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RESEARCH ARTICLE

Composting of Sweet Sorghum Bagasse and its Impact on Plant Growth Promotion

Subramaniam Gopalakrishnan¹ · Vadlamudi Srinivas¹ · Are Ashok Kumar¹ · Akula V. Umakanth² · Uma Addepally³ · Pinnamaneni Srinivasa Rao¹

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Abstract The present study was carried out to optimize a protocol to rapidly decompose sweet sorghum bagasse and to evaluate the bagasse compost for plant growth promotion (PGP) in sweet sorghum. A total of ten cellulose-degrading microbes were screened for decomposing sweet sorghum bagasse, of which three (*Myceliophthora thermophila* ATCC 48104, *Aspergillus awamori* and *Bacillus subtilis* ATCC 6633) decomposed bagasse efficiently in 60 days. When these potential microbes were characterized for their in vitro PGP traits, all were found to produce indole acetic acid, cellulase, lipase (except *M. thermophila*) and siderophore (only *A. awamori*) and solubilize phosphorous (except *M. thermophila*). The bagasse compost prepared with the three microbes was evaluated for PGP on sweet sorghum under greenhouse conditions. The results showed that the bagasse compost prepared with potential microbes significantly and consistently enhanced PGP traits including the plant height (37–44%), leaf weight (63–81%), shoot weight (38–66%), root weight (87–89%), leaf area (75–83%) and root length (37–48%) at 35 days after sowing (DAS); shoot weight (40–58%) and root weight (24–38%) at 70 DAS; and shoot weight (30–46%), panicle weight (40–51%), seed number (20–62%) and seed weight (37–65%) at harvest over the bagasse compost

prepared without microbes. Among the three potential strains, *A. awamori* and *M. thermophila* significantly and consistently enhanced all the PGP traits compared to *B. subtilis*. It is concluded that sweet sorghum bagasse can be decomposed rapidly and the bagasse compost prepared with microbes can be successfully used for PGP in sweet sorghum.

Keywords Sweet sorghum · Bagasse · Cellulose-degrading microbes · Plant growth promotion · Yield traits · Micronutrients

Introduction

Biofuels produced from renewable energy sources are gaining importance in the light of rising fossil fuel prices, depleting oil reserves and increasing ‘greenhouse effect’ associated with the use of fossil fuels. Sweet sorghum (*Sorghum bicolor* L. Moench) is one such alternative feed stock that has been used in recent years for cultivation under rain-fed agriculture not only for food and feed security but also for ethanol production. Sweet sorghum accumulates sugars in the stalks that can be crushed to extract juice and processed into bioethanol. Sweet sorghum has the potential to yield up to 8000 L ha⁻¹ of ethanol or approximately twice the ethanol yield potential of corn and 30% greater than the average sugarcane productivity. It is best suited for ethanol production because of its higher total reducing sugar content and more sugar content compared to sugarcane juice (Huligol et al. 2004). The presence of reducing sugars in sweet sorghum prevents crystallization. Some sweet sorghum cultivars have recorded over 90% fermentation efficiency (Ratnavathi et al. 2004; Kumar et al. 2013). The bagasse, the cellulosic residue

✉ Subramaniam Gopalakrishnan
s.gopalakrishnan@cgiar.org

¹ International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana 502 324, India

² ICAR-Indian Institute of Millets Research (IIMR), Rajendranagar, Hyderabad, Telangana 500 030, India

³ Centre for Biotechnology, Jawaharlal Nehru Technological University (JNTU), Kukatpally, Hyderabad, Telangana 500 085, India

leftover after extraction of juice from the stalk, representing about 30% of the plant fresh weight, can be used as livestock feed and for cogeneration of power. The other option for bagasse utilization is soil amendment. However, the direct incorporation of bagasse into the soil is not advisable because they may cause immobilization of soil nitrogen and phytotoxicity (Swaragaonkar et al. 2013). Composting of sweet sorghum bagasse is one of the best ways of transforming them into more stable end product, which is safe and beneficial to plant growth.

Composting is the biochemical transformation of organic wastes by naturally occurring or inoculated microbes to organic fertilizer and soil conditioners through biological processes (Gautam et al. 2010). It is done by diverse microorganisms including bacteria, fungi and actinomycetes such as *Bacillus* sp., *Pseudomonas* sp., *Aspergillus* sp., *Streptomyces rectus*, *Thermomonospora fusca*, *Thermopolyspora bisporea*, *Thermomonospora curvata* and *Thermoactinomyces* sp. (Zeng et al. 2011). The composting process involves three phases. First phase is done by mesophilic microbes where the substrate is reduced due to the degradation of sugar and proteins (Novinsak et al. 2008). Second phase is done by thermophilic microbes where the temperature of the compost piles increases from 45 to 70 °C (Schloss et al. 2003). The third phase begins with the decrease in temperature of the compost pile where beneficial microbes are added to enhance the quality of compost. The quality of compost produced by microbes is mostly dependent on its raw materials (Wang et al. 2004). However, other traits that can make good-quality compost are C:N ratio, pH, moisture content and the presence of plant growth-promoting microbes (Fourti et al. 2011). Application of compost not only benefits crop plants as it contains beneficial microbes that help the plants to mobilize and acquire nutrients but also promotes plant growth and inhibits plant pathogens (Perner et al. 2006). The objective of this study was to develop protocol for rapid decomposition of sweet sorghum bagasse and to evaluate the bagasse compost for plant growth promotion (PGP) in sweet sorghum.

Materials and Methods

Microbes Used

A total of six bacteria such as *Bacillus subtilis* ATCC 6633, *B. subtilis* BCB-19 (GenBank accession number: MG832888), *B. aquimaris* MP-4 (GenBank accession number: MG980053), *B. licheniformis* MP-6 (GenBank accession number: MG980062), *B. infantis* MP-7 (GenBank accession number: MH005066) and *B. flexus* MP-9 (GenBank accession number: MH005063), one

Actinobacteria *Streptomyces* sp. MA-1 (GenBank accession number: MG980058) and three fungi such as *Aspergillus awamori* (GenBank accession number: MH011355), *Myceliophthora thermophila* ATCC 48104 and *Chaetomium virescens* ATCC 32319 reported earlier to have cellulose-degrading capabilities were used in this study. Out of the ten cellulose-degrading microbes, three (*B. subtilis* ATCC 6633, *M. thermophila* ATCC 48104 and *C. virescens* ATCC 32319) were acquired from American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA, while the rest were collected from microbial gene bank, ICRISAT, Patancheru, India, and previously reported as cellulose-degrading microbes (Rupela et al. 1998; Chander et al. 2018).

