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Cell Wall Hydrolase Producing Microbial Consortium Modulates Soil Carbon Dynamics with its Ensuing Effect on Productivity of Wheat under Straw Incorporated Conditions

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Abstract

Abundance and improper disposal of agricultural residues often leads to loss of biomass resources and environmental health. Straw is the major residue of rice-wheat cropping system and a potential source of soil and plant nutrients. However, natural decomposition of rice straw is slow owing to its structural composition lignocellulose and silica), therefore, often left unutilized regardless of different management practices. To subdue this problem an elite alternative would be to manage rice straw in the field itself. So, the present work emphasizes on microbial bioconversion of rice straw into soluble nutrients through the action of cell wall hydrolases. Henceforth, selection and characterization of efficient cellulase and laccase producing microbes; and consortia development using compatible microbes was targeted for speeding the decomposition process under straw incorporated field conditions. The selected microbial consortium Cons16; comprising Phanerochaete chrysosporium, Trichoderma harzianum, Trichoderma asperellum and Bacillus sp.) was subjected for evaluation of its decomposition efficacy and the concomitant effects on soil health and carbon dynamics along with the growth of subsequent wheat crop. Soil carbon dynamics studied in terms of total carbon; methane and carbon dioxide emission depict carbon sequestration in treatment with Cons 16. Our inimitable findings showed that apart from distinct improvement in soil physicochemical and biological properties increased yield of wheat was observed.

Keywords: Rice straw; Microbial consortia; Biodegradation; Straw incorporation; Soil carbon dynamics

Introduction

Asian countries contribute to almost 80-90% of world's rice cultivation with subsequent production of bulk straw. Rice straw contains high amount of silica, cellulose, hemicellulose, and lignin content 6, 27, 33 and 36% respectively). However, due to high lignocellulosic component, it shows slower rate of decomposition resulting in prolonged degradation time period. Straw is either burnt or incorporated in-situ) in fields by farmers. Incorporation method is usually not adopted by farmers due to delayed decomposition of rice straw and subsequent losses to wheat production; rather they choose on-farm stubble burning. Farmer's choice of on-farm stubble burning cause huge carbon losses >213.15 Tg C) from biomass ~40% of straw); thus, adding to greenhouse gas load of the environment. This practice although much criticized yet not subsided, and farmers still retort to straw burning due to i) shortage of labor and high labor wages, ii) high cost of residue removal by transportation Pathak, iii) mechanized harvesting lack of proper residue recycle technology Singh and Kaskaoutis [1].

Crop residue CR) act as slow-release nutrient source which gradually mineralize soil nutrients for plant and microbial uptake Chen. Ability of crop residue in improving soil C sequestration and SOC has been illustrated by many workers Pathak. Moreover, the potency of agriwaste like straw) to provide different nutrients has also been extensively reported Singh. Soil incorporation of CR in soil has been reported to improve physical, chemical, and biological properties of soil with further stimulation of soil enzymes and microbial population Tejada and Benítez. However, its direct incorporation cause nitrogen immobilization and reduced seed germination; prolonged degradation due to lignin and silica content; reduced productivity and increased gas emissions. Composting of agricultural residues through the action of ligno-cellulolytic microorganisms is one of the up-coming technologies to manage and recycle the agriwaste. It is one among the direct approaches to manage straw burning just by the application of composted organic matter from plant residues to the soil. Soil its health, structure, and stability) is the vital component of sustainable agriculture. Intense use of chemical fertilizers to endure higher food demand results in deterioration of soil health Kumar. Soil carbon content is one among the important indicators of soil quality which is declining gradually. However, different measures pertaining to use of organic manure and incorporation of agriwaste have been adopted to improve soil quality. Among different agriwaste, rice straw being an abundant and economic source of nutrients can serve as safe alternative to chemical fertilizers for restoration of soil fertility. Rice straw, rich in carbon, when incorporated to soil would not only solve the problem of straw management, but also reduce carbon losses and environmental pollution due to burning. Straw incorporation mediated CO_2 exchange measured in terms of soil respiration builds positive correlation with

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soil organic carbon. Direct incorporation of straw in soil cause nitrogen immobilization and reduced seed germination; prolonged degradation due to lignin and silica content; reduced productivity and increased gas emissions [2]. Therefore, demand for exploitation of economical, convenient and environment friendly methods for efficient utilization of rice straw necessitates the utilization of potential microbes which aid in faster decomposition of incorporated straw. Microbial activities would not only bring about the straw assimilation but also boost the soil physical, chemical, and biological status which was otherwise affected due to continuous cropping.

Microbes have great potential to improve the soil health with the use of rice straw as compost. Decomposition of the complex biomass releases the assimilated nutrients into an available form through intervention of microorganisms. Some useful bacteria Azotobacter chroococcum, Azospirillium brasilense, Bacillus megaterium and Bacillus circulans) and fungi Trichoderma viride, Phanerochaete chrysosporium) are known for CR decomposition). Addition of rice straw along with potential microbes improve physical, chemical, and biological properties of soil and increase crop productivity. Despite the vast potential, there is insufficient knowledge about the impact of microbial processes for efficient decomposition of rice straw and its effect on soil health and productivity of the subsequent crop. Therefore, the study aims to develop an effective rice straw decomposing microbial consortia with better yield of subsequent crop wheat. The objectives were screening microbes i) for production of hydrolase enzymes ii) for better soil health iii) for better productivity and iv) development of consortia with all above attributes for soil health and productivity [3].

