 replications) were taken. Nine treatments included 2 bacteria; 2 fungi; four bacteria-fungi consortium and one control (no microbial strain) were imposed in the experiment. In each pot 600gm of oven dried rice straw (2-3 cm size) were added. Then all the microbial broth with consortium was inoculated to different pots having straw. Optimum moisture content was maintained during the decomposition process (15-20%). The samples were collected during straw decomposition from different treatments at seven days interval for estimating the enzymatic activities (β -glucosidase, cellulase, laccase), lignin contents, C: N ratio and weight loss.

2.2.1 β -glucosidase activities

The β -glucosidase activities was estimated based on the released by the detection of p-nitrophenol (PNP) from p-nitro phenyl- β -D-glucopyranoside (PNG, 0.05 M) as substrate (Eivazi and Tabatabai, 1988). Briefly, 1g of sample from different treatments were added with 0.25mL of toluene, 4mL of MUB buffer (pH-6.5) and 1mL of PNG solution and mixed with in a 50mL Erlenmeyer flask. Then flasks were mixed properly, stoppered and incubated for 1 hour at 37°C. After that, 1 mL of 0.5M CaCl_2 was added and tris-hydroxymethyl amino methane (THAM) was added stop the reaction. Flasks were swirled for few second and the suspension was filtered through Whatman no. 41. The concentrations of the filtrates were measured at 420 nm on a spectrophotometer.

2.2.2 Cellulase activities

In order to estimate cellulase enzymatic activity, first 1 gm sample was taken. After that 10mL of 1% carboxy methyl cellulose (CMC) was added as substrate followed by 10 mL of acetate buffer (0.2M solution of acetic acid+0.2M solution of sodium acetate, pH-5.9). After that, the solution was incubated for 24 hours and the releasedsugar was estimated by di-nitrosalicylic (DNS) acid method (Miller 1959).

2.2.3 Laccase activities

Laccase activity of the samples were done by preparing the crude enzyme extract from moist soil (Songulashvili et al. 2007). First, 10 ml of distilled water was added to 1g of soil and shaken for 30 minutes. After shaking, the samples were filtered and centrifuged at 10000rpm for 15 min. Then, 25 μ L of the supernatant was taken and 1.5 mL of 0.2% ABTS in sodium acetate buffer was added. It was incubated at 30°C for 30 min. After incubation, absorbance was taken at 436nm by using spectrophotometer.

2.2.4 Cellulose, hemicellulose and lignin content

Ultrasound assisted alkaline extraction method was used for the estimation of cellulose, hemicellulose lignin content of samples collected from straw decomposition. The samples were collected at regular intervals during rice-straw decomposition were dried and grinded into powder. One gram of sample was taken in centrifuge tube. Then samples were treated with 2M NaOH solution, and there after it were placed in water bath at 90°C for 1.30 hrs. Then each tubewas placed in the ultra sonicator for 30 minutes. After sonication, those samples were centrifuged and the residue and filtrate were separated out. Then residue was washed with water for repeated times until clear or transparent filtrate was reached.After washing the tubes were placed in inverted position for 24hrs and the residues were transferred to petriplates and placed in hot air oven at 50-60°C for estimation of cellulose. After the cellulose was

collected, the filtrate was acidified with HCl to pH 5.5. Then it was precipitated with 90% ethanol solution (1:3:sample:ethanol). It was again centrifuged and residue and filtrate were separated out. Residues were washed with 70% ethanol solution and after washing the tubes were placed inverted position for 24 hrs and then similar to cellular estimation the residues were transferred to petriplates and placed in hot air oven at 50-60°C and hemicellulose content were obtained. Then filtrates were acidified with HCl to pH 1.5 and placed for evaporation of ethanol. Samples were centrifuged after evaporation of ethanol. The residues were washed with HCl (pH 2.0) and lyophilized. After lyophilization, lignin contents were obtained after weighing of tubes taking empty tube as control.

2.2.5 Estimation of carbon: nitrogen (C: N) ratio

Total carbon and nitrogen content were estimated by using the CN Analyzer (Model No. SNC100-IC, Skalar Primacs). The samples were air dried and grinded by using 2mm sieve. After that, 0.5g of samples were taken in the auto sampler.

2.2.6 Weight loss

At the beginning of the composting 600g of oven dried rice straw were taken in each pot. After adding constant amount of water in each treatment for moisture maintenance, the weight was measure. At an interval of seven days during composting, the weight loss of the compost was measured.