Composting Protocols

The sweet sorghum (variety SSV 74) bagasse was obtained after juice extraction from the stalk. The composition of the bagasse includes moisture (85%), carbon (34%), nitrogen (1.0%), C:N ratio (34%) and pH 6.9. Composting of sweet sorghum bagasse was done by testing it in pilot-scale (15 kg) as well as large-scale (200 kg) studies.

Pilot-Scale Study

The experiment had one un-inoculated control and 10 microbial treatments: T1 = control- sweet sorghum bagasse (SB) + rice straw (RS); T2 = SB + RS + *A. awamori*; T3 = SB + RS + *B. subtilis*; T4 = SB + RS + *M. thermophila*; T5 = SB + RS + *C. virescens*; T6 = SB + RS + MA-1; T7 = SB + RS + BCB-19; T8 = SB + RS + MP-4; T9 = SB + RS + MP-6; T10 = SB + RS + MP-7; T11 = SB + RS + MP-9. Another set of 11 treatments were made with farm yard manure (FYM); here, rice straw was replaced with FYM. Hence, a total of 22 treatments were made.

Pilot-scale study was performed in cement tanks (75 cm wide × 75 cm deep) buried in the soil and covered with galvanized iron lids. In each tank, 15 kg of sun-dried sweet sorghum bagasse (without chopping) and 5 kg of rice straw/FYM were placed after moistening with water containing cow dung slurry (10 kg in 15 L water). The selected microbes were formulated as peat-based inoculants and added @ 70 g (10⁸ CFU g⁻¹) per tank in their respective treatments. Rock phosphate (5% of bagasse; powdered Mussoorie rock phosphate) and nitrogen (0.38% of bagasse; in the form of urea) were mixed thoroughly along with the contents in the tanks, including those in controls. The tank was covered with rice straw bundle in nets (so that it will not mix with the experimental part), and the lid was left undisturbed for 20 days. Moisture content of the pile was maintained between 60 and 70% throughout the experiment. At the end of 20 days, the contents in the tank

were mixed thoroughly and water was added as per requirement. The tanks were undisturbed for another 10 days. At the end of 30 days, locally available earthworms (*Eudrilus eugeniae*; 50 numbers) were released in each tank (including controls) after thorough mixing and left undisturbed for another 30 days. At 60 days after start (DAS), the experiment was terminated. Samples were taken at both 30 and 60 DAS for observing degradation % ($100 - (\text{fresh weight} - \text{dry weight}) / \text{fresh weight} \times 100$). Strength of strands of bagasse was recorded at both 30 and 60 DAS on a 1–4 rating scale. If the strings of bagasse were difficult to break they were rated 1, while a well-composted bagasse was rated 4. At 60 DAS, samples were also collected, oven-dried at 45 °C, ground into fine powder (using blender) and stored in airtight containers at 4 °C until further analysis. The powdered samples were digested as per AOAC (AOAC, 2000) and analyzed for minerals such as Fe, Zn, Cu, Ca, Mn and Mg using inductively coupled plasma-optical emission spectroscopy (ICP-OES) by the Prodigy High Dispersion ICP-OES instrument (Teledyne Leeman Labs) against known standards.

Large-Scale Study

The most promising three microbes, which degraded the sweet sorghum bagasse quickly, in the pilot-scale study, were further evaluated for their degradation potential in a large-scale study, where 200 kg sun-dried sweet sorghum bagasse was scaled up. This study was conducted on the soil surface in a field. In this experiment, a total of four treatments were made: T1 = control- sorghum bagasse (SB) + rice straw (RS); T2 = SB + RS + *A. awamori*; T3 = SB + RS + *B. subtilis*; T4 = SB + RS + *M. thermophila*. Another set of four treatments were made with FYM; here, rice straw was replaced with FYM. Hence, a total of eight treatments were made. All other experimental protocols and observations remained the same as done in pilot-scale studies.

In Vitro Plant Growth-Promoting (PGP) and Biocontrol Traits of the Selected Cellulose-Degrading Microbes

The selected 10 cellulose-degrading microbes were characterized for their enzymatic activities and secondary metabolite production traits including cellulase, lipase, protease, phosphate solubilization, chitinase, hydrocyanic acid (HCN), indole acetic acid (IAA) and siderophore. Cellulase, lipase and protease production were studied as per the methodologies in cellulose congo red agar (CCRA), Tween 80 agar (T80A) and casein agar (CA), respectively (Bhattacharya et al. 2009; Hendricks et al. 1995). Phosphate solubilization was done on National Botanical Research Institute's phosphate growth medium (NBRIP) as per the

protocols of Fiske and Subbarow (1925). Chitinase and HCN production was tested in minimal media (with 5% colloidal chitin) and yeast extract mannitol agar (YEMA with glycine by sulfocyanate method), respectively (Lorck 1948; Hirano and Nagao 1988). IAA and siderophore were quantified in yeast extract mannitol (YEM) broth (supplemented with L-tryptophan @ $1 \mu\text{g mL}^{-1}$) and King's B broth, respectively (Schwyn and Neilands 1987; Patten and Glick 2002).

The selected cellulose-degrading microbes were evaluated for their antifungal activity against *Macrophomina phaseolina* (which causes charcoal rot in sorghum) by dual-culture assay as per the protocols of Anjaiah et al. (1998). *M. phaseolina* was acquired from cereal pathology division, ICRISAT. In brief, a disk of actively growing *M. phaseolina* (5–6 mm) was placed on one edge (about 1 cm from the corner) of the glucose casamino acid yeast-extract (GCY) agar plate and selected cellulose-degrading microbe was streaked on the other edge of the plate (about 1 cm from the corner). The plates were incubated at 28 ± 2 °C for 4 days or until the *M. phaseolina* covered the entire plate in the control plate. Halo zone around the cellulose-degrading microbe was noted as positive, and the inhibition zone measured in mm.