Methods

Characterization of microbes for production of cell wall hydrolases

Microbes were screened for high cellulolytic and lignolytic property as described earlier Teather and Wood. Efficient strains were selected by quantitative estimation of cellulolytic and lignolytic enzymes by growing them in Czapex dox broth. To determine the cellulolytic activity, the culture extract after 48h of incubation) was subjected to carboxymethyl cellulase enzyme quantification using the DNS 3, 5-dinitrosalicylic acid) method Miller, 1959). In brief, 900 µl of 1% CMC prepared in 10 mM sodium phosphate buffer pH 7.0) was added to 100 µl culture supernatant and incubated at 40°C for 60 min. The reaction was terminated with the addition of 1.0 ml 3, 5-dinitrosalicylic acid reagent and 2.5ml distilled water. The reaction mixture was boiled for 15 min in boiling water bath and was cooled rapidly. Development of orangish-red colour signifies the enzyme activity and was measured against a reagent blank at 540 nm. A control was also run along with the test which contained distilled water instead of culture extract. The concentration of glucose released by enzyme was determined by comparing against a standard curve constructed similarly with known concentrations of glucose. One unit of enzyme activity is defined as the amount of enzyme that liberates 1 µg of glucose per minute under the assay conditions.

Laccase, indicator of lignolytic enzyme activity, was determined by growing them in Czapex dox broth. The culture supernatant 100µl), after 48h of incubation, was mixed with 100µl ABTS 10mM) and 800µl sodium acetate buffer in test tubes, followed by its incubation at 28˚C for 30min. Furthermore, the absorbance of enzyme activity was measured at 420nm [4]. The control set was also run along with the test which contained distilled water in place of culture. One unit of enzyme activity is the amount of laccase which oxidize 1 µmol of ABTS per minute.

Development of fast decomposing microbial consortia through microcosm experiments

Microbial potential for fast decomposition of straw was evaluated through the microcosm in-vitro) experiment performed in polythene bags. The sterile moistened rice straw was inoculated with the selected microbes alone and combination). The bags were incubated at 40°C and the experiment was run for 1 month; straw decomposition was determined in 15 and 30 dpi samples by estimation of cellulase, laccase enzyme activities and nutrient release Si, P and sugar). Briefly, for FPase assay the straw extract was mixed with equal volumes of sodium citrate buffer 0.05M; pH 4.8) and one filter paper strip 1cm x 1cm) followed by incubation of reaction mixture at 50°C for 1h. In CMCase, 1 volume of straw extract was mixed with 9 volumes of substrate CMC prepared in 10mM sodium phosphate buffer, pH 7) and reaction mixture was incubated at 50°C for 30min. Both the reactions were stopped with the addition of DNS and mixture was boiled for 15-20min [5]. On cooling, distilled water was added volume makeup) and mixed well followed by its absorbance at 540nm against blank. Further consortia of microbes were characterized for faster decomposing potential through ex-situ and in-situ method piling heaping) and litterbag burying of mesh bags)

Methods

In the piling method, there were four layers formed with moistened straw, each inoculated with microbial formulation cow dung used as carrier material). In the litterbag method, moistened rice straw 40% moisture, ~3 inches in size; 300g in each) inoculated with microbial formulation was filled in mesh bags. The mesh bags were buried two bags/pot) in pots 12×8") filled with soil. The treated straw, after 60 days post inoculation dpi), was screened for saccharification, textural changes, mass reduction, microbial activity, and lignin decomposition [6].

Evaluation of cell wall decomposition under microcosm conditions

Cellulose degradation

Cellulosic breakdown in rice straw was determined in terms of endoglucanase, exoglucanase and β glucosidase enzyme activities. Methods for endoglucanase CMCase) and exoglucanase FPase) have been described earlier. β glucosidase activity in straw extract was determined according to the method of Dietz. In brief, straw extract was mixed with sodium citrate buffer 100mM, pH 4.8) and PNG p-nitrophenyl- β-D-glucopyranoside) substrate prepared in dimethyl formamide 200mg/ml). The reaction mixture was incubated for 1 hour at 37°C and terminated with the addition of sodium carbonate 200mM). Absorbance of end-product p-nitrophenol was measured at 405nm. Total sugar was measured as described earlier. Dehydrogenase activity under both the conditions was measured according to the standard method of Alef &Nannipieri as described earlier. Straw mass reduction was measured after 2 months of experiment as per the formula [7],

$$\frac{m_0 - m_n}{m_0} \times 100$$

Where, m_0 is the initial weight of rice straw and m_n is the weight of straw dry) harvested at 30 dpi.

Lignin degradation

Lignin content in rice straw was determined following the protocol of Moreira-Vilar. Before the estimation of lignin, the tissue was first made protein-free to avoid any hindrance during quantification. The

process involves different steps of washing after the homogenization of dried tissue 0.3 g) in potassium phosphate buffer 50mM, 7ml, pH 7.0). The mixture was centrifuged and pellet was subjected to successive washings with: 1) phosphate buffer pH 7.0, 7ml; 2 times); 2) 1% v/v) Triton X 100 mixed in phosphate buffer pH 7.0, 7ml; 3 times); 3) 1M NaCl mixed in phosphate buffer pH 7.0, 7ml; two times); 4) distilled water 7 ml; 2 times); and 5) acetone 5ml; 2 times). The obtained pellet was dried in oven for 24 h at 60°C, and cooled. Protein-free cell wall tissue was used for lignin content estimation by acetyl bromide method. In brief, 20mg of this sample was mixed with 25% w/v) acetyl bromide prepared in glacial acetic acid followed by incubation for 30 minutes at 70°C for digestion. The sample was cooled on ice bath and mixed with 2M NaOH, 5M hydroxylamine HCl and glacial acetic acid in amount sufficient to totally dissolve the digested extract. The solution was centrifuged and absorbance was measured at 280 nm [8]. The results were quantified $\varepsilon = 22.9 g^{-1} Lcm^{-1}$) and expressed in unit mg lignin/g cell wall.