2.3 Greenhouse gas (GHGs) fluxes measurement

The gas samples were collected from different treatments at seven days interval by using plastic funnel chamber (with volume 5306.6 cm³ and area of 0.053 m²) fitted with rubber cork at the top. The funnel is air tight with the upper part of the pot (Figure 1). The gas was collected by using 50 ml syringe and measured the GHGs i.e. methane (CH₄), carbon dioxide (CO₂) and nitrous oxide (N₂O) in gas chromatograph (GC) (Model no. Trace 1110, Thermo Scientific). This GC was fitted with Porapak Q column and having flame ionization detector (FID) and electron capture detector (ECD) for measuring the GHGs concentrations. The GHGs fluxes were calculated by using this formula (Equations 1, 2, 3).

See formulas 1, 2, and 3 in the supplementary files.

Results

3.1 Isolation and identification of efficient lignin decomposing microbial consortium

3.1.1 Isolation of microbes from potential sources

In order to identify lignin decomposing microbial population, culturable bacterial and fungal population was isolated from potential natural sources like, elephant dung, vermicompost, compost, and dead plant bark by using nutrient agar (for bacteria) and potato dextrose agar (for fungi). Bacterial population (10⁻³ and 10⁻⁴ dilution) was highest in elephant dung followed by vermicompost > compost > dead plant bark (Table 1). Similarly, fungal population (10⁻² and 10⁻³ dilution) was also highest under elephant dung (5.4 and 5.2 Log cfu g⁻¹) and lowest in dead plant bark (5.1 and 4.9 Log cfu g⁻¹) (Table 1).

3.1.2 Screening and efficiency test of lignin degrading microbes

After screening by using methylene blue, total of 21 bacteria and 9 numbers of fungal isolates were selected. The efficiency tested of lignin decomposing bacterial isolates were estimated by measuring the quantity of glucose through DNS method produced during decomposition process. The sugar production was increased from 1st days of incubation to 7 days and then it was declined. Compared to all the bacterial isolates, LB 18 and LB 8 degraded the lignin more on each days of incubation. The highest sugar was produced by LB 18 (154.8 µg ml⁻¹) and LB 8 (149.9 µg ml⁻¹) at the 7th days of incubation (Figure 2a). However, the glucose production of the fungal isolates was increased from initial days of incubation and was observed maximum at 9th days of incubation. Therefore, the most two efficient fungi were selected for further study i.e. LF 3 and LF 9, which produced 171.3 and 147.0 µg ml⁻¹ respectively (Figure 2b).

3.1.3 Morphological characterization of the isolates

After the conformation test, all the bacterial and fungal isolates were morphologically evaluated (Table 2). The sequences of selected efficient microbial strains were registered to NCBI. The NCBI accession numbers of LB 8, LB 18 and LF 3, LF 9 were MN784664, MN784667 and MK855473, MK855476, respectively.

3.2 Evaluation of efficient microbial consortium for rice-straw decomposition

The efficient bacterial (LB 8, LB 18), fungal (LF 3, LF 9) strains and their consortium were tested in net house condition for evaluating their efficacy of decomposition. The efficacy was tested by quantifying the enzymatic activities, weight loss, reduction of lignin content and decrement of C:N ratio during the composting.

3.2.1 Enzymatic activity

β -glucosidase activities

The β -glucosidase activities were increased in the treatments where microbial consortium were used up to 28th days after composting (DAC) and then steadily decreased up to 42th DAC in all the treatments (Figure 3a). In 28th DAC, the β -glucosidase activities were ranged from 423.3 to 630.1 $\mu\text{g PNP g}^{-1} \text{ h}^{-1}$ in the treatments. The higher β -glucosidase activities were observed under the microbial consortium, LB 18+LF 3 (290.4 $\mu\text{g PNP g}^{-1} \text{ h}^{-1}$) followed by LB 8+LF 3 (280.3 $\mu\text{g PNP g}^{-1} \text{ h}^{-1}$) than the other treatments.

Cellulase activities

Similar to β -glucosidase activities, the cellulase activities were higher at 28th DAC and then decreased up to 42th DAC in all the treatments (Figure 3b). The average cellulase activity throughout the decomposition process, was also higher in LB 18+LF 3 (64.7 $\mu\text{g glucose g}^{-1} \text{ h}^{-1}$) followed by LB 8+LF 3 (62.8 $\mu\text{g glucose g}^{-1} \text{ h}^{-1}$) than the other treatments and least in control (without microbial inoculation) (45.4 $\mu\text{g glucose g}^{-1} \text{ h}^{-1}$).