Value Addition of Bagasse Compost with PGP Actinobacteria

In order to enhance the value of the bagasse compost, a set of five strains of *Streptomyces* (CAI-24, CAI-78, CAI-155, KAI-27 and MMA-32), and their consortia, known for their PGP, antagonistic and entomopathogenic traits (Gopalakrishnan et al. 2011a, 2011b, 2014, 2016a; Vijayabharathi et al. 2014) were incorporated with the bagasse compost and checked for their viability and count at the interval of one month for a duration of five months. For this, the five *Streptomyces* strains were inoculated on bagasse compost and allowed to multiply for 2 weeks at room temperature (28 ± 2 °C). At the end of two weeks, bagasse compost was enumerated for *Streptomyces* survival and longevity and this was considered as 0 months. The *Streptomyces* colonies were represented as colony-forming units (CFU), and the CFU was enumerated at one-month interval for a period of five months.

Evaluation of Bagasse Compost for PGP Potential on sweet Sorghum Under Greenhouse Conditions

The bagasse compost prepared with the three most promising cellulose-degrading microbes was further evaluated for their PGP traits on sweet sorghum under greenhouse conditions. In this experiment, a total of eight treatments were made: T1 = control- compost prepared with only sorghum bagasse (SB) + rice straw (RS);

T2 = compost prepared with SB + RS + *A. awamori*; T3 = compost prepared with SB + RS + *B. subtilis*; T4 = compost prepared with SB + RS + *M. thermophila*; T5 = control- compost prepared with only SB + farm yard manure (FYM); T6 = compost prepared with SB + FYM + *A. awamori*; T7 = compost prepared with SB + FYM + *B. subtilis*; and T8 = compost prepared with SB + FYM + *M. thermophila*. Three replications were made for each treatment, and the experiment was conducted in a completely randomized design (CRD). Three sets of pots were maintained for samplings at 35 DAS, 70 DAS and harvest. Soil mixture containing Vertisol, sand and bagasse compost (3:2:1) was prepared and filled in plastic pots (8"). The seeds of ICRISAT sweet sorghum variety ICSV-93046 (ICRISAT-bred high-yielding and salinity-tolerant variety released in Kazakhstan in 2016; acquired from sorghum breeding unit, ICRISAT) were surface-sterilized with 2.5% sodium hypochlorite (for 5 min) and planted. Three seeds were sown (at 2–3 cm depth) in each pot and 1 week later thinned to retain one seedling. At 35 DAS, PGP traits including plant height, leaf area, leaf weight, root weight, stem weight, root length and root volume were recorded. At 70 DAS, shoot weight and root weight, while at harvest, plant height, panicle length, shoot weight, panicle weight, seed weight and seed number were recorded. After harvest, soil samples were also collected from the pots and analyzed for microbial biomass carbon (by fumigation method), microbial biomass nitrogen (by Kjeldahl distillation method) and dehydrogenase activity (by triphenylformazan production method) (Casida 1977; Brooks et al. 1985; Anderson and Domsch 1989).

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) (GenStat 10.1, version 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) to standardize the cellulose-degrading microbes for composting sweet sorghum bagasse (in the field studies) and to evaluate the efficiency of bagasse compost application (in the greenhouse studies). Significance of differences between the treatment means was tested at $P = 0.05$.

Results

Composting Abilities of Selected Cellulose-Degrading Microbes

Pilot-Scale Study

At 30 DAS, profuse microbial growth was clearly visible in the treatments compared to un-inoculated control in both

bagasse + rice straw and bagasse + FYM treatments. Further, degradation of bagasse was found higher in the microbial treatments over un-inoculated control; however, these were not statistically significant. At 60 DAS, microbial treatments were found to significantly enhance the composting of sweet sorghum bagasse, as more than 71% of the bagasse was degraded. This was true in both rice straw- and FYM-amended treatments. Among the ten microbes studied, three (*A. awamori*, *B. subtilis* ATCC 6633 and *M. thermophila* ATCC-48104) were found to enhance the composting of sweet sorghum bagasse over other microbial treatments in both rice straw- and FYM-amended treatments. Visual rating of compost, based on the strength of bagasse strands, was also found higher in selected three microbial treatments over the other microbial and un-inoculated controls (Table 1).

At 60 DAS, in both rice straw- and FYM-amended treatments, the macro- and micronutrient contents were found more in the microbial treatments over the un-inoculated controls. Of the ten microbial treatments, the three (*A. awamori*, *B. subtilis* ATCC 6633 and *M. thermophila* ATCC-48104) were also found to enhance most of the macro- and micronutrients. Further, rice straw-amended microbial treatments showed clear advantage (more significance) of macro- and micronutrients including total N, total P, Fe, Mn, Mg, B and S over FYM-amended microbial treatments (Table 2).

Large-Scale Study

The three best performed microbes in the pilot-scale study, such as *A. awamori*, *B. subtilis* ATCC 6633 and *M. thermophila* ATCC-48104, were further evaluated in a large-scale study, where 200 kg bagasse was used. As observed in the pilot study, rice straw was found better than FYM as amendment. Further, degradation and visual ratings were found significantly better in *A. awamori* and *M. thermophila* ATCC-48104 treatments over *B. subtilis* ATCC 6633 treatment (Table 3). As observed in the pilot-scale study, the microbial treatments enhanced macro- and micronutrient contents over the un-inoculated control, but these were not significant in the large-scale study (Table 4).

In Vitro PGP and Biocontrol Traits of the Selected Cellulose-Degrading Microbes

When the ten cellulose-degrading microbes were evaluated for their enzymatic activities and secondary metabolite production, all of them produced cellulase, lipase (except *M. thermophila* ATCC-48104 and *C. virescens*), protease (except BCB19, *A. awamori* and *C. virescens*) and HCN (except *A. awamori* and *M. thermophila* and *C. virescens*).