Si estimation in rice straw

Silicon content in decomposed rice straw was quantified using the modified molybdenum blue method of Nayar. In brief, decomposed sample supernatant was mixed with 2 volume of ammonium molybdate 10%) and incubated at room temperature for 5 min, followed by addition of 2 volume of 0.5% ascorbic acid and equal volume of 10% oxalic acid for removal of phosphorous interference followed by 5 volumes of 1:1 dil. HCl. The reaction volume was made upto 3 ml followed by 15 min incubation at RT. Absorbance was taken at 600 nm against water blank Bist.

Total Sugar

The rice straw extract was subjected to estimation of reducing sugar by phenol-sulphuric acid method of Dubois. To estimate the released sugar, supernatant straw extract) was mixed with equal volumes of phenol solution 5%) and 1.25ml concentrated sulphuric acid H_2SO_4) in test tubes. The prepared reaction mixture was incubated for 10-15min and absorbance was measured at 640nm. The control set contained DW in place of rice straw extract.

Plant growth promotion activity

Initially, composted straw:soil mixture 1:1) was checked for phytotoxicity inhibition assays by determining the germination percent. Furthermore, the combinations causing significant changes in soil physico-chemical properties and promotes plant growth were selected for further experimentation. Potential microbial consortia were assessed in micro-plot trials at Distance Research Centre CSIR-NBRI, Banthara in a field having rice stubble remains. Talc based formulation of the selected combinations after mixing with dried cow dung powder was applied to the fields followed by ploughing and water irrigation. Microplots $2x2m^2$) already used for rice cultivation were utilized for the RBD experiment conducted for two consecutive years 2015-16 and 2016-17) [9]. Rice straw remains ~ 1 feet) were mulched in the beds having around 30-40% moisture. After 10-15 days of mulching, wheat seeds were sown in rows with row spacing of 30 cm and plant to plant spacing of 15 cm. Micro-plots were broadcasted with selected microbial combinations 40% moisture-based talc formulation).

Identification of the selected Strains

For molecular identification of bacterial and fungal strains 16S rDNA and ITS sequencing was performed as described earlier Nautiyal. In brief 16S rRNA gene of bacteria was amplified under standard PCR conditions Initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 50° C for 40 s, extension at 72°C for 90 s, and final extension at 72°C for 7 min) using forward 27F 5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse 1492R 5′-AAGGAGGTGATCCAGCCGCA-3′) primers. While the internal transcript spacer ITS) region of fungal DNA was amplified using ITS-1 and ITS-4 primers in PCR at PCR conditions of, initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 30s, 56°C annealing) for 45s, and extension at 72 °C for 1 min, with final extension at 72 °C for 10 min. Purified PCR product ~1.4 kb) was sequenced at Central Instrument Facility at National Botanical Research Institute, Lucknow using ABI 3730XL capillary DNA sequencer 50 cm capillary). The obtained sequences were aligned and identified using BLAST alignment tool.

Effect of microbial consortia on growth of subsequent crop wheat) under conditions of straw incorporation

Potential microbial consortium Cons 16 was assessed in microplot 1x1 sq. m. trials at CSIR-NBRI, for improved soil health and growth of subsequent crop wheat during straw incorporation conditions. A RBD Randomized Block Design experimental setup was conducted for two consecutive years, with 3 replicates of each treatment. Each microplot was sectioned into 5 rows with the row spacing of 30 cm and plant to plant spacing of 15 cm. The field comprise of sandy loam soil with following physio-chemical properties: pH-7.5±0.2, EC-136.71 µS/cm, total K- 6.36 mg/g, total N- 0.2412%, total P- 61.63 µg/g, Si- 33.49 mg/kg, TOC-0.743%. Microbial formulation after mixing with dried cow dung manure was applied to the fields incorporated with straw 1.5 Kg/plot). After 10 days of application; fields were tilled followed by sowing of wheat seeds. The treatments were Soil C no straw), Straw C uninoculated straw), Urea C straw+urea), CD C straw+cowdung manure) and Cons 16 straw inoculated with microbes). Plant physical parameters were measured after uprooting plants at flag-leaf stage and were determined in terms of shoot and root length, number of tillers and spikes, spike length and weight, dry biomass, spacing between spikelet and flag leaf, and total yield after harvesting. Plants were harvested at the time of maturity [10].