Laccase activities

The laccase activities were ranged from 4.9 to 73.8 $\mu\text{g g}^{-1} \text{ h}^{-1}$ in all the treatments (Figure 3c). Higher laccase activities were observed at 28th DAC followed by 21st DAC. At 28th DAC, higher laccase activities were observed in LF 18+LF 3 (73.8 $\mu\text{g g}^{-1} \text{ h}^{-1}$) as compared to other treatments and least in control (14.04 $\mu\text{g g}^{-1} \text{ h}^{-1}$).

3.2.2 Cellulose, hemicellulose and lignin content

The initial content of cellulose, hemicellulose and lignin of the rice straw were 39.4, 20.4 and 9.3%, respectively. After inoculation of microbes and their consortium to the rice straw, the cellulose, hemicellulose and lignin content was decreased significantly to 18.4, 17.8 and 7.2%, respectively at 28th DAC (Figure 4a, b, c).

3.2.3 C: N ratio

The C and N contents were ranged from 45.6 to 52.6% and 0.71 to 0.82%, respectively at initial date of composting and 31.8 to 36.1% and 1.27 to 1.42%, respectively at 28th DAC (Table 3). Similarly, the C: N ratio were reduced from initial day of composting (60.0 to 67.3%) to 28th DAC composting (24.8 to 27.7%) in all the treatments. Moreover, highest degree of reduction of C: N ratio was found at 28th DAC in the consortium LB 18+LF 3 (60%) as compared to control treatment.

3.2.4 Weight loss

Weight reduction were significant from initial to 28th DAC (59.5 to 64.3%) and after wards (68.0 to 77.0%) at 42nd DAC. Highest degree of weight reductions (26.8 to 36.1%) were found t 28th DAC and higher reductions were observed in LF 8+LF 3 (253.1%) followed by LF 18+LF 3 (220.4%) consortium than control (Figure 5).

3.3 The GHGs fluxes during composting

The GHGs fluxes were measured during rice straw composting in all the treatments. The CH_4 fluxes increased from 7th DAC to 28th DAC and then decreased (Figure 6a). Maximum CH_4 flux was observed at 28th DAC in all the treatments. At 28th DAC, the CH_4 flux was

ranged from 0.11 to 1.36 mg m⁻² h⁻¹. Higher CH₄ flux was observed in LB 18+LF 3 treatment (1.36 mg m⁻² h⁻¹) followed by LB 8+LF 3 (1.13 mg m⁻² h⁻¹). Higher CO₂ flux was also observed at 28th DAC followed by 14th DAC (Figure 6b). At 14th and 28th DAC the CO₂ fluxes were in the range of 37.9 to 191.9 mg m⁻² h⁻¹ and 84.5 to 201.4 mg m⁻² h⁻¹, respectively. However, there was no significant differences were observed in N₂O fluxes (Figure 6c).

Discussion

Huge amount of lignocellulosic rice straw is generated worldwide have the potential to reuse for bioethanol, biochar and pulp production (Bhattacharyya et al. 2020; Huang and Lo 2019). Lignin is a polymer and high molecular weight that gave it rigidity and resistant against decomposition by different kinds of microorganisms, (Wang et al. 2011). However, lignin at the same time causes hindrance for making compost or the value-added product due to their slower decomposition rate. Therefore, microbial decomposition is a viable option to make use of this resource (rice straw) for better alternatives in eco-friendly manner. White-rot and brown-rot fungi are the well-known for lignin-degradation by generating extracellular oxidative enzymes. White-rot fungi like *Ceriporia lacerata*, *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Cyathus stercolerus*, *Pleurotus streausand* *Pycnoporus cinnabarinus* have been found effective for decomposing different lingo-cellulosic biomass (Shi et al. 2008). It was reported that, the fungi have higher potential but sometimes natural or extreme environmental conditions and substrate restriction is not suitable for lignin degradation (Sharma et al. 2014). Another school of thought that the bacteria, in particular are better microbes having immense environmental adaptability and biochemical versatility for lignin degradation (Maki et al. 2009; Dini et al. 2018). A variety of bacterial species have been reported to be able to degrade lignin and cellulosic materials such as *Bacillus*, *Pseudomonas* and *Acintobacteria* (Maki et al. 2011); *Bacillus*, *Clostridium* (Wang et al. 2011), and *Acintobacteria* (Woo et al. 2014). So there is a potential to use microbial consortium using both fungi and bacteria for eco-friendly decomposition of rice straw for cleaning up environments and to use the value added end products like, bio-compost, biofuel, lignin for producing dyes, paper pulp and generating silica.

