Rice Straw Degradation with Mixed Cultures of Microfungi And Their Enzymes

Abstract

Huge quantities of agricultural residues are generated every year but it is neither converted into energy nor allowed to go back to the soil and sometimes burnt that leads to air pollution and loss of soil biology and fertility. Cellulolytic microfungi secrete extracellular enzymes that degrade lignocellulosic biomass in nature and this ability of microbes may be exploited to enhance the rates of degradation of agriculture residues to recycle carbon, nitrogen and minerals in the soil. This study aimed to find out the effect(s) of co-cultivation of high cellulolytic microfungi in various combinations on rice straw under field conditions to enhance its microbial decomposition to discourage the farmers from burning it in the fields. The effectiveness of five fungal cultures in different combinations was tested for efficient degradation of rice straw. Five dominant species of fungi that have been earlier shown to secrete high amounts of cellulases in our own laboratories, were cultured on medium containing yeast powder (2g/L), jaggery (5g/L) and urea (1g/L) at room temperature. Release of reducing sugars from 1 g of rice straw treated with 10IU/mL of fungal extracellular enzymes showed that *Penicillium chrysogenum* released highest amount of mono and oligomers (96 mg/g) followed by *Aspergillus flavus* (80 mg/g) and *A. oryzae* (78 mg/g), *A. fumigatus* (72 mg/g) and *Trichoderma viride* (70 mg/g) within 24 h that increased with increasing temperature and increasing period of incubation. Treatment of rice straw with fungal cultures of *Penicillium chrysogenum, Aspergillus flavus* and *A. oryzae* revealed that the co-inoculation of all these species decomposed approximately 75% of the total rice straw as assessed by weight loss method. The application extracellular secretory enzymes on rice straw, though, revealed to release of reducing sugars, but the rate of reducing sugars was not sufficient enough to be used for degradation of rice straw under field conditions, hence, the used of mixed cultures of microfungi was planned and tested experimentally, which allowed the decomposition of rice straw much faster than control which were treated with heat-killed dead cultures of test microfungi. Hence, it is recommended that additional spray of mixed cultures of microfungi on rice straw may facilitate its degradation under field conditions.

Key words: Mixed Cultures, cellulases, rice straw, degradation, microfungi

1. Introduction

Lignocarbohydrate is the major renewable biomass on this earth that is produced by various agricultural crops as residues [1,2]. It is usually composed of cellulose (40-50%), hemicellulose (20-30%) and lignin (11-18%). Rice straw is relatively difficult to decompose in nature because of the presence of high amounts of silicon (9-14%) [3,4]. Approximately, 700 million tons of lignocarbohydrate is generated by various agricultural practices and agro-based industries [5] of which 122.6 million tones of residue is generated by paddy [6]. In India rice is grown as a major crop with approximately 130.84 million metric tons production of grain in 2022-23.The natural decomposition of rice straw is very slow and for the immediate requirements of the land for next crop, the farmers generally burn the rice straw in the field that create smog and pollution problems leading to several health hazards, cause loss of the soil fertility and nutrients [7, 8, 9].

Several microbes (bacteria, fungi and actinomycetes) are known to degrade lignocellulose in nature. Several microfungi are known to degrade lignocellulose in nature (10-16]. We have previously isolated a large number of microfungi from naturally degrading rice straw [17, 18] and also analyzed the production of cellulases using solid-state fermentation technology [18]. The microbial enzymes accelerate the rice straw decomposition in the field and improves the soil quality and fertility [19].

The treatment of lingo-carbohydrates with high activity cellulases, xylanases and ligninases complex mix of enzymes from different cellulolytic microbes may facilitate the breakdown of complex molecules of cellulose, hemicelluloses and lignins [20,21] and using this principle of microbial technology, various enzyme combinations in the past, have been used to enzymatically digest agricultural residue [22].

Previously *Aspergillus* and *Trichoderma reesi* were utilized to break down raw rice straw and sugarcane bagasse, respectively [23, 24]. Combination of cellulolytic enzymes secreted by the white rot fungus was used for saccharification of wheat and rice straw [25] and maize stalk was hydrolyzed using lignocellulolytic enzyme cocktails from *Auricularia auricular* [26].