Estimation of Plant physiological parameters under straw incorporated microplot conditions

Plant physiological parameters were measured in terms of photosynthetic rate A: µmol $m^{-2}s^{-1}$), transpiration rate E: mol m^{-2} s^{-1}) and stomatal conductance gs: mol m^{-2} s^{-1}) at different growth stages of wheat seedling SS), flag-leaf FS) and ripening MS)) during the second year of the same experiment. The estimations were performed using LI-6400 portable photosynthesis system Li-Cor, Lincoln NE, USA) equipped with red and blue LED light sources, in leaves clamped on the leaf chamber Li-Cor 6400-40, fluorometer). The conditions during measurements were light intensity 999.86±0.09 µmol m^{-2} s^{-1} at all three stages), temperature 22.68±0.14, 33.70±0.65, 28.01±0.02 °C at SS, FS and MS respectively), CO_2 Ci) concentration 292.83±1.94, 232.52±5.04, 252.31±7.71 µmol mol^{-1} at SS, FS and MS respectively), and relative humidity 45.62±0.34, 32.32±1.46, 49.98±0.16 % at SS, FS and MS respectively). Everytime the measurements of six leaves per treatment were taken at morning time 07.00-09.00am.

Estimation of Soil carbon under straw incorporated microplot conditions

Estimation of total carbon TC) in soil was performed in multi N/C 2100S duo analyser using the direct method in the consecutive year

of the experiment. The analyser is equipped with high temperature furnace HT 1300 and a solid sampler FPG 48. Soil samples were weighed in ceramic sample boats and carbon determination took place by combustion of sample at 1200°C in the ceramic combustion tube having pure oxygen atmosphere. The resultant CO_2 formed was detected through a non-dispersive infrared NDIR) detector.

Estimation of Soil Respiration under straw incorporated microplot conditions

To elucidate the effect of microbial inoculation on CO_2efflux from fields incorporated with rice straw, soil respiration was measured with an automated CO_2 flux system LI-6400 Li-Cor, Lincoln, NE, USA) equipped with a portable infra-red gas analyser IRGA) and Licor soil survey chamber 100 mm diameter). Two PVC collars 11 cm in diameter and 5 cm in height) were inserted into the soil 3 cm) at opposite corners of each plot. All plants inside the collars were removed prior to measurements for exclusion of plant respiration. Measurements were taken by placing LI-6400 chamber on PVCcollars for 1–2 min. Soil CO_2 efflux µmol CO_2 m^{-2} s^{-1}) was calculated using the default Li-Cor software afterchecking the fluxes to be linear during 120-second measurement period.

Estimation of Methane emission CH_4 flux under straw incorporated microplot conditions

Soil carbon dynamics was also determined in terms of CH_4 flux which was measured using the closed chamber technique.The rectangular 68x50 cm^2 area) chambers, equipped with a battery-operated air circulation homogenization) fan, were used to collect the air samples. The air was collected in syringes from top of the chamber openings covered with rubber stoppers); and sampling was done at intervals of 0, 15 and 30 minutes morning hours 11.00-12.00 AM). The gas samples were analysed through Thermo Scientific Trace GC 600 gas chromatography) using Flame ionization detector FID) consisting of hydrogen-air diffusion flame. The method employed during analysis includes: oven temperature at 80°C, injector at 230°C, detector at 250°C; and nitrogen was used as carrier gas flow rate 30ml/min). Standard curve for methane was prepared from 10ppm pure) methane. Rate of emission was studied for two consecutive years and result is the average data of two years.

The rate of methane emission was calculated using the formula suggested by Mallick and Dutta 2009):

$$CH_4 \text{ flux mg/m}^2\text{/h)} = \frac{BV_{stp} \times C_{CH4} \times 16 \times 1000 \times 60}{106 \times 22400 \times A \times t}$$

Where, $BV_{stp} = \frac{BV \times BP \times 273)}{[273+T) \times 760}$ [BV_{stp} is Box air volume in cc at STP],

$$BV = H \times L \times W - \text{volume of biomass inside chamber)},$$

[W = width of the chamber, H = height of the chamber, L = length of the chamber in cm; volume of biomass inside the chamber was calculated by water displacement method]

C_{CH4} = Difference in CH_4 concentration ppm) between t_0 and $t_{15/30min)}$ sampling,

A= area m^2) covered by the chamber

SEM analysis

Straw was sampled from microplots at time intervals of 1st and 3rd month post microbial inoculation and its structural analysis was performed by scanning electron microscopy SEM). For analysis, the samples were twice washed with water to remove dirt and soil particles, further washing thrice) was done by 70 % ethanol for dehydration to remove left-out water. The dehydrated samples were mounted on aluminium stubs, the stubs were then coated with gold by sputter coater Q150TES High Vacuum coating unit). These coated stubs were then analysed under Scanning Electron Microscope Quanta 250, Thermo Fischer, FEI) operated at an accelerating voltage of 15kV.

Statistical analysis

Results were subjected to statistical analysis one-way ANOVA) using SPSS 16.0 software. The data obtained is presented as mean values with significant differences at $p<0.05$, evaluated by Duncan's multiple range test DMRT).