Lignin protects cellulose, hemicelluloses and other cell wall constituents in rice straw for easy decomposition but a few microorganisms can act on complex structure of lignin by secreting extra cellular enzymes. Ligno-cellulolytic fungi have benefits in bioconversion of solid waste as they are filamentous and produce high-volume of spores (Datta et al. 2017). Mixed microbial cultures (bacteria and fungi) have greater effect on substrate accumulation through resistance to environmental contamination and increased enzyme production (de Lima et al. 2016). Compatibility of microbial consortium is another important aspect which influences the distribution, density, association and ecological steadiness of communities (Mishra and Malik 2014; Martínez-Sanz et al. 2014; Vázquez et al. 2015). Thus, a better compatible microbial consortium might have plays an important part for hastening rice straw bio-conversion. In our experiment, the lignin degrading bacterial and fungal strains were isolated from the natural sources like, elephant dung, dead plant-bark, vermicomposting pits and compost pits. We got two most efficient bacterial (LB 8, *Bacillus cereus*; LB 18, *Enterobacteriaceae bacterium*) and two fungal (LF 3, *Penicillium sp.*; LF 9, *Alternaria alternata*) isolates, which was used for rice straw composting. These isolates alone and their consortium were used in rice straw composting. Hence isolation of the lignin degrading microbes from natural lignin reach sources is effective due to higher lignin content. The elephants are herbivorous and their dung is reach in lignin. Similarly, the dead plant bark is slowly degraded by the lignin decomposing microbes. Hence, there is a possibility to find efficient isolates from this lignin reach sources.

Composting of agricultural organic wastes through bio-augmentation of biodegradable microbes is an efficient process (Sánchez-Monedero et al. 2010; Maeda et al. 2010; Yamamoto et al. 2011; Sharma et al. 2014; Wang et al. 2016). In recent years, social demand for economic and eco-friendly decomposing technology of rice straw have drawn attention of researcher. The sustainable resources for the production of biofuel have also increased interest because of cost effectiveness and readiness in the recent past (Chen and Dou 2016). Among them, agricultural byproducts are the emerging source of renewable and eco-friendly raw materials which provide substitute resource biomass for facilitation of high demanded woody materials (Liu et al. 2013; Chen and Dou 2016). As rice straw is a renewable agricultural residue and rich in nutrients, resources, hence it can also be resolve the future energy requirements (Zuroff and Curtis 2012). Recycling of organic matter through agriculture residues (rice straw) also highly adopted methods (Antil and Raj 2012).

Greenhouse gases emission could also increase with low C: N ratio of substrate biomass during this biological composting process (Jiang et al. 2011). Biomass burning is a global phenomenon which responsible towards air pollution in worldwide (Yang et al. 2008). Globally, due to high carbon density, forest burning is the major source and agricultural biomass burning (approximately 2020 Tg) is reported the second for the carbon loss, emission of GHGs and other air pollutants (Crutzen and Andreae 1990; Andreae et al. 2001;

Jain et al. 2014). In that context, compost is a valued source for agricultural soil due to its local availability as a rich source of nutrients (Khaliq et al. 2006). In nature, frequency of bio-conversion of rice straw is slow and it takes more time to composting in natural field micro-flora due to moisture limitation (Brethauer and Studer et al. 2014). Increase use combine-harvester further aggravated the problem of *in-situ* straw decomposition by spreading the straw all over the field. Many a times these cause field burning of straw on large scale. In those context, *ex-situ* decomposition of rice straw through lignin degrading microbial inoculation is an effective way to hasten the composting process with ecofriendly manner. The practice of lingo-cellulolytic microorganisms inoculation has been widely used as an worthy approach that could potentially hasten up the decomposition (Zeng et al. 2010; Okeke and Lu 2011) and ultimately improve the quality of compost (Zeng et al. 2009; Wang et al. 2011). Hence, microbial decomposition is an effective, environmentally sound alternative for the recycling of straw (Groyal and Sindhu 2011; Meena et al. 2016).