This paper aims to evaluate reducing sugars releasing abilities of five dominant species isolated previously by the authors [17, 18] and to find out the possibilities of using mixed cultures as co-inoculants to facilitate the degradation of rice straw under field conditions.

2. Materials and Method

2.1 Microbial cultures

Rice straw was collected from a Rawali village, Muradnagar Ghaziabad. It was cut into small pieces of 2-3 cm and used without pretreatment. *Aspergillus flavus ,A .fumigatus,* A. *oryzae, Penicillium chrysogenum* and *Trichoderma viride* were isolated from decaying rice straw as dominant species earlier in our laboratories and their CMCase and FPase activities were determined [17,18]. Pure cultures of these dominant species were maintained on PDA (Potato Dextrose Agar) at -20ºC and the cultures were revived on PDA whenever required for further use.

2.2 Release of reducing sugars from rice straw by fungal enzymes at different temperatures

The authors previously prepared the enzyme from *Aspergillus flavus, A. fumigatus, A. oryzae, Penicillium chrysogenum* and *Trichoderma viride* having 10.2, 6.49, 7.6, 37.0 and 5.6 IU/mL CMCase activity respectively using solid-state fermentation technology [18]. These enzymes were used to find out the release of reducing sugars. 1g of powdered rice straw was added in 100 mL sterile Erlenmeyer flask to which the total amount of 20 mL of buffer + 10 IU of the enzyme was added. The flasks were incubated on rotary shaker at 100 r.p.m. for 24 and 48 h at 30, 40 and 50ºC temperatures. The reducing sugars were assayed with the DNS method [27] and the release of reducing sugars in mg/g of rice straw are shown in Table 1 along with the volumes of enzyme and buffers used for all 5 species.

2.3 Mass culture of microfungi

The species were cultured separately in liquid media which was prepared using yeast powder (2g/L), jaggery (5g/L) and urea (1g/L). 2 L of medium was sterilized at 15 p.s.i. in 10 L Erlenmeyer flask for 15 minutes for each species separately and two colonies of one week old culture on PDA were aseptically inoculated and allowed to grow at room temperature until full growth of the fungus was observed. Occasional shaking of the contents were made. Each species was harvested after 7 days of incubation when there was good biomass of the fungus was produced. The contents were shaken vigorously with the help of sterile glass rod to produce a good mix of the culture.

2.4 Rice straw decomposition by mixed cultures under filed conditions

Rice straw samples were collected from rice field of Rawali village near Muradnagar (U.P.) and cut into 2-3 cm small pieces. 20 gm sun dried rice straw was taken into 100 mL Erlenmeyer flask, sterilized at 15 p.s.i. for 15 minutes and soaked into live liquid cultures of different combinations of microfungi as shown in Table 2. The control was treated similarly but with dead cultures prepared by boiling the live cultures on water bath for 10 min. After treatment the rice straw was put into nylon bags and buried into the field. For each fungal combination, 12 sets were prepared and three replicates of each set were removed after every 15 days, soil particles and fungal mycelium from the surface were removed carefully with the help brush, washed thoroughly to remove remnant of growing fungus and all soil matter and sun dried followed by final dry in oven at 70ºC for 12 h. Each combination was prepared in triplicate. Rice straw treated with heat killed fungus in similar way served as a control. Nylon bags were taken out at 15, 30, 45 days for sample analysis of degradation process.