Results

Evaluation of potential microbes for their hydrolase activity

Approximately 100 bacterial and 90 fungal strains were screened and characterized for their cellulase and laccase enzyme activity. Following their screening, three bacterial 12.10P, 7.7P, 12.14P) and four fungal Ph, 7AN6, K-14, Fx) strains were selected for further experimental proceedings. Furthermore, to develop microbial consortia, different parameters reflecting faster degradation viz., enzyme activity CMCase and FPase), reducing sugar, released silicon and phosphate were quantitatively measured to draw conclusion about microbial action. The selected microbes were characterized for their straw degrading activity at 15th and 30th dpi to determine the faster saccharification of cellulose in rice straw. Higher activity of β-1, 4 exoglucanase FPase) and β-1,4 endoglucanase CMCase) prevailed during earliest phase of decomposition, i.e., 15th dpi whereas declined until 30th dpi. Microbial strains showed higher FPase activity viz., 12.14P 28-88%), 7.7P 18-54%), Fx 26-145%), and 7AN6 16-57%) in comparison to control both at 15th and 30th dpi, however, reduced activity was observed on 30th dpi, irrespective of treatments. CMCase has been found to be higher in 12.14P 11-50%), 7.7P 17-22%), Fx 21-50%), and Ph 7-61%) in comparison to control both at 15th and 30th dpi. Incorporation of straw and its consequent decomposition also enhance the soil nutrient pool; therefore, the release of sugar, Si and P was also determined during this experiment. Released sugar was found to be higher in 12.14P 1.75-41%) and 7.7P 8-95%) as mentioned. Estimation of silicon showed higher release in 12.14P 50-291.86%), 7.7P 2.70-324%), K14 32-201.84%), Ph 23-68%) and Fx 5.39-65.90%); while, increased release of P in almost all treatments with remarkable increase on 30th day was found in K14 174.20%), Ph 334.80%), and 7AN6 87.40%) in comparison to control.

Ex-situ degradation monitored using piling method showed that in the heaps piles), CMCase activity was remarkably high in Cons 16 50-567%), 17 24-221%) and 24 30-332%); whereas, FPase was found to be higher in Cons 16 42-280%) and 17 28-103%) in comparison to control. Both the enzyme activities were higher during 30 and 45 days of decomposition, although in Cons 16 initial commencement of cellulase activity was found 15th day) showing the fast hydrolysis of cellulose. β glucosidase was found to be higher in Cons 16 81-204%), 17 48-119%) and 24 22-98%). Reducing sugar, the resultant product of saccharification, was produced highest in Cons 16 23-397%). Under piling method, Cons 11 41.31%), 16 37.49%) and 24 42.59%) showed reduced lignin content in composted straw. Even silicon content was high in Cons 16 30-86%) under heaping method. Loss in straw weight was estimated after two months of microbial inoculation which was found to be higher in Cons 11 38.89%), 16 41.11%) and 17 38.89%) under piling method.

In-situ degradation estimated by burying the microbe treated rice straw in litterbags) under live soil in 20" pots. All the three cellulolytic enzymes exhibit higher activity under the treatment with Cons 11, 16 and 17. CMCase was higher in Cons 11 1.80-57%) and 16 21-127%); FPase showed higher activity in Cons 11 18-95%), 16 22-149%) and 17 8-193%); and β glucosidase activities were found to be higher in Cons 11 6-57%), 16 26-56%) and 17 40-60%) as compared to control. Total sugar was largely produced in Cons 16 33-240%) and 17 36-233%) as shown in. Microbial activity assessed in terms of dehydrogenase DHA) was higher in Cons 16 230-1632%) and 17 192-1576%) when compared to untreated control in the litterbags condition. Lignin content was less in Cons 16 49.03%) and 17 48.58%), with high silicon in Cons 16 61-104%) and 17 33-101%) as compared to control. Loss of straw mass showed maximum reduction under treatment with Cons 16 462.00%) in comparison to control when subjected to decomposition in litterbags.

Selection of microbial consortia and identification of selected strains

Effect of selected microbial consortia on plant growth was monitored under straw incorporated conditions in-situ) in microplots. Different plant parameters in terms of shoot length indicated increased growth under the treatment of Cons 11 9.53%) and 16 7.93%) in comparison to control. Number of spikelets was found to be highest in Cons 16 27.27%); and dry plant biomass was more in order of Cons 16 137.08%) > Cons 24 116.72%) > Cons 17 111.47%) as compared to control. Seed weight measured as plant yield parameter was found to be highest in Cons 16 675.63%) > Cons 17 465.55%) > Cons 11 396.64%) > Cons 24 278.15%). Seed:straw ratio was found to be maximum in Cons 16 showing its efficient role in decomposition of straw and subsequent effect on plant growth .Microbial combination Cons 16 among all other combinations have shown better effect on plant growth. Thus, with all the analysis of this formulation, we identified the microbes of Cons 16 viz., Trichoderma asperellum, Trichoderma harzianum, Bacillus sp., and Phanerochaete chrysosporium as mentioned in Furtheron, second year onwards Cons16 was selected as best consortia and tested for following different parameters.

Effect of microbial consortia Cons 16 on plant physical growth characteristics under microplot conditions

Improved plant growth was found in treatment with Cons 16, consisting of Trichoderma asperellum, Trichoderma harzianum, Bacillus sp., and Phanerochaete chrysosporium when compared to soil C and other farmer's practice methods CD C and Urea C. Although comparable dense vegetative growth was observed in treatment with Urea control, however, yield was higher in Cons 16 treated plots. Plant height was found to be higher in Cons 16 by 3.26% when compared to control and 1-7.36% in comparison to other treatments except for slight decrement 6.56%) in comparison to Urea C treatment. Results revealed improved root growth in Cons 16 with higher root length 3.68-14.96%) in comparison to control and other treatments. Higher number of tillers 13.38-132.88%), number of spikes 7.26-135.04%), increased mass of spikes 12.68-103.91%), and dry biomass 4.43-44.11%) was found in Cons 16 as compared to control and other treatments. Spike length of Cons 16 was less in comparison to urea control but higher in comparison to control. Interestingly, total yield in first year of experiment was highest in Urea C 172.47g) followed by Cons 16 162.94g); however, subsequent application of formulation Cons 16) in the same plot for second year, showed increment in crop yield 26-114%) in comparison to all treatments. The growth and physical parameters of wheat plants for the first year of experiment has been shown and mentioned.

