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Evaluating efficacy of lignocellulolytic bacterial consortia for decomposition of different spent mushroom substrate under laboratory condition

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ABSTRACT

In the present study, bacterial consortia are developed to facilitate quick composting process for mushroom SMS decomposition. In total 9 treatments were developed and tested for composting. *In vitro* setups indicated that treatments with bacterial consortia, T7 (SMS of paddy straw inoculated with AAU PG 23) and T6 (SMS of paddy and wheat straw [1:1] + ABBC 2), exhibited significant improvements in decomposition efficiency and compost quality. These treatments resulted in rapid SMS degradation, substantial reductions in dry weight and notable chemical enhancements, including decreased C:N ratio. Furthermore, both treatments displayed a substantial increase in pH and a decrease in EC, indicating improved compost quality.

Keywords: Mushroom, spent mushroom substrate, lignocellulolytic bacteria, decomposition, weight loss

Agricultural waste and SMS are types of biomasses made up of lignocellulose, which are the most plentiful form of unused biomass. They consist mainly of cellulose (30–50%), hemicellulose (15–35%) and lignin (10–20%). Cellulose and lignin are abundant parts of plant biomass and pose difficulties in waste management because of their complex structures and are tough to break down (Mielenz, 2001; Girio *et al*., 2010). Cellulose is a linear polysaccharide consisting of β-1,4-linked D-glucose residues, whereas hemicellulose is a hetero-polysaccharide made up of different hexoses, pentoses and gluconic acid. Lignocellulolytic bacteria produce a variety of cellulases that work together to break down cellulose by hydrolyzing the β-1,4-glycosyl linkages of the cellulose chain, producing cello-oligosaccharides. This enzyme family is roughly grouped into endolytic enzymes (endo-1,4-β-glucanase) and exolytic enzymes (cello-biohydrolase). Complete depolymerization of cellulose to glucose requires another kind of cello-oligosaccharide degrading enzyme, 1,4-β-glucosidase (Nishida *et al.*, 2007). Lignin, the second most abundant renewable biopolymer in nature, is the primary aromatic polymer found in the biosphere. It serves a vital role in plant cell walls by providing rigidity and protecting cellulose from pathogens. However, due to its complex structure and non-hydrolysable bonds, lignin is more challenging to break down compared to cellulose or hemicellulose. It forms a matrix around cellulose in the plant cell

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wall, further contributing to its resistance against degradation. Therefore, the identification of bacteria having lignin-oxidizing enzymes would be significant (Raghukumar *et al*., 2008).

Modern agriculture heavily relies on hybrid seeds and high-yielding varieties that are responsive to chemical fertilizers and irrigation. However, the indiscriminate use of synthetic fertilizers has resulted in soil pollution and contamination, water table pollution and the destruction of microorganisms and beneficial insects. This has made crops more susceptible to diseases and reduced soil fertility. Additionally, the continuous use of chemical fertilizers depletes fossil fuel resources, leading to an energy crisis and escalating fertilizer costs, which small farmers may not be able to afford. The widening gap between nutrient removal and supply further intensifies the decline in soil fertility (Kumar *et al*., 2017). Biofertilizers, on the other hand, are natural fertilizers consisting of living microbial inoculants that can convert unavailable nutrients into available forms through biological processes such as nitrogen fixation, phosphate solubilization, excretion of plant growth-promoting substances and biodegradation in soil, compost and other environments. They include bacteria, algae and fungi, either alone or in combination and play a vital role in enhancing nutrient availability to plants. In the current context of rising chemical fertilizer costs and their harmful effects on soil health, the use of biofertilizers has become particularly significant.