Carbon sources for energy transformation to microbes during composting, and CO₂ emission as by product are the indicators of microbial activity and composting stability (Li et al. 2017). In our study, initially the CO₂ emission was more (maximum at 28th DAC followed by 14th DAC) and then reduced. The practice of fresh rice straw incorporation to the soil significantly increases methane emissions, whereas decomposed straw emits relatively less emissions (Corton et al. 2000; Bhattacharyya et al. 2012, 2013; Gang et al. 2019).

Inadequate *in situ* straw decomposition in relation to GHGs emission data comparing with different microbial consortium are available. In addition, emissions from rice straw composting processes should be combined with lignin decomposing enzymatic activities to assess the whole process of composted manures. Similar to CO₂, the CH₄ fluxes increased from 7th DAC to 28th DAC and then decreased in our study. Applying of rice straw compost in the rice field reduced GHGs emission as compared to straw incorporation and open-field-straw burning (Table 4). As an aerobic process, composting converts organic substrates into a humus-like material which impedes methanogenesis and increases CH₄ oxidation (Chen et al. 2010). As the organically generated compost application is gradually decreasing, hence agricultural soil is largely depend upon straw recycling to get rid of straw burning menace and restore soil carbon eventually better soil health. Although burning of straw in the field condition ensures the quick land preparation to subsequent crops and also restricts the nitrogen immobilization menaces during straw decomposition with wider C/N ratio, yet incomplete carbon-combustion generates large amounts of GHGs and adversely affects the air quality (Abdel-Rahman et al. 2016; Hussain et al. 2015). Zschornack et al. (2011) indicated that CH₄ and N₂O emission decrease by 69 and 81%, respectively in surface retention when compared to straw incorporation in rice fields (Hussain et al. 2015). Apart from this, open straw burning is an unrestrained combustion practice, in which CO₂, N₂O, CH₄, CO, other hydrocarbons (NMHC), NO_x, SO₂, particulate matter (PM) were also emitted to the atmosphere. It is estimated that quantity of annual rice straw open burnt (13.92 Tg) in India would represent about 15% of the total amount of crop residues (84 Tg) (Streets et al. 2003). On the other hand, applying the rice-straw-compost could reduce the CH₄ emissions from irrigated paddy fields compared with other fertilizers uses at the same time sustain the yield. Therefore, composting of rice straw by use of efficient lignin degrading microbial consortium is an intelligent step towards mitigating GHGs emission and combat with the menace of straw burning.

Conclusion

Isolation of lignin decomposing microbial strain from natural sources like elephant dung, dead plant-bark, vermicomposting pits and compost pits found effective for rice straw decomposition in faster time i.e. 28 days. Combination of fungus (LF 3, *Penicillium sp.*; LF 9, *Alternaria alternata*) and bacteria (LB 8, *Bacillus cereus*; LB 18, *Enterobacteriaceae bacterium*) for hastening *ex-situ* rice straw decomposition is more effective than single microbial inoculation. Production of β -glucosidase, cellulase and laccase during decomposition were positively correlated with better quality compost (suitable C: N ratio, lignin and cellulose content). However, quicker decomposition relatively triggers higher CO₂ and CH₄ fluxes, but if we take the process as a whole, it could be balanced. Future endeavor should be in the direction of *in-situ* straw decomposition in faster time with this valuable microbial consortium in order to curb the menace of on-field straw burning.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

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Tables

Table 1: Lignin degrading bacteria and fungus population of different lignin reach natural sources.

Natural sources	Bacteria		Fungus	
	10 ⁻³	10 ⁻⁴	10 ⁻³	10 ⁻⁴
Compost	5.9±0.05	5.6±0.05	5.2±0.02	5.0±0.05
Vermicompost	6.0±0.05	5.8±0.03	5.3±0.03	5.1±0.02
Elephant dung	6.2±0.02	6.0±0.03	5.4±0.03	5.2±0.02
Bark composing soil	5.6±0.05	5.3±0.1	5.1±0.04	4.9±0.04

Table 2: Morphological characterization and NCBI Accession number of the efficient lignin degrading bacterial isolates

Lignin degrading bacterial isolates								
Isolates	Source	Colour	Elevation	Margin	Consistency	Shape	Accession number	Organism
LB 8	Vermicompost	White	Flat	Entire	Non gummy	Irregular	MN784664	<i>Bacillus cereus</i>
LB 18	Elephant dung	White	Flat	Entire	Non gummy	Regular	MN784667	<i>Enterobacteriaceae bacterium</i>
Lignin degrading fungal isolates								
Isolates		Source	Colour		Accession number		Organism	
LF 3		Compost	Whitish		MK855473		<i>Penicillium sp.</i>	
LF 9		Dead plant bark	Blackish		MK855476		<i>Alternaria alternata</i>	

Table 3: Carbon, nitrogen content and C: N ratio during rice straw composting under different treatments.