Effect of microbial consortia Cons 16 on physiological growth of plant under microplot conditions

Plant physiological growth was measured in terms of photosynthesis, transpiration, and stomatal conductance during three stages of wheat, i.e., seedling SS), flag-leaf FS) and ripening or milking stage MS). Microbial inoculant was found to induce higher photosynthesis in wheat plant as compared to all other treatments. It was 32-57% higher in SS stage, 1-32% higher in FS stage and 4-35% higher in MS stage when compared to other treatments. Transpiration rate was variable among treatments and growth stages. In SS stage, Cons 16 showed higher transpiration 42-77%) in comparison to others. In FS stage, transpiration was low 22-44%) in Cons 16 when compared to all treatments except control. In MS stage, lowered transpiration was found in Cons 16 in comparison to all treatments except for CD C. Stomatal conductance was higher 16-178%) in Cons 16 at SS stage in comparison to other treatments). In FS stage, higher stomatal conductance was also found in Cons 16 when compared to other treatments except for Straw-C treatment. In MS stage, almost comparable conductance was found in all treatments except for control which showed higher stomatal conductance.

Effect of microbial consortia inoculation on soil enzymes activity under straw incorporated conditions

Being the determinant of soil biological activity, different soil enzymes were measured for two consecutive years at three growth stages of wheat to assess the role of microbe mediated straw decomposition on soil microbial ecology. Dehydrogenase DHA) activity was found to be higher in Cons 16when compared to control by 136.52% at seedling SS) stage, 231.11% at flag-leaf FS) stage and 274.12% at milking MS) stage. It was also higher than other treatments in Cons 16, like the results found in the previous year of experiment. Our study showed higher activity of cellulase in Cons 16 by 40.94% at SS stage, 24.07% at FS stage and 21.69% at MS stage in comparison to control and other treatments as shown in. Similarly, β glucosidase was also highly estimated in Cons 16 at all three growth stages, i.e., SS 91.66%), FS 60.51%) and MS 22.18%) when compared to control, like the outcomes of first year. Acidic Acid P) and alkaline phosphatase Alk P) was found to be higher in Cons 16 with some exceptions. Acid P was 18.00%, 17.47% and 4.04% higher in Cons 16 at SS, FS and MS growth stages respectively, but at MS stage the activity was higher in straw C and urea C treatments. Alk P activity was higher in Cons 16 by 16.46% SS), 7.70% FS) and 1.43% MS) at different stages in comparison to control, while higher activity was found in urea C at FS stage. Urease and protease soil enzymes were found to be higher in Cons 16 in comparison to control and all other treatments except for higher urease activity in straw-C and urea-C treatments at SS stage. The variation in different soil enzyme activities thus indicates the microbial functions and ecology thereby influencing their population composition. The modulation in soil enzymes during the first year of experiment has been mentioned in.

Effect of microbial consortia inoculation on soil carbon dynamics under straw incorporated conditions

Soil carbon content total carbon) was found to be higher in treatment with Cons 16 during all stages of wheat growth. Soil sampled at seedling SS) stage showed 78.72% higher carbon in Cons 16 when compared with soil C; whereas 10.53-58.49% higher soil carbon in comparison to other treatments. During flag leaf FS) stage, ~16% higher carbon content was found in Cons 16 when compared to soil C and approximately 5-8% higher carbon total carbon; TC) in comparison to other treatments. At the ripening stage, soil TC in Cons 16 was 45%

higher than soil C treatment and almost 34-75% higher in comparison to other treatments.

Effect of microbial consortia inoculation on GHG emission under straw incorporated conditions

While determining methane emission through GC analysis, results showed less emission in treatment with Cons 16 during morning hours. Almost 66% reduced emission was measured in Cons 16 when compared to control. Similarly, ~57-85% less emission was found in Cons 16 when compared to other treatments. In afternoon, less 37-64%) emission was found in Cons 16 in comparison to all treatments. Unlike morning and afternoon, CH_4 emission was higher in Cons 16 ~19-53%) when compared to all treatments other than CD C during evening time. However, less emission was estimated in Cons 16 35.27% less) when compared to CD C treatment.

Effect of microbial consortia inoculation on soil respiration under straw incorporated conditions

Soil respiration was also measured during FS stage and it was found that amount of CO_2 emission was less in treatment with Cons 16. Approximately 34% reduced soil respiration was found in Cons 16 when compared to control, and 14-67% reduced CO_2 emission occurred in Cons 16. Soil respiration is directly proportional to soil temperature; therefore, lowered respiration was also correlated to lowered soil temperature in Cons 16 22.30±0.18°C); although little difference in temperature was found among treatments.

Surface structure morphology

The structural changes in straw sampled from different microplots were observed by SEM analysis which reveal the surface differences between untreated and treated rice straw at time intervals of 1month I) and 3months II) post inoculation of microbes mpi). This study mark the conspicuous results of decomposition as treatment with Cons 16 Cons 16 I) showed the structural damage initiated since 20-30 days post inoculation in comparison to straw Straw C I) and urea Urea C I) treatments which exhibit intact surface. At the time of wheat harvesting after 3 mpi), again the straw sample was analysed for surface structural changes and it showed complete disintegration of rice straw structure by Cons 16 Cons 16II) in comparison to other treatments Straw C II and Urea C II). These results thus validate with other degradation properties of straw inoculated with Cons 16.