With the increasing demand for sustainable agriculture and the environmental challenges of disposing of spent mushroom substrates (SMS), combining biofertilizers with lignocellulolytic bacteria shows great promise. Lignocellulolytic bacteria can efficiently break down cellulose and lignin, abundant in SMS, converting them into simpler compounds that release nutrients and enhance mineralization. Since a substantial amount of SMS is produced as waste during mushroom cultivation, finding sustainable management strategies is crucial. Enriching the compost derived from SMS with biofertilizer becomes vital as it further boosts compost productivity. The beneficial microbes in the biofertilizer can enhance soil nutrient levels, leading to improved plant growth and overall soil health. This enrichment process creates fertile compost, serving as an eco-friendly alternative to chemical fertilizers, supporting healthier crop growth and contributing to environmental preservation

To address these challenges, the present study aims to isolate, screen and characterize lignocellulolytic bacteria from SMS and develop a bacterial consortium that can offer a viable solution for SMS disposal.

MATERIALS AND METHOD

Isolation of Bacterial isolates for SMS of mushrooms

Bacterial colonies were isolated from the SMS of mushroom using dilution plating technique at the dilution of 10^{-6} and 10^{-7} on nutrient agar plates and maintained on nutrient agar slants. The bacterial isolates were purified using streaking method.

In vitro compatibility assessment and consortium preparation of selected isolates

To evaluate the *in vitro* compatibility of the selected isolates, a cross-streaking method was employed. The selected isolates were streaked perpendicular to each other on nutrient agar plates. Throughout the incubation period, the plates were regularly monitored to observe any signs of interaction or inhibition at the point of intersection (Liu, 2019). These observations were recorded to determine the compatibility among the selected isolates.

Subsequently, to prepare the consortium, the selected isolates were separately inoculated into nutrient broth and incubated at a temperature of 30°C. Once the isolates reached their optimum growth, an equal volume of broth from each isolate was mixed under aseptic conditions and consortium formulation was developed with a population of 5 x 10^8 CFU ml^{-1}. The prepared formulation was monitored for microbial population at monthly intervals for shelf-life evaluation up to 6 months.

Evaluating efficacy of lignocellulolytic bacterial consortia for decomposition of different SMS under laboratory condition

Efficacy of consortia for degradation of different SMS in the flask: The efficacy of consortia for the degradation of different SMS in flasks was studied through the following experiment details:

Design	Completely Randomized Design
Flask size	**500 ml**
Quantity of SMS (g/flask)	**50**
No. of repetitions	**3**
No. of treatments	**9**
T_1	SMS of rice straw
T_2	SMS of wheat straw
T_3	SMS of rice and wheat straw (1:1)
T_4	SMS of rice straw + ABBC 2*
T_5	SMS of wheat straw + ABBC 2
T_6	SMS of rice and wheat straw (1:1) + ABBC 2
T_7	SMS of rice straw + AAU PG 23**
T_8	SMS of wheat straw+ AAU PG 23
T_9	SMS of rice and wheat straw (1:1) + AAU PG 23

*ABBC 2 - *ANUBHAV* bacterial bio-degrader consortium; **AAU PG 23 – Consortium of compatible isolates of lignocellulolytic bacteria; (ABBC 2 and AAU PG 23 will be applied at 1 L/tonne of SMS)

Substrate preparation, inoculation and cultural conditions for SMS degradation in flask: Different samples of SMS were collected from the mushroom laboratory and processed to ensure uniform size. For each treatment, a specific type of SMS was selected and a quantity of 50g was mixed with R.O. water to achieve a moisture content of approximately 40% (w/w). The resulting moistened SMS was suspended in a 500 ml flask. Each treatment was inoculated with respective lignocellulolytic consortium having a concentration of 5 x 10^8 cells/ml and three replications were maintained. Subsequently, the inoculated substrates were placed for incubation at a temperature of 30±2°C for 80 days.

Days to degradation of SMS in flask

Regular inspections were carried out on the flasks to monitor any changes in color, odour, compaction of the mass and reduction in particle size, which were used to assess degradation using physical indicators.