Table 1 Composting capabilities of selected cellulose-degrading microbes on sweet sorghum bagasse—pilot-scale study

Treatment	Degradation % at 30 DAS	Visual rating# at 30 DAS	Degradation % at 60 DAS	Visual rating# at 60 DAS
SB + RS (control)	67	2	70	2
SB + RS + <i>A. awamori</i>	74	3	78***	4
SB + RS + ATCC 6633	71	3	80***	4
SB + RS + <i>M. thermophila</i>	74	3	80***	4
SB + RS + <i>C. virescens</i>	68	2	77***	3
SB + RS + MA-1	70	3	80***	4
SB + RS + BCB-19	68	2	78***	4
SB + RS + MP-4	68	2	78***	4
SB + RS + MP-6	72	2	77***	3
SB + RS + MP-7	72	3	78***	4
SB + RS + MP-9	71	3	78***	4
Mean	77	3	78	4
LSD (5%)	7.9	0	2.2	0
CV %	5	0	1	0
SB + FYM (control)	63	2	65	2
SB + FYM + <i>A. awamori</i>	59	3	69***	3
SB + FYM + ATCC 6633	62	3	73***	3
SB + FYM + <i>M. thermophila</i>	64	2	71***	3
SB + FYM + <i>C. virescens</i>	65	2	75***	3
SB + FYM + MA-1	68*	3	73***	3
SB + FYM + BCB-19	65	2	71***	2
SB + FYM + MP-4	62	2	69***	2
SB + FYM + MP-6	66	3	68***	2
SB + FYM + MP-7	65	3	70***	2
SB + FYM + MP-9	65	3	67***	2
Mean	64	3	70	2
LSD (5%)	3.9	0	2.5	0
CV %	3	0	2	0

Visual rating # 1 = not possible to break the string of bagasse; 2 = possible (with difficulty) to break the string of bagasse; 3 = easy to break the string of bagasse; 4 = very easy to break the string of bagasse

SB sorghum bagasse; RS rice straw; FYM farm yard manure; LSD least significant deviation; CV coefficient of variation

*Statistically significant at 0.05; ***statistically significant at 0.001

Chitinase was produced by only *B. subtilis* ATCC 6633 and MA-1, whereas P was solubilized by only *B. subtilis* ATCC 6633, MP-9 and *A. awamori*. All the microbes produced IAA, but MP-9 and MA-1 produced more than 50 µg ml⁻¹ of culture filtrate, whereas only *A. awamori* produced siderophore. In the dual-culture assay, only three microbes (*B. subtilis* ATCC 6633, BCB-19 and MA-1) were found to inhibit *M. phaseolina* (Table 5).

Value Addition of Bagasse Compost with PGP Actinobacteria

The results showed that all five PGP *Streptomyces* strains and their consortia not only survived but also multiplied (from 10⁶⁻⁷ CFU/ml to 10⁷⁻⁸ CFU/ml) in the 5-month

study (Table 6). Hence, it is concluded that value addition of bagasse compost could be done with PGP microbes.

Evaluation of Bagasse Compost for PGP Potential on Sweet Sorghum

At 35 DAS, bagasse compost prepared with the selected cellulose-degrading microbes, *A. awamori*, *B. subtilis* ATCC 6633 and *M. thermophila* ATCC-48104 with rice straw amendments, significantly enhanced the agronomic traits including the plant height (59–77%), leaf area (294–486%), leaf weight (167–440%), root weight (154–197%), shoot weight (89–181%), root length (52–93%) and root volume (7–11%) over the control bagasse compost, prepared without cellulose-degrading

Table 2 Micronutrient analysis of bagasse compost prepared using selected cellulose-degrading microbes—pilot-scale study (at 60 DAS)

Treatment	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Ca (ppm)	Mg (ppm)	B (ppm)	S (ppm)	Total (N%)	Total (P%)	Total (K%)	Ash (%)
SB + RS (control)	2930	72	9.8	192	27758	4974	13.9	1815	2.05	1.00	2.23	25.9
SB + RS + <i>A. awamori</i>	3419	73	10.5	268***	33,657	5450***	19.2***	1925***	2.21***	1.24***	2.64***	33.3***
SB + RS + ATCC 6633	4683***	72	11.0	303***	38,792**	5732***	24.6***	2073***	2.21***	1.35***	2.09	31.7***
SB + RS + <i>M. thermophila</i>	5054***	78**	10.3	315***	42,264**	5293***	24.7***	1874	2.19***	1.34***	1.92	35.7***
SB + RS + <i>C. virescens</i>	6246***	77	12.3*	226***	32,311	6421***	29.4***	1917***	1.88	1.01	1.68	32.8***
SB + RS + MA-1	4473***	72	11.0	250***	26,473	6312***	25.4***	1960***	1.92	0.98	1.71	29.3***
SB + RS + BCB-19	5581***	72	10.9	227***	27,994	6265***	28.5***	2044***	2.02	1.03	1.89	33.2***
SB + RS + MP-4	6770***	74	13.4*	234***	29,568	6720***	33.2***	1970***	2.05	1.05	1.90	34.9***
SB + RS + MP-6	7480***	75**	12.3*	234***	32,598	6001***	33.4***	1721	1.87	1.04	1.72	35.7***
SB + RS + MP-7	4646***	73	11.8*	217	29,094	6914***	25.5***	1919***	2.11	1.11	1.99	30.3***
SB + RS + MP-9	5523***	73	11.1	216	28,558	6536***	27.7***	2017***	2.09	1.10	2.12	33.2***
Mean	5164***	74	11.3	244	31,733	6047	26.0	1930	2.05	1.11	1.99	32.4
LSD (5%)	1360.9	2.9	1.70	26.3	6366.3	244.8	1.96	85.6	0.080	0.109	0.085	2.59
CV %	12	2	7	5	9	2	3	2	2	4	2	4
SB + FYM (control)	7111	73	14.1	206	33,639	4644	30.8	1560	1.55	1.04	1.57	44.7
SB + FYM + <i>A. awamori</i>	7248	85	14.9	252***	34,866	5223**	36.7*	1960***	1.84*	1.19**	1.68***	46.2
SB + FYM + ATCC 6633	7148	74	14.4	219	35,178	4710	32.1	1582	1.58	1.12	1.62	45.2
SB + FYM + <i>M. thermophila</i>	9975***	73	13.8	276***	38,148	4680	38.7*	1588	1.51	0.97	1.31	51.9**
SB + FYM + <i>C. virescens</i>	6689	87	14.6	205	26,490	5596**	34.5	1528	1.66	0.95	1.49	46.0
SB + FYM + MA-1	7630	95**	14.5	223	29,950	5568**	37.1*	1538	1.44	0.98	1.29	45.4
SB + FYM + BCB-19	7889	74	13.4	200	24,208	4843	36.4*	1528	1.39	0.77	1.78***	49.3**
SB + FYM + MP-4	8520***	93**	15.0	231***	32,945	5360**	40.2*	1538	1.50	0.98	1.73	51.0**
SB + FYM + MP-6	7832	82	13.6	192	30,524	5067	36.9*	1408	1.58	0.97	1.77***	54.3**
SB + FYM + MP-7	7640	95**	12.9	196	35,850	5545**	34.9*	1470	1.55	0.94	1.51	48.1
SB + FYM + MP-9	7472	84	14.0	201	28,355	5576**	35.3*	1572	1.74	0.93	1.73	45.7
Mean	7741	83	14.1	218	32,105	5165	35.8	1570	1.57	0.98	1.59	48.0
LSD (5%)	884.9	13.2	1.08	20.9	6976.6	488.3	4.13	130.4	0.204	0.141	0.089	3.66
CV %	5	7	4	4	10	4	5	4	6	6	3	3