Discussion

Characterization of fast decomposing microbial consortia

Most natural environments comprise synergistic microbial communities where individual species cooperate to survive and thrive together. Under such conditions, distinct microbes in combination achieve complex tasks probably through division of labor which individual strains struggle. Henceforth, to apprehend the role microbial consortium in decomposition, the microcosm experiment was conducted with developed combinations intending to accelerate the straw decomposition under microcosm conditions.

Numerous microorganisms like bacteria, fungus and actinomycetes are known for their cellulolytic activity triggering biodegradation of cellulose. Cellulase is the principal enzyme causing its saccharification and comprise of exoglucanase, endoglucanase and β-glucosidase for conversion of cellulose into sugar. Straw incorporation, through many studies, has been reported to improve soil nutrients thereby affecting soil quality. For that reason, the release of silicon and phosphate was quantified during this experiment as decomposition rate is also related to nutrient release. Silicon is one among the major components of rice straw and therefore its release was measured and found to be higher in treatments 12.14P, 7.7P, K14, Ph and Fx; thereby signify increased microbial degradation of straw

Results also indicate higher P levels during later stages of decomposition 30th day) in K14, Ph, and 7AN6, which distinctly reveal decomposition with subsequent release of nutrients when added in soil as earlier reported by.

Selection of efficient microbial combinations for enhanced decomposition efficiency

Furthermore, decomposition efficiency of microbial combinations was monitored using two different strategies of heaping and litterbags to decipher the degradation dynamics under insitu and exsitu conditions. Enzymatic hydrolysis of cellulosic component of rice straw occurs by saccharification thereby converting cellulose into sugar. Therefore, enzymes of cellulase complex were quantified and higher activity of Cons 16, 17 and 24 was obtained, thus signifying the involvement of microbial processes in decomposition through the action of cellulases as also shown by Peng. Moreover, the findings also depict the enzymatic saccharification of straw through the synergistic action of microbes inoculated in consortium. The bioconversion action was also established from the results of released sugar which again mark the cellulose hydrolysis to sugar Sangrila and Maiti.

Different pre-treatment methods have been employed for straw degradation; but the eco-friendly nature of biological process makes it the most desirable one. Longevity of decomposition diminishes the magnetism of this microbial method; however, the combination of efficient microbes would intensify the rate of decomposition in comparison to their individual action Arnthong. Similar outcomes have been obtained in results of lignin and silica content estimation, both under heaps and litterbags conditions. Lignin and silica are the cellular recalcitrant components of rice straw and hinder its digestibility Kaur and Phutela, but application of microbes altered their content with concomitant loss in straw mass as indicated.From the aforementioned microcosm experiments, we obtained promising results with Cons 11, 16, 17 and 24; whether it is saccharification, delignification, silicon release, improved microbial status or reduction in straw mass, these combinations hence manifest their role in degradation of straw both under in-situ and ex-situ counterfeit) conditions.

Considering the need of the hour to adopt sustainable methods, efficacy of selected microbial formulations for insitu straw decomposition was evaluated in microplot experiment. Improved plant growth effects have been significantly found in our study under different microbial treatments of Cons 11, 16, 17 and 24 as reported earlier Patel. Furthermore, distinctive yield effects spikelets, seed weight, seed:straw ratio) with Cons 16 thus proclaim the microbial conversion of straw resulting in soil enrichment with its ensuing effects on wheat crop yield.

All the phases of selection and analysis discussed above reveal the exceptional decomposition potential of Cons 16 in saccharification, delignification as well as straw mass reduction. Furthermore, in-situ decomposition dynamics indicate the subsequent role of Cons 16 on improved plant growth. Therefore, next microplot experiment was performed to infer the microbial efficacy of Cons 16 for in-situ management of leftover rice straw in comparison to different uninoculated treatments under conditions of straw incorporation. Our findings manifest the promising effect of Cons 16 on straw decomposition and its succeeding effect on wheat plant growth.

Incorporation of rice straw and its decomposition have significant effects on plant physical) growth which was measured in terms of shoot, root, spike length; number of spikes and tillers; weight of spike; dry biomass; distance between spike and flag-leaf; and total yield. Being the foremost trait of cropping system, grain yield unlike other parameters, is related to growth of upper plant parts. Flag leaves are one among them, known to play essential role in photosynthesis which in-turn regulate grain filling Tambussi. Therefore, while measuring different plant parameters, the spacing between flag leaf and spikelet was also measured to examine the role of flag leaf if any) on wheat productivity, and it was found to be highest in wheat plants treated with Cons 16.

Growth and crop yield attributes are also the resultant of different plant physiological variables like photosynthesis, transpiration and stomatal conductance, and these systemic plant effects have been recorded in our work throughout the growth stages seedling, flag-leaf and ripening or milking stage) of wheat. Being the principal physiological process, photosynthesis results in carbon-di-oxide assimilation and its conversion into organic matter thus correlating to higher plant dry matter and crop yield Alpha. Similar increased photosynthetic effects have been observed in our outcomes under treatment with Cons 16 at SS, FS and MS stages of wheat. Different fertilizers and their application rates also alter the plant photosynthetic parameters, similar evidences indicated from the distinct rates obtained during amendment with untreated straw, cow-dung, and urea. Photosynthetic activity of plant is also determined by transpiration and stomatal conductance, as they mark the fitness of plant. Altered transpiration and stomatal conductance under different treatments signify the gaseous exchange and water balance, thus emphasizing on the physiological adaptation of wheat plants at different growth stages. Higher transpiration and stomatal conductance during early stages of growth got lowered at later stages, hence modulating the wheat growth and yield.