Reduction in dry weight of SMS in flask

The reduction in dry weight of the SMS was determined by initially drying 50g samples at 105°C for 24 hours to obtain the initial dry weight. After the 80-day incubation period, the SMS samples were dried again at 105°C for 24 hours to obtain the final dry weight. The reduction in dry weight was then calculated using the formula:

$$\text{Reduction (\%)} = \frac{\text{Initial Dry Weight} - \text{Final Dry Weight}}{\text{Initial Dry Weight}} \times 100$$

C:N ratio (at initial, 40th day, 80th day of treatment) of SMS degradation in flask

The C: N ratio was determined by dividing the percentage of total organic carbon by the percentage of total nitrogen.

RESULTS AND DISCUSSION

Efficacy of Consortia for Degradation of Different SMS in the Flask

Days to degradation of SMS in flask: The degradation of different SMS types varied significantly across treatments, with notable differences in degradation times. T7 (SMS of paddy straw inoculated with AAU PG 23) exhibited the most rapid degradation, with an average of 72 days to degrade completely. Following closely were T4, T6 and T9, which showed comparable degradation times of 78, 79 and 80 days, respectively (Table 1). On the other hand, treatments involving wheat straw generally degraded slower, with T2 (SMS of wheat straw alone) taking the longest at 122 days. Mixed substrate treatments demonstrated intermediate degradation rates, reflecting a combination of the individual straw types' degradation characteristics (Fig 1).

The degradation times observed in our study align with findings reported by Abdel-Rahman *et al*. (2016). They noted that compost piles inoculated with mixed cultures matured within 51–58 days, while those inoculated with a single strain completed maturation within 72–79 days. In contrast, control piles took significantly longer, maturing within 89–96 days.

Reduction in dry weight of SMS in flask

The percentage reduction in dry weight of SMS showcased diverse degradation efficacy across treatments. T7 (SMS of paddy straw inoculated with AAU PG 23) with the highest reduction (44.65%), indicating robust decomposition. Following closely were T4 and T6, achieving reduction percentages of 41.47% and 41.95%, respectively (Table 1). On the other hand, wheat straw treatments generally displayed lower reduction percentages, notably T2 with the lowest at 21.65% (Fig 1). Mixed substrate

Table 1. Reduction in dry weight and days to degradation of SMS in flask study

Treatments	Percentage reduction in dry weight	No. days to degrade
T_1 SMS of paddy straw alone	26.17^{e}	108^{d}
T_2 SMS of wheat straw alone	21.65^{f}	122^{f}
T_3 SMS of paddy and wheat straw (1:1) alone	24.37^{e}	114^{e}
T_4 SMS of paddy straw + ABBC 2*	41.47^{b}	78^{b}
T_5 SMS of wheat straw + ABBC 2	39.42^{cb}	86^{c}
T_6 SMS of paddy and wheat straw (1:1) + ABBC 2	41.95^{b}	79^{b}
T_7 SMS of paddy straw + AAU PG 23**	44.65^{a}	72^{a}
T_8 SMS of wheat straw + AAU PG 23	37.64^{d}	88^{c}
T_9 SMS of paddy and wheat straw (1:1) + AAU PG 23	40.51^{bc}	80^{b}
S.Em ±	0.604	1.100
C.D. at 5 %	Significant	Significant
C.V.	2.96	2.08

Note: Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5 % level of significance. (ABBC 2 and AAU PG 23 can be applied at 1 L/tonne of SMS)
*ABBC 2 - *ANUBHAV* bacterial biodegrader consortium. **AAU PG 23 – Consortium of compatible isolates of lignocellulolytic bacteria

Fig. 1. Efficacy of consortia for degradation of different SMS in flasks. (A) Treatment flasks at day 0. (B) Treatment flasks at day 80

treatments exhibited intermediate reduction rates. Although the reduction percentages varied among treatments, they followed a similar trend to the degradation rates. The reduction in dry weight of SMS observed in our study aligns with findings reported by Bahatkar (2023). They observed a maximum loss in weight of wheat stalk in flask assays by cellulolytic bacteria, reaching 74.88%. Additionally, our results are comparable to those of Wang *et al*. (2011), who isolated bacterial strains capable of bioremediating paddy straw with degradation rates ranging from 60% to 75%.