Treatment	DAC	LB 18	LB 8	LF 3	LF 9	LB 18 + LF 9	LB 8 + LF 9	LB 18 + LF 3	LB 8 + LF 3	Control
Carbon content (%)	0	48.2 ± 0.94	47.6 ± 0.93	49.5 ± 0.97	45.6 ± 0.89	50.4 ± 0.99	52.6 ± 1.03	48.4 ± 0.95	46.7 ± 0.92	49.8 ± 0.98
	7	46.3 ± 0.91	45.8 ± 0.90	48.1 ± 0.94	43.6 ± 0.85	49.3 ± 0.97	51.4 ± 1.01	47.1 ± 0.92	45.2 ± 0.89	49.2 ± 0.96
	14	43.7 ± 0.86	42.6 ± 0.83	46.3 ± 0.91	42.4 ± 0.83	45.8 ± 0.90	48.2 ± 0.94	43.3 ± 0.85	42.2 ± 0.83	48.6 ± 0.95
	21	39.4 ± 0.77	36.7 ± 0.72	40.2 ± 0.79	36.3 ± 0.71	41.7 ± 0.82	42.9 ± 0.84	37.5 ± 0.73	36.4 ± 0.71	46.2 ± 0.91
	28	35.4 ± 0.69	34.6 ± 0.68	35.7 ± 0.70	31.8 ± 0.62	36.1 ± 0.71	39.1 ± 0.77	34.8 ± 0.68	33.2 ± 0.65	45.5 ± 0.89
	35	32.7 ± 0.64	31.6 ± 0.62	34.7 ± 0.68	30.7 ± 0.60	33.2 ± 0.65	36.4 ± 0.71	31.1 ± 0.61	31.3 ± 0.61	42.8 ± 0.84
	42	31.2 ± 0.61	30.6 ± 0.60	32.6 ± 0.64	29.1 ± 0.57	30.6 ± 0.60	33.5 ± 0.66	29.9 ± 0.59	27.4 ± 0.54	41.2 ± 0.81
Nitrogen content (%)	0	0.74 ± 0.01	0.71 ± 0.01	0.82 ± 0.02	0.76 ± 0.01	0.82 ± 0.02	0.76 ± 0.01	0.74 ± 0.01	0.75 ± 0.01	0.74 ± 0.01
	7	0.82 ± 0.02	0.86 ± 0.02	0.94 ± 0.02	0.84 ± 0.02	0.86 ± 0.02	0.84 ± 0.02	0.87 ± 0.02	0.82 ± 0.02	0.79 ± 0.02
	14	1.04 ± 0.02	1.01 ± 0.02	1.12 ± 0.02	1.06 ± 0.02	1.12 ± 0.02	1.06 ± 0.02	1.08 ± 0.02	1.05 ± 0.02	0.82 ± 0.02
	21	1.24 ± 0.02	1.18 ± 0.02	1.32 ± 0.03	1.21 ± 0.02	1.37 ± 0.03	1.34 ± 0.03	1.24 ± 0.02	1.28 ± 0.03	0.87 ± 0.02
	28	1.36 ± 0.03	1.27 ± 0.02	1.39 ± 0.03	1.28 ± 0.03	1.42 ± 0.03	1.41 ± 0.03	1.33 ± 0.03	1.32 ± 0.03	0.92 ± 0.02
	35	1.48 ± 0.03	1.41 ± 0.03	1.43 ± 0.03	1.38 ± 0.03	1.52 ± 0.03	1.47 ± 0.03	1.38 ± 0.03	1.46 ± 0.03	0.98 ± 0.02
	42	1.52 ± 0.03	1.47 ± 0.03	1.48 ± 0.03	1.54 ± 0.03	1.61 ± 0.03	1.54 ± 0.03	1.55 ± 0.03	1.56 ± 0.03	1.06 ± 0.02
C:N ratio	0	65.1 ± 1.28	67.0 ± 1.31	60.4 ± 1.18	60.0 ± 1.18	61.5 ± 1.20	69.2 ± 1.36	65.4 ± 1.28	62.3 ± 1.22	67.3 ± 1.32
	7	56.5 ± 1.11	53.3 ± 1.04	51.2 ± 1.0	51.9 ± 1.02	57.3 ± 1.12	61.2 ± 1.20	54.1 ± 1.06	55.1 ± 1.08	62.3 ± 1.22
	14	42.0 ± 0.82	42.2 ± 0.83	41.3 ± 0.81	40.0 ± 0.78	40.9 ± 0.80	45.5 ± 0.89	40.1 ± 0.79	40.2 ± 0.79	59.3 ± 1.16
	21	31.8 ± 0.62	31.1 ± 0.61	30.5 ± 0.60	30.0 ± 0.59	30.4 ± 0.60	32.0 ± 0.63	30.2 ± 0.59	28.4 ± 0.56	53.1 ± 1.04
	28	26.0 ± 0.51	27.2 ± 0.53	25.7 ± 0.50	24.8 ± 0.49	25.4 ± 0.50	27.7 ± 0.54	26.2 ± 0.51	25.2 ± 0.49	49.5 ± 0.97
	35	22.1 ± 0.43	22.4 ± 0.44	24.3 ± 0.48	22.3 ± 0.44	21.8 ± 0.43	24.8 ± 0.49	22.5 ± 0.44	21.4 ± 0.42	43.7 ± 0.86
	42	20.5 ± 0.40	20.8 ± 0.41	22.0 ± 0.43	18.9 ± 0.37	19.0 ± 0.37	21.8 ± 0.43	19.3 ± 0.38	17.6 ± 0.34	38.9 ± 0.76

