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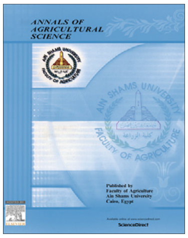

Biotechnological Application of Thermotolerant Cellulose-Decomposing Bacteria in Composting of Rice Straw

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Compost additives;
Composting process

Abstract The main objective of this study is to investigate the potentiality of cellulase producing isolates, *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v, as microbial additives on composting of rice straw materials. Different pyramid shape piles were constructed, and each contained 80 kg of rice straw and 288 kg of cattle manure, with/without 5 kg of feldspar supplementation (0.75%, *w/w*). *C/N* ratio was adjusted to 35.8 before microbial inoculation. The piles were inoculated with either single strain or mixed cultures. The physical, chemical and biological parameters indicating the decomposition of organic material, maturation and quality of the compost product were investigated during the composting process. A rapid increase in compost temperature was obtained in inoculated piles. Moreover, piles containing mixed inoculants exhibited longer time at high temperatures >55 °C for 15 consecutive days compared to control treatments that lasted for only 5 consecutive days. The microbial inoculation had greatly reduced the composting time by 40–43% (from 89–96 days to 51–58 days). Additionally, it resulted in a higher decrease in the total organic carbon and carbon-to-nitrogen ratio, as well as increase in compost quality by an increase in total nitrogen, phosphorus, and potassium content. The analysis of moisture content, bulk density, pH value, electrical conductivity, phytotoxicity, and pathogenic bacterial content of the final compost products exhibited maturation and good quality of final product to be used without any limitations.

Introduction

Egypt is the largest producer of rice in Africa with supplying of 5.9 million tons in 2013. It is estimated that rice straw was the most abundant lignocellulosic agro-residues in Egypt of about

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3.1 million tons are produced annually (Abdelhady et al., 2014). With the increase in rice production, more straws are being left unused that are poorly disposed as waste or burnt directly in fields producing high amount of dust particles and ashes constituting serious pollution problems. To lessen the disposal problem on the environment and solve the environmental pollution, fast and efficient utilization of rice straw has become quite important. Rice straw contains principally cellulose and hemicellulose which are biopolymers together with lignin; therefore, various value added products can be produced via microbial fermentation processes by using these materials either directly or after pretreatments (Abdel-Rahman et al., 2011; Sakdaronnarong and Jonglertjunya, 2012).

Composting is one of the useful methods that can directly consume large amount of these wastes for compost production that can be used as a source of nutrients to improve soil structure, increase its organic matter, and enhance plant growth. Traditionally, composting process is a time-consuming process that goes through four phases: (1) preparatory stage (mesophilic phase, 20–40 °C) which initiates organic matter decomposition; (2) thermophilic phase (40–60 °C) due to the extensive metabolic activities by endogenous microorganisms; (3) second mesophilic phase allowing re-establishment of the heat resistant microbes; and (4) maturity phase of constant nutrient contents (Liu et al., 2011).

The compost must be in a high degree of maturity and stability for safe application in agriculture without any adverse effects on plants (Qian et al., 2014). There are several factors affecting the maturation of composting process including carbon to nitrogen ratio (C/N), surface area and raw material particle size, aeration, moisture content, temperatures, pH, chemical and biological additives, and toxic substances (Bernal et al., 2009; El-Haddad et al., 2014; Jiang et al., 2015; Kausar et al., 2014; Savage and Diaz, 2007; Zeng et al., 2012). Of these factors, the inoculation of microbial additives that can tolerate composting condition, accelerate the composting process and increase nutrients is worthy to study. Jusoh et al. (2013) reported an increase in the mineralization in composting of rice straw with goat manure by the application of commercial effective microorganisms (EM·1®) from Japan (consisted of consortia of beneficiary microorganisms). Lim et al. (2014) found that inoculation of microbial additive (EM·1™) improved the efficiency of oil palm empty fruit bunches decomposition in composting process.

The aim of this study was to evaluate the application of efficient thermotolerant cellulase producing Bacilli, *Bacillus licheniformis* 1-1v and *Bacillus sonorensis* 7-1v (Abdel-Rahman et al., 2015a,b), either as single strain or mixed cultures (bacterial consortium) on the maturation and quality of compost produced by composting of rice straw with cattle manure.