LSD least significant deviation; CV coefficient of variation; SB sorghum bagasse; RS rice straw; FYM farm yard manure

*Statistically significant at 0.05; **statistically significant at 0.01; ***statistically significant at 0.001

Table 3 Composting capabilities of selected promising cellulose-degrading microbes on sweet sorghum bagasse—large-scale study

Treatment	Degradation % at 30 DAS	Visual rating# at 30 DAS	Degradation % at 60 DAS	Visual rating# at 60 DAS
SB + RS (control)	63	1	68	2
SB + RS + <i>A. awamori</i>	71*	3	74**	4
SB + RS + ATCC 6633	65	2	69	2
SB + RS + <i>M. thermophila</i>	68*	2	74**	4
Mean	67	2	71	3
LSD (5%)	4.9	0	2.3	0
CV %	2	0	1	0
SB + FYM (control)	61	1	63	2
SB + FYM + <i>A. awamori</i>	61	2	64	3
SB + FYM + ATCC 6633	61	1	66	3
SB + FYM + <i>M. thermophila</i>	63*	3	68	3
Mean	62	2	65	3
LSD (5%)	2.0	0	5.9	0
CV %	1	0	3	0

Visual rating # 1 = not possible to break the string of bagasse; 2 = possible (with difficulty) to break the string of bagasse; 3 = easy to break the string of bagasse; 4 = very easy to break the sting of bagasse

*Statistically significant at 0.05; **statistically significant at 0.01; *SB* sorghum bagasse; *RS* rice straw; *FYM* farm yard manure; *LSD* least significant deviation; *CV* coefficient of variation

Table 4 Micronutrient analysis of bagasse compost prepared using selected promising cellulose-degrading microbes—large-scale study at 60 DAS

Treatment	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Ca (ppm)	Mg (ppm)	Total (N%)	Total (P%)	Total (K%)	Ash (%)
SB + RS (control)	6268	60.5	11.1	777	40,132	4986	1.71	1.07	1.45	34.1
SB + RS + <i>A. awamori</i>	6507	60.7	11.0	880	50,818*	5006	1.96**	1.29*	1.57*	35.2
SB + RS + ATCC 6633	8096*	66.2*	14.0*	830	40,854	5338*	1.83**	1.12	1.59*	37.2*
SB + RS + <i>M. thermophila</i>	5362	62.0	13.4*	710	44,368	4734	1.86**	1.11	1.46	34.8
Mean	6558	62.2	12.4	800	44,043	5016	1.84	1.14	1.51	35.3
LSD (5%)	1732.8	3.19	2.10	311.3	5671.2	302.2	0.095	0.151	0.107	1.8
CV %	8	2	5	12	4	2	2	4	2	2
SB + FYM (control)	5088	76.4	21.0	720	31,798	5010	1.61	0.98	1.07	43.6
SB + FYM + <i>A. awamori</i>	5404***	81.3	20.5	734	32,302	5519**	1.61	1.10*	1.19*	56.0**
SB + FYM + ATCC 6633	12,177***	77.6	20.2	1116***	36,781	5400**	1.54	0.94	1.27*	52.0**
SB + FYM + <i>M. thermophila</i>	7034***	106.4***	21.9	712	35,025	5521**	1.97**	1.21*	1.13	48.2**
Mean	7426	85.4	20.9	820	34,102	5362	1.68	1.05	1.16	49.9
LSD (5%)	371.0	5.17	1.66	61.3	5647.7	218.8	0.175	0.152	0.091	4.05
CV %	2	2	3	2	5	1	3	5	2	3

SB sorghum bagasse; *RS* rice straw; *FYM* farm yard manure; *LSD* least significant deviation; *CV* coefficient of variation

*Statistically significant at 0.05; **statistically significant at 0.01; ***statistically significant at 0.001

microbes. However, such enhancement was not found with FYM and cellulose-degrading microbial combinations (Table 7).

At 70 DAS, bagasse compost prepared with rice straw and cellulose-degrading microbes significantly enhanced

root weight (32–58%) and shoot weight (66–136%) over the control bagasse compost. When the FYM was used, significant enhancement was found only with *A. awamori* (56% for root weight and 20% for shoot weight) and *B. subtilis* ATCC 6633 (47% for root weight and 8% for shoot

Table 5 *In vitro* PGP and biocontrol traits of selected cellulose-degrading microbes

Isolate	Production score for						IAA (µg/ml)	Siderophore %	<i>M. phaseolina</i> (Inhibition zone in mm)
	Cellulase	Lipase	Protease	P	Chitinase	HCN			
ATCC 6633	4	2	3	1	1	3	8.7	0	3
BCB-19	4	3	0	0	0	2	13.9	0	6
MP-4	3	3	2	0	0	1	13.2	0	0
MP-6	3	3	2	0	0	2	12.3	0	0
MP-7	3	2	3	0	0	3	9.3	0	0
MP-9	3	3	3	2	0	1	77.5	0	0
MA-1	4	5	4	0	3	2	57.3	0	14
<i>A. awamori</i>	3	5	0	4	0	0	8.1	36	0
<i>M. thermophila</i>	4	0	2	0	0	0	16.5	0	0
<i>C. virescens</i>	3	0	0	0	0	0	12.0	0	0
Mean	3	3	2	1	0	1	22.9	4	2
LSD (5%)	0	0.3	0.4	0.3	0	0	0.50	0.8	0.3
CV %	0	7	12	25	0	0	1	13	8