Soil enzymes determine the metabolic processes modulated by its physico-chemical and biological properties. Among all types of enzymes, microbial enzymes in soil are most abundant and participate in crucial biochemical processes like mineralization, nutrient cycling and organic matter decomposition thus serving ecosystem functions. Dehydrogenases DHA) are the intracellular enzymes which denote the oxidative metabolism in soil and therefore are the bioindicators of soil health. Higher DHA of soil inoculated with Cons 16 indicates higher soil metabolic ability, i.e., biological oxidation of soil organic matter and further reflect the higher microbiological activity. Cellulases and β glucosidases are the principal enzymes playing role in organic matter decomposition and nutrient cycling. Their higher activity in soil amended with Cons 16 thus reveal microbial role in bioconversion and carbon cycling thus serving as energy source for soil microbes. Higher acidic and alkaline phosphatase of soil amended with Cons 16 again implies microbe mediated phosphorus transformation organic phosphorus to inorganic) in accordance to earlier reports Nannipieri. Likewise, mineralization of organic nitrogen by urease and protease was also higher under treatment with Cons 16 in comparison to all other untreated soils.

Crop residues are known to maintain soil carbon balance and nutrients recycling, resulting in carbon sequestration and improved soil health Casado-Murillo and Abril. Soil carbon C content is regulated by crop rotations, rate of decomposition and tillage types, which in-turn is influenced by microbes decomposers), type of crop residue and environment Hättenschwiler. Among these residues rice straw is another abundant and economic carbon source, used in present study to give an insight into the effect of straw incorporation on soil carbon content. Improved C content with Cons 16 distinctly evidences the microbial action on straw degradation with its resulting effect on nutrient acquisition in soil. Results also mark the effect of decomposition on soil carbon status which got depleted during straw or residue removal after rice harvesting. Thus, our results clearly reveal that straw incorporation not only improves soil nutrient balance but also regulate C sequestration which increases soil microbial biomass, with latter effect on soil physical and chemical properties as found under treatment with Cons 16.

While studying soil carbon dynamics, it is pertinent to measure carbon fluxes methane and carbon-di-oxide) as addition of straw in soil results in increased methane emission potent greenhouse gas) adversely affecting global warming. However, methane emission indicates the decomposition to be anaerobic and therefore it urges for sustainable management practices to enhance soil carbon pool but lower the levels of methane CH_4) emission. Likewise, our outcomes display reduced effects on release of methane during microbial treatment thus proposing the novel means of straw management to curb the emerging adverse effects on the environment. As flag-leaf FS) stage contributes to almost 70-75% of final wheat yield, gaseous CH_4 and CO_2) emission was measured in FS stage at different time spans of the day morning, afternoon, and evening) during incorporation and decomposition study in microplots. Lowered emission during before and after) noon in Cons 16 signify the potential of microbes in sustainable straw management leading to improved carbon stocks of soil. Higher methane emission during late afternoon in Cons 16 is in accordance to the results of Parashar. Thus, our findings indicate the decomposition by Cons 16 to be aerobic with complete breakdown of organic matter.

Among the sequestration traits, soil respiration CO_2 flux) is another governing factor for carbon mineralization, carbon stabilization and nutrient cycling during straw incorporation in fields Radicetti. Our results exemplify lowered soil respiration under treatment with Cons 16, resulting in reduced CO_2 emission thereby fostering on soil carbon sequestration and conserved soil organic matter. Soil respiration is also affected by soil temperature which was lowered in our treatment, as high temperature would increase microbial metabolism and thus increased CO_2 flux would cause carbon losses. This suggests that the use of microbial consortium Cons 16) for straw decomposition enhances soil carbon pool, its stability and sequestration.

Besides all findings related to effects of straw incorporation on soil and plant growth, there was a lack of any observation indicating morphological alterations in rice straw. This led to examination of structural deformity in straw samples collected during this experimental work in order to detect surface morphological changes on treatment with Cons 16. Resulting structural alterationsthus signifies role of microbes and their enzymatic activities which breakdown lignin with further hydrolysis of cellulose ensuing complete breakdown of rice straw.

Conclusion

Our findings thus dispense complete characterization of microbial combinations for accelerated decomposition of rice straw. All the phases of characterization and selection demonstrated through gradual screening methods reveal the decomposition potential of selected microbial combinations. However, Cons 16 among all microbial consortia has shown the exceptional role in saccharification, delignification, straw mass reduction; and subsequent role in improved soil health and plant growth. Even the surface structural changes of straw substantiate the decomposition potential of Cons 16

under conditions of straw incorporation. Loss of nutrients through continuous cropping can also be rectified through these methods, as evident from our significant outcomes with Cons 16. Interestingly, the microbial decomposition of incorporated straw enhanced the soil carbon pool significantly indicated by total carbon and its fluxes CH_4 and CO_2). Both ex-situ and in-situ decomposition strategies thus introducepioneer methods for poor/marginal farmersto eliminate the gap between production and sustainable management of surplus crop residues like rice straw).Owing to its in-situ decomposition, increased nutrient pool would result in improved soil fertility, biodiversity, productivity and soil re-carbonization, thus enabling the effective use of agro-resources for sustainable agriculture.

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