C:N ratio of SMS degradation in flask

One of the often-used parameters to assess the rate of decomposition in the composting process is the C:N ratio, as it can reflect the maturity of the compost. At day 40 of the flask study, the C:N ratio of SMS treatments displayed noticeable shifts, indicating ongoing degradation processes. Treatments inoculated with bacterial consortia (T4-T9) exhibited early reductions in C:N ratio compared to treatments with SMS substrate alone (T1-T3). Notably, T7 (SMS of paddy straw inoculated with AAU PG 23) showcased the most substantial reduction, falling from 70.03 to 33.03, followed by T4 and T6, which experienced reductions from 68.89 to 35.86 and 72.10 to 39.65, respectively (Table 2), signifying rapid carbon utilization facilitated by the bacterial consortium.

By day 80, the degradation process had progressed further. Organic carbon decreased due to substrate decomposition and the increase in total N during composting was caused by the decrease in substrate carbon resulting from the loss of CO_2

Table 2. Changes in C:N ratio of SMS treatments over time in flask study

Treatment	C:N ratio		
	Initial	**Day 40**	**Day 80**
T_1 SMS of paddy straw alone	71.03[b]	54.62[h]	36.81[f]
T_2 SMS of wheat straw alone	66.94[a]	49.44[f]	35.25[e]
T_3 SMS of paddy and wheat straw (1:1) alone	72.54[b]	52.50[g]	35.52[e]
T_4 SMS of paddy straw + ABBC 2*	68.89[ab]	35.86[b]	17.64[b]
T_5 SMS of wheat straw + ABBC 2	66.93[a]	41.93[de]	22.42[d]
T_6 SMS of paddy and wheat straw (1:1) + ABBC 2	72.10[b]	39.65[c]	17.05[b]
T_7 SMS of paddy straw + AAU PG 23**	70.03[ab]	33.03[a]	14.27[a]
T_8 SMS of wheat straw + AAU PG 23	66.70[a]	42.40[e]	22.91[d]
T_9 SMS of paddy and wheat straw (1:1) + AAU PG 23	72.10[b]	40.42[dc]	18.55[c]
S.Em. ±	1.132	0.509	0.259
C.D. at 5 %	Significant	Significant	Significant
C.V. %	2.81	2.04	1.83

Note: Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5 % level of significance. (ABBC 2 and AAU PG 23 will be applied at 1 L/tonne of SMS)
*ABBC 2 - *ANUBHAV* bacterial biodegrader consortium. **AAU PG 23 – Consortium of compatible isolates of lignocellulolytic bacteria

(Tajbakhsh *et al*., 2008). Significant reductions in C:N ratio were observed across all treatments, with treatments inoculated with bacterial consortia (T4-T9) exhibiting the most pronounced reductions, indicative of efficient carbon utilization and decomposition. T7 (SMS of paddy straw inoculated with AAU PG 23) maintained the lowest C:N ratio (14.27), followed by T6 (17.05), which was at par with T4 (17.64), suggesting continued microbial activity and decomposition efficiency (Table 2). In contrast, control treatments T1, T2 and T3 showed relatively slower reductions in C:N ratios, with values of 36.81, 35.25 and 35.52, respectively.

The observed reduction in C:N ratio during the degradation of SMS in our study is consistent with findings reported by Kausar (2011) and Pan (2012). Kausar (2011) noted that after 6 weeks *in vitro* (flask), the C:N ratio of inoculated paddy straw compost was 18.1, indicating sufficient maturity for field application. Similarly, Pan (2012) observed that when a consortium of three isolates was used, the initial high organic carbon content (128:1 for wheat straw and 76:1 for paddy husks) reduced to around 25–30:1 at 75–90 days, reflecting a faster rate of decomposition.

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