The rating scale for cellulase, lipase, protease, phosphate solubilization and chitinase is as follows: 1 = 0–10; 2 = 11–20; 3 = 21–30; 4 = 31–40; 5 = 41–50. The rating scale for HCN is as follows: 0 = no color change; 1 = light reddish brown; 2 = medium reddish brown; 3 = dark reddish brown

P phosphate solubilization; HCN hydrocyanic acid; IAA indole acetic acid; ATCC 6633 = *Bacillus subtilis* ATCC 6633; BCB-19 = *Bacillus subtilis* BCB-19; MP-4 = *B. aquimaris* MP-4; MP-6 = *B. licheniformis* MP-6; MP-7 = *B. infantis* MP-7; MP-9 = *B. flexus* MP-9; MA-1 = *Streptomyces* sp. MA-1; *A. awamori* = *Aspergillus awamori*; *M. thermophila* = *Myceliophthora thermophila* ATCC 48104; *C. virescens* = *Chaetomium virescens* ATCC 32319; SE standard error; LSD least significant deviation; CV coefficient of variation

Table 6 Value addition of the bagasse compost with plant growth-promoting Actinobacteria

Strains	Colony-forming units (CFU/ml) at different months (values are mean of 3 replications)					
	0th day	1st month	2nd month	3rd month	4th month	5th month
<i>Streptomyces</i> sp. CAI-24	0.92×10^{-6}	0.37×10^{-8}	0.39×10^{-8}	0.13×10^{-8}	0.12×10^{-8}	0.12×10^{-8}
<i>Streptomyces</i> sp. CAI-78	0.21×10^{-7}	0.72×10^{-7}	0.16×10^{-8}	0.14×10^{-8}	0.29×10^{-8}	0.66×10^{-8}
<i>Streptomyces</i> sp. CAI-155	0.2×10^{-7}	0.15×10^{-7}	0.10×10^{-8}	0.11×10^{-8}	0.83×10^{-8}	0.53×10^{-8}
<i>Streptomyces</i> sp. KAI-27	0.2×10^{-6}	0.3×10^{-8}	0.12×10^{-8}	0.4×10^{-8}	0.59×10^{-8}	0.56×10^{-8}
<i>Streptomyces</i> sp. MMA-32	0.36×10^{-7}	0.43×10^{-7}	0.11×10^{-8}	0.14×10^{-8}	0.15×10^{-8}	0.5×10^{-8}
Consortia	0.52×10^{-6}	0.28×10^{-8}	0.34×10^{-8}	0.14×10^{-8}	0.17×10^{-8}	0.35×10^{-7}
Control	0.7×10^{-7}	0.4×10^{-7}	0.6×10^{-7}	0.73×10^{-7}	0.7×10^{-7}	0.63×10^{-7}

weight) but not with *M. thermophila* ATCC-48104 over the control bagasse compost (Table 8).

At harvest, bagasse compost prepared with rice straw and cellulose-degrading microbes significantly enhanced plant height (5–18%), shoot weight (42–85%), panicle dry weight (66–104%), seed weight (58–188%) and seed number (25–160%) over the control bagasse compost. When the FYM was used, such enhancement was found only with FYM and *A. awamori* treatments (Table 8). At harvest, microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activity of the pot soils were also found significantly enhanced by 15–158%,

793–1993% and 55–142%, respectively, in the rice straw-amended treatments and by 24–105%, 32–220% and 6–74%, respectively, in the FYM-amended treatments. The enhancement was found higher in rice straw-amended treatments over FYM-amended treatments (Table 9).

Discussion

Sweet sorghum bagasse has multiple uses including a source for energy cogeneration, animal feed, lingo cellulosic biofuel feedstock and manure. In the production-to-

Table 7 Effect of sweet sorghum bagasse compost (prepared with rice straw and farm yard manure as amendments and cellulose-degrading microbes) on growth performance of sweet sorghum under greenhouse conditions—at 35 days after sowing

Compost prepared with	Plant height (cm)	Leaf area (cm ²)	Leaf weight (g plant ⁻¹)	Root weight (g plant ⁻¹)	Shoot weight (g plant ⁻¹)	Root length (cm m ⁻³)	Root volume (cm ⁻²)
SB + RS (control)	50	79	0.207	0.147	0.190	3.76	1.33
SB + RS + A. <i>awamori</i>	86**	407***	1.117**	0.437**	0.400*	7.24*	1.48**
SB + RS + ATCC 6633	79**	311***	0.553	0.373**	0.360	6.00*	1.43**
SB + RS + M. <i>thermophila</i>	89**	463***	1.103**	0.410**	0.533*	5.72*	1.43**
Mean	76	315	0.745	0.342	0.371	5.68	1.42
LSD (5%)	13.6	99.6	0.374	0.156	0.184	1.81	0.067
CV %	9	16	25	23	25	16	2
SB + FYM (control)	98	738	1.900	0.500	1.273	8.16	1.39
SB + FYM + A. <i>awamori</i>	100	764	1.903	0.593	1.387	8.44	1.43
SB + FYM + ATCC 6633	100	738	1.930	0.540	1.510***	8.31	1.41
SB + FYM + M. <i>thermophila</i>	104	772	2.023	0.637	1.993***	8.83*	1.45
Mean	101	753	1.940	0.568	1.541	8.43	1.42
LSD (5%)	10.2	107.0	0.884	0.158	0.216	0.49	0.079
CV %	5	7	23	14	7	3	3

LSD least significant deviation; CV coefficient of variation; SB sorghum bagasse; RS rice straw; FYM farm yard manure

*Statistically significant at 0.05; **statistically significant at 0.01; ***statistically significant at 0.001

consumption sweet sorghum value chain, it is important to work out the trade-offs between its multiple uses, as a source of biofuel and carbon balance. In this context, recycling of sweet sorghum bagasse into manure/compost and using it in agriculture is an environmentally safe option of sequestering carbon in the soil (Prakasham et al. 2012; Swaragaonkar et al. 2013). The major difficulty associated with utilization of sweet sorghum bagasse is the rigid barrier of lignin that sandwiches the cellulose underneath its recalcitrant structure (Mishra et al. 2017). Hence, there is a need for identification of good decomposing microbe. The ability of microbes to produce ligninolytic enzymes such as laccase, polyphenol oxidase, lignin peroxidase, aryl alcohol oxidase and manganese peroxidase and cellulolytic enzymes such as cellulase plays a greater role in decomposing lignocellulosic biomass (Meehnian et al. 2016; Mishra et al. 2017). Therefore, in the present study, a total of 10 lignin and cellulose-degrading microbes were selected.