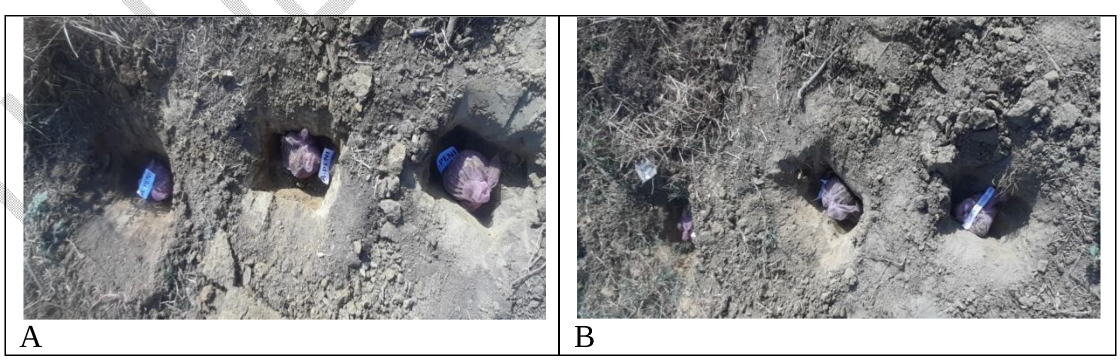

Fig. 1 A: One set of treated rice straw with fungal cultures in nylon bags buried into the soil
B: Second set of treated rice straw with fungal cultures in nylon bags buried into the soil

3. Results and Discussion

Enzymatic depolymerization of lignocellulosic residue requires the involvement of several hydrolases, cellulases, hemicellulases and ligninases [28, 29].The major component of the lignocarbohydrate is cellulose which is hydrolyzed by enzyme complex cellulases that are commonly measured and expressed in terms of CMCase and FPase [30]. Filamentous fungi have the advantage of penetration of plant tissues from their hyphal tips and the extracellular secretion of the enzyme complex cellulases break down the β 1, 4 linkages and release the monomers and oligomers of hexoses [31, 32, 33].These sugar molecules facilitate the fungus to grow and multiply further and the enzyme complex hemicellulases is secreted which is most generally measured and expressed in the form of xylanases. Lignin is the most difficult part of the lignocellulosic material which is attacked by a highly complex group of enzymes, commonly referred as ligininases that are measured and expressed in the form of peroxidases [34]. All these 3 sets of enzyme complexes degrade, recycle and mineralize this natural lignocellulosic residue in the soil, but the presence of high amounts of silicon in rice straw makes it difficult to degrade under field conditions, hence, the farmers are tempted to burn it in the field to make it free for next crop. The objective of the present investigation was to isolate and characterize high cellulases producing microfungi from naturally degrading rice straw in the field [17,18] have earlier isolated 10 dominant species of microfungi that showed high cellulolytic activity on CMC (Carboxymethyle cellulose) agar plates and on further screening finally five species were selected that produced high CMCas and FPase activity enzymes under solid-state fermentation conditions. The treatment of rice straw with 10 IU/g enzyme (CMCase) at 30,40 and 50 ºC temperature for 24 and 48 h on rotatory shaker revealed that *Penicillium chrysogenum* released highest amount of reducing sugar followed by *Aspergillus flavus*, *A. oryzae*, *A. fumigatus* and *Trichoderma viride* (Table 1). The release of reducing sugars further increased with increasing temperature and duration of incubation that suggest that the enzyme complex released by the microfungi is not de-activated on longer periods of incubation and increasing temperature [35,36,37]. Further the experiments were designed to evaluate the degradation potential of *Penicillium chrysogenum*, *Aspergillus flavus* and *A. oryzae* singly and in combination of each other under field conditions. It was found that of all these three test species *Penicillium chrysogenum* was highly active followed by *Aspergillus flavus* and *A. oryzae* in degradation of rice straw under field conditions (Table 2) but when these were used as mixed inoculants the degradation of rice straw fastened synergistically and the highest rate of decomposition was recorded with mixed cultures of all three species of *Penicillium chrysogenum*, *Aspergillus flavus* and *A. oryzae* that degraded approximately 75% of the rice straw under field conditions while only approx. 20% of the heat killed treated rice straw (control) was degraded under similar conditions of incubation (Table 2) [38,39,40]. The endophyte fungus *Chaetomium globosum* DX-THS3 has been reported to produce lignocellulolytic enzymes and glycyrrhizic acid 3-O-mono-D-glucuronide under SSF conditions utilizing licorice straw as substrate that is able to degrade the lignocarbohydrates [41]. The release of reducing sugars suggest that rice straw may be used as useful agro-waste for the production bio-ethanol and the spray of mixed cultures of microfungi may facilitate the biodegradation of rice straw in the field and may help in recycling of carbon and other nutrients in the soil, that may attract the farmers and they may not burn the rice straw in the field.