Table 4: Comparison GHGs emission from different straw management process.

Straw management	Seasonal/ Annual/ days	CH ₄	N ₂ O	CO ₂	GWP (CO ₂ equivalent)	Unit	References
Rice straw incorporation	Annual	440	0.71	2903	-	kg ha ⁻¹	Liang et al., 2007
	Annual	790	0.39	545	-	kg ha ⁻¹	Koga and Tajima, 2011
	Seasonal	107.1	-	-	-	kg ha ⁻¹	Hou et al., 2013
	Seasonal	77	1.8	-	-	kg ha ⁻¹	Romasanta et al., 2017
	Seasonal	115.4	0.84	-	-	kg ha ⁻¹	Bhattacharyya et al., 2012
Rice straw burning (India)	Annual	16,253	0.8	13	-	Gg	Gadde et al., 2009
Composting	Rice straw	90 days	333	-	-	mg g ⁻¹	Goyal and Sindhu, 2011
		45 days	-	-	83	kg t ⁻¹	Duan et al., 2019
		28 days	37.8	2.34	8647	mg kg ⁻¹	In this study
	Garden waste	Annual	-	-	-	kg t ⁻¹	Andersen et al., 2010
					3038		

Figures

Figure 1

Greenhouse gas collection and measuring the GHGs concentration during rice straw composting.

[Note: LF= lignin degrading fungus; LB= lignin degrading bacteria]

Figure 2

Lignin decomposition efficiency test of different (a) bacterial and (b) fungal strains isolated from different lignin rich natural sources.

[Note: LF= lignin degrading fungus; LB= lignin degrading bacteria; DAI= date after inoculation]

Figure 3

(a) β-glucosidase and (b) cellulase (c) laccase activity of the rice straw at different days after composting (DAC) by using bacterial and fungal strains alone and also their respective consortium.

[Note: LF= lignin degrading fungus; LB= lignin degrading bacteria]

Figure 4

(a) cellulose (b) hemicellulose and (c) lignin content of the rice straw at different days after composting (DAC) by using bacterial and fungal strains alone and also their respective consortium.

[Note: LF= lignin degrading fungus; LB= lignin degrading bacteria]

Figure 5

Weight reduction during rice straw composting in all the treatments.

[Note: LF= lignin degrading fungus; LB= lignin degrading bacteria]

Figure 6

(a) Methane (CH_4) (b) carbon dioxide (CO_2) and (c) nitrous oxide (N_2O) flux measurement at different days after composting (DAC) by using bacterial and fungal strains alone and also their respective consortium.

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