Sweet sorghum bagasse is composed of cellulose (up to 25%), hemicellulose (up to 50%) and lignin (up to 25%) and calorie up to 4125 kcal Kg⁻¹ (ash-free) (Grassi 2001). However, the composition varies with the genotype, whereas the most elite genotypes possess 35–40% cellulose

and 30–35% hemicellulose and 20–25% lignin. The sweet sorghum bagasse contains low levels of NPK and micronutrients but rich in organic C resulting in high C:N ratio (De Bertoldi et al. 1985). The direct application of bagasse into the soil causes immobilization of soil nitrogen (Sawargaonkar et al. 2013). Therefore, N-rich organic material needs to be added for better decomposition of sweet sorghum bagasse. Negro et al. (1999) used pig slurry and sewage sludge as raw material/amendment for composting sweet sorghum bagasse. In the present investigation, in order to bring down the C:N ratio, organic wastes which are easily available such as rice straw or FYM (at 25%) were used while composting sweet sorghum bagasse. Rupela et al. (1998) advocated the usage of phosphorous and nitrogen for rapid composting of crop residues. Therefore, in the present study, rock phosphate (at 5% of bagasse as Mussoorie rock phosphate) and nitrogen (at 0.38% of bagasse as urea) were incorporated while composting sweet sorghum bagasse.

Vermicomposting is known to hasten the decomposition of organic residues through physical breakdown of the raw biomass by the introduced earthworms. Further, the microbes present in the gut of earthworms have higher potential in digesting organic residues (Aira et al. 2007).

Table 8 Effect of sweet sorghum bagasse compost (prepared with rice straw and farm yard manure as amendments and cellulose-degrading microbes) on growth performance of sweet sorghum under greenhouse conditions—at 70 days after sowing and harvest

Compost prepared with	At 70 DAS		At final harvest					
	Root weight (g plant ⁻¹)	Shoot weight (g plant ⁻¹)	Plant height (cm)	Panicle length (cm)	Shoot weight (g plant ⁻¹)	Panicle weight (g plant ⁻¹)	Seed weight (g plant ⁻¹)	Seeds (plant ⁻¹)
SB + RS (control)	1.79	12.97	167	11.7	12.74	4.97	2.71	131
SB + RS + <i>A. awamori</i>	2.83*	30.65**	198**	14.7***	22.07**	10.12*	7.80***	341**
SB + RS + ATCC 6636	2.36*	29.29**	175	11.7	18.11**	8.29*	4.41***	164
SB + RS + <i>M. thermophila</i>	2.79*	21.54**	179**	11.7	23.54**	8.25*	4.27***	169
Mean	2.45	23.60	180	12.4	19.11	7.91	4.80	201
LSD (5%)	0.638	7.44	10.6	0.99	5.184	2.918	1.331	95.0
CV %	13	16	3	4	14	19	14	24
SB + FYM (control)	4.71	64.31	207	15.7	37.43	13.77	11.04	421
SB + FYM + <i>A. awamori</i>	7.34**	77.23**	208	16.0	42.82	17.67*	14.11*	552**
SB + FYM + ATCC 6633	6.91**	69.74**	210	17.0*	59.63***	17.00*	14.45*	429
SB + FYM + <i>M. thermophila</i>	5.04	67.22	227***	16.3	42.05	13.85	11.23	447
Mean	6.00	69.63	213	16.3	45.48	15.57	12.71	462
LSD (5%)	1.459	5.307	5.1	0.75	6.893	2.964	2.278	47.4
CV %	12	4	1	2	8	10	9	5

LSD least significant deviation; CV coefficient of variation; SB sorghum bagasse; RS rice straw; FYM farm yard manure

*Statistically significant at 0.05; **statistically significant at 0.01; ***statistically significant at 0.001

Therefore, in the present investigation, locally available earthworms were introduced into the composting heap at 30 days after start of the experiment.

In the present study, 68–70% and 63–65% of degradation of sweet sorghum bagasse were observed when selected microbes with rice straw and/or FYM were added, respectively, in the pilot- and large-scale studies. Among the ten microbes studied, *A. awamori*, *B. subtilis* ATCC 6633 and *M. thermophila* ATCC-48104 were found to enhance the composting process over other microbes in both rice straw- and FYM-amended treatments and in both pilot- and large-scale studies. In the present investigation, degradation % of bagasse was found higher than those reported in the literature by other researchers. For instance, while using sewage sludge and/or pig slurry with sweet sorghum bagasse, Negro et al. (1999) reported 60–63% of degradation at the end of fermentation and maturation. In the present study, higher macro- and micronutrient contents were observed in the cellulose-degrading microbes inoculated treatments over un-inoculated control. This may be due to better degradation of bagasse as a result of higher microbial activity. Shah et al. (2015) reported

vermicomposting of sugar industry wastes (including bagasse) with FYM producing best-quality manure with enriched nutritional status comprising more OC (4%), N (3%), P (2%), K (7%), Ca (3.5%), Na (2.5%) and B (twofold) when compared to natural composting. Microbes are reported widely to enhance macro- and micronutrient contents of the harvested grains of many crops including wheat, cowpea, faba bean, lentil, chickpea and pigeon pea (Vidal-valverde et al. 1998; Adebooye and Singh 2007; Hefnawy 2011; Gopalakrishnan et al. 2016b; Sathya et al. 2016). It is concluded that the three selected microbes used in this study help to decompose sweet sorghum bagasse efficiently and hence could be formulated, mass-multiplied and supplied to farmers for degradation of sweet sorghum bagasse under on-farm conditions.