Table-1 Release of reducing sugars (mg/g) from rice straw using enzymes produced from five dominant species at Different Temperature

Fungus Species	Enzyme concentration (IU/ml)	Volume of enzyme (10IU/g) + buffer	30 ^{0}C Temperature		40 ^{0}C Temperature		50 ^{0}C Temperature	
			24 h	48 h	24 h	48 h	24 h	48 h
Aspergillus flavus	10.2	0.980mL+19.02mL =20ml	80	112	98	134	108	146
A. fumigatus	6.49	1.540mL+18.46mL =20mL	72	98	88	120	98	112
A. oryzae	7.6	1.315mL+ 18.685mL=20ml	78	102	92	128	106	132
Penicillium chrysogenum	37	0.270mL+19.73mL = 20ml	96	128	112	148	134	160
Trichoderma viride	5.6	1.785mL+ 18.215mL=20ml	70	86	84	108	96	106

Table-2: Weight Loss of rice straw under field conditions when buried in soil after treatment with different combinations of microfungi. (Each figure is an average of 3-independent replicates).

	Loss of biomass (Rice straw) weight in grams after inoculation of different combinations of microfungi at different interval time period						
Fungus Species	**0 Day**	**15 Day**		**30 Day**		**45 Day**	
	Control	Control	Treatment	Control	Treatment	Control	Treatment
Penicillium chrysogenum	20.10 ± 1.20	19.60 ± 0.55	18.65 ± 1.20	18.310 ± 0.58	16.090 ± 0.270	16.690 ± 1.10	13.120 ± 0.120
Aspergillus oryzae	20.20 ± 1.30	19.3 ± 0.45	18.680 ± 1.30	18.110 ± 0.450	16.250 ± 0.500	16.990 ± 1.25	14.200 ± 0.60
Aspergillus flavus	20.15 ± 1.10	19.45 ± 0.35	18.920 ± 0.062	17.90 ± 0.960	16.760 ± 0.200	16.800 ± 0.960	14.510 ± 0.300
P. chrysogenum + *A. oryzae*	20.23 ± 1.50	19.70 ± 0.60	17.856 ± 1.857	18.110 ± 0.50	12.096 ± 0.268	16.220 ± 1.115	8.126 ± 0.328
P. chrysogenum + *A. flavus*	20.21 ± 1.450	19.0 ± 0.60	18.286 ± 1.544	17.860 ± 0.650	15.050 ± 0.580	16.110 ± 1.200	7.286 ± 0.603
A. oryzae + *A. flavus*	20.10 ± 1.20	19.20 ± 0.35	18.620 ± 0.066	17.50 ± 0.860	13.883 ± 0.201	15.860 ± 0.980	10.410 ± 0.364
P. chrysogenum + *A. oryzae* + *A. flavus*	20.11 ± 1.11	19.10 ± 0.25	14.283 ± 0.319	17.250 ± 1.120	9.333 ± 0.227	15.350 ± 1.450	5.253 ± 0.340

A-*Penicillium chrysogenum*; **B**- *Aspergillus oryzae*; **C**- *Aspergillus flavus*
Values present a Mean±SD of three replication under field condition

Conclusions

In order to hydrolyze lignocelluloses in laboratory or under field conditions, a set of enzyme combinations is required that are produced by microbes. Microfungi are the primary degraders of lignocelluloses under aerobic conditions and play a significant part in the biogeochemical cycling of organic carbon. Though, *Penicillium chrysogenum*, *Aspergillus flavus* and *A. oryzae* are individually able to degrade rice straw in the field conditions but when their combinations were used as co-inoculants, the degradation potential of these species increased synergistically as revealed from the 75% weight loss of rice straw after treatment with mixed cultures. Though, the microbial extracellular enzymes have capabilities to break down the lignocellulosic materials into monomers and oligomers but their rates of breakdown is so slow that these may not be commercially exploited. It is, therefore, suggested that the mixed cultures fungi should be used to enhance the rates of biodegradation of rice straw in the field that may discourage the farmers to burn it in the field that will save the environment.

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