In the present study, the selected 10 cellulose-degrading microbes were found to produce IAA, cellulase, lipase (except two), protease (except three) and HCN (except three). Further, siderophore was produced only by *A. awamori* and chitinase by *B. subtilis* ATCC 6633 and MA-1. P was solubilized only by *B. subtilis* ATCC 6633, MP-9 and *A. awamori*. Microbes producing IAA are widely

Table 9 Effect of sweet sorghum bagasse compost (prepared with rice straw and farm yard manure as amendments and cellulose-degrading microbes) on soil biological traits at the harvesting stage in sweet sorghum

Treatment	Microbial biomass C ($\mu\text{g g}^{-1}$ soil)	Microbial biomass N ($\mu\text{g g}^{-1}$ soil)	Dehydrogenase activity ($\mu\text{g TPF g}^{-1}$ soil 24 h^{-1})
SB + RS (control)	1.22	1.2	8.1
SB + RS + <i>A. awamori</i>	2.00***	16.7***	12.5***
SB + RS + ATCC 6633	3.15***	24.5***	13.1***
SB + RS + <i>M. thermophila</i>	1.40***	10.5***	19.6***
Mean	1.94	13.2	13.3
LSD (5%)	0.141	1.20	1.04
CV %	4	5	4
SB + FYM (control)	1.09	13.2	10.7
SB + FYM + <i>A. awamori</i>	1.54***	42.2***	11.4
SB + FYM + ATCC 6633	2.24***	17.4***	18.7***
SB + FYM + <i>M. thermophila</i>	1.36***	24.7***	12.6***
Mean	1.56	24.4	13.4
LSD (5%)	0.09	3.1	0.67
CV%	3	6	3

LSD least significant deviation; CV coefficient of variation; SB sorghum bagasse; RS rice straw; FYM farm yard manure

***Statistically significant at 0.001

reported to stimulate germination of seed and promotion of roots and shoots. This helps the plant to access water and nutrients from deeper soil (Khamna et al. 2009). Microbes producing extracellular enzymes including cellulase, lipase and protease are reported to control plant pathogens, by acting on their cell walls (Ellis et al. 2000; Lynd et al. 2002; Haas and Defago 2005). Cell walls of plant pathogenic fungi and insect pests contain chitin. Microbes producing chitinase enzyme are widely reported to cleave/degrade the chitin and thus help to control plant pathogens and insect pests (Shapira et al. 1989; Yandigeri et al. 2015). HCN-producing microbes are reported to help the plants in disease suppression (Haas et al. 1991; Siddiqui 2006). Siderophore-producing microbes function as solubilizing agents for iron (act as iron chelators) under conditions of iron limitation and thus help the plants to inhibit the growth of phytopathogens (Tokala et al. 2002). Phosphorous (P) is abundant in most agricultural soils, but it is unavailable to plants, due to low level of soluble in nature. P-solubilizing bacteria such as *Burkholderia cepacia* are reported to solubilize total soil P through mineralization (Zhao et al. 2014). When these microbes were tested for their antagonistic potential against *M. phaseolina* by dual-culture assay, only three microbes (*B. subtilis* ATCC 6633, BCB-19 and MA-1) were found to inhibit *M. phaseolina*. It is concluded that the cellulose-degrading microbes used in this study produced one or more extracellular enzymes and

growth-promoting hormones and thus can be exploited for PGP and biocontrol of plant pathogens.

Beneficial microbes when introduced into a new environment and/or field conditions often find it hard to establish a niche for their survival among the predators and competitors (such as native microbes) in addition to unpredictable environmental conditions. Several other factors including soil type, plant species, sunlight and inoculant density also play an important role in survival and functions of the introduced beneficial microbes (Hughes et al. 1997; Shapiro and Argauer 1997; Yu and Brown 1997; Slininger et al. 2003). Hence, the viability of introduced PGP microbe in an appropriate formulation for a certain length of time is essential for their success. For instance, *Bacillus*, *Pseudomonas* and *Ochrobactrum* formulations are reported to survive up to one year in bioformulations including compost (Trivedi et al. 2005; Chakraborty et al. 2009, 2013). In the present study, different bagasse composts were evaluated for their compatibility with well-known PGP microbes for enhancing their value addition. These results clearly demonstrated that the selected five PGP *Streptomyces* strains and their consortia not only survived but also multiplied in the 5-month study. It is concluded that value addition of bagasse compost could be done with PGP microbes. Further, bagasse compost could be exploited for field application of PGP microbes.

When the bagasse compost prepared with the cellulose-degrading microbes with rice straw amendments was evaluated for their PGP potential on sweet sorghum, significant enhancement was observed on the agronomic performances including the plant height, leaf area, leaf weight, root weight, shoot weight, root length, root volume, panicle weight, seed weight and seed number over the control bagasse compost (prepared without microbes). However, such enhancement was not consistently visible with the cellulose-degrading microbes and FYM combinations. Further, at harvest, microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activity of the pot soils were also found significantly enhanced in both microbes with rice straw- and/or FYM-amended treatments. Application of vermicompost not only benefits crop plants as it contains beneficial microorganisms that help the plants to mobilize and acquire macro- and micronutrients, but also promotes plant growth and inhibits phytopathogens. This has been demonstrated by many researchers including Suthar et al. (2005), Perner et al. (2006) and Nath and Singh (2009).

Conclusions

This study was carried out to standardize the protocol to degrade the sweet sorghum bagasse quickly and to evaluate the bagasse compost for PGP traits in sweet sorghum. Among the ten cellulose-degrading microbes studied in this investigation, *A. awamori*, *B. subtilis* ATCC 6633 and *M. thermophila* ATCC-48104 were found to enhance the bagasse composting process over other microbes in both rice straw- and FYM-amended treatments and in both pilot- and large-scale studies. The selected three microbes produced several hydrolytic enzymes and hormones. The usefulness of bagasse compost for PGP in sweet sorghum was also demonstrated under greenhouse conditions. Therefore, the selected three microbes could be formulated, mass-multiplied and supplied to farmers for degradation of sweet sorghum bagasse under on-farm conditions after checking the effectiveness of bagasse compost under field conditions. Further, the value addition of bagasse compost could also be done with well-known PGP microbes.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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