Materials and methods

Bacteria and medium used

Thermotolerant cellulase producing isolates, *B. licheniformis* 1-1v and *B. sonorensis* 7-1v were cultivated at 50 °C for 20 h. Modified inorganic salts starch nitrate medium into which starch was replaced by the cellulose substrates (Carboxy methyl cellulase, CMC) was used for cell cultivation (Kuster and Williams, 1964). CMC-mineral medium was composed of (g/L): CMC, 10; K_2HPO_4, 1.0; $MgSO_4{\cdot}7H_2O$, 0.5; KCl, 0.5; $FeSO_4{\cdot}7H_2O$, 0.01; yeast extract 1.0. pH of the medium was adjusted to 6.0 and 5.0 for the isolates 1-1v and 7-1v, respectively as described by Abdel-Rahman et al. (2015a,b).

Composition

Eight compost piles (T_1–T_8) were constructed by mixing cattle wastes and rice straw, with/without 5 kg of feldspar ore powder supplementation. The piles were inoculated with either single strain or mixed culture as described in each experiment. Control piles are not inoculated.

Fresh cattle (cow) dung was collected and moved from dairy cattle farms of Sakha Agricultural Research Station, Agricultural Research Center, Kafr El-Sheikh governorate, Egypt, to the composting unit. The cow dung was immediately mixed with rice straw just after received at the composting unit. Rice straw was air-dried and used directly without chopping.

Feldspar (total potassium, 11.6%) was provided by Al-Ahram Company, Maadi, Egypt, to be used as a source of potassium in compost piles. The physical and chemical composition of feldspare is shown in Table 1. Analysis was performed by Microbiol. Dept., Soil, Water and Environmental, Res. Inst., A.R.C., Giza, Egypt.

Pure culture of *B. licheniformis* 1-1v and *B. sonorensis* 7-1v were grown separately in CMC-mineral broth medium at 50 °C for 20 h under static condition. The incubated media (contained 6.8×10^7 and 3.6×10^7 for isolate 1-1v and 7-1v, respectively) were then diluted with water (20-fold) before inoculation into the compost piles at 1%. Inoculation was applied three times, at days 1, 11, and 23 of composting process. Microbial inoculation was supplemented separately or in mixture (1:1).

Compost procedure

The composting process was conducted on an open site at the Sakha Agricultural Research Station, Agricultural Research Center, Kafr El-Sheikh Governorate, Egypt, during winter

Table 1 Physical and chemical characteristics of feldspar.

Characteristics	Value/content
pH	8.50
EC (dS/m)	0.53
Moisture content (%)	0.95
Dry matter (%)	99.1
Total nitrogen (%)	0.025
Total phosphorus (%)	0.01
Total potassium (%)	11.6
Chemical composition (%)	
SiO_2	70.9–71.4
TiO_2	0.02
Al_2O_3	14.2–14.6
Fe_2O_3	0.27–0.30
MnO	0.01
MgO	<0.01
CaO	$0.30–<0.01$
Na_2O	1.36–1.65
K_2O	10.1–12.1
P_2O_5	0.09

Table 2 The content of piles (T_1–T_8) used for composting process.

Treatment No.	Pile content
T_1	Rice straw[a] + cattle dung[b]
T_2	Rice straw + cattle dung + 5 kg from feldspar ore powder (0.75%, *w*/*w*)
T_3	Rice straw + cattle dung + 5 kg from feldspar ore powder + isolate 1-1v
T_4	Rice straw + cattle dung + isolate 1-1v[c]
T_5	Rice straw + cattle dung + 5 kg from feldspar ore powder + isolate 7-1v
T_6	Rice straw + cattle dung + isolate 7-1v[d]
T_7	Rice straw + cattle dung + 5 kg from feldspar ore powder + Mixture of isolates 1-1v and 7-1v (1:1)
T_8	Rice straw + cattle dung + Mixture of isolates 1-1v and 7-1v (1:1)

[a] Rice straw, 80 kg.
[b] Cattle dung, 288 kg.
[c] Isolate 1-1v, *Bacillus licheniformis* 1-1v.
[d] Isolate 7-1v, *Bacillus sonorensis* 7-1v.

season. Eight piles were constructed with different compositions as shown in Table 2. Carbon-to-nitrogen ratios were adjusted at 35.8:1.0 on dry weight basis. Each pile contains basics ingredients of 288 kg cattle dung and 80 kg rice straw. The piles have pyramid shapes with about 2.0 m length, 1.90 m width and 0.75 m height.

Compost analysis

Physical parameters

Ambient temperature and piles temperatures were measured using a digital thermometer. Temperature measurements were taken at different depths around pile center when the ambient temperature was fairly stable in late morning. The moisture content was determined by drying the samples at 105 °C until the weight becomes constant. Bulk density of compost is defined as its weight per unit volume. The odor was assessed by smelling. Color change was assessed visually.

Chemical analysis

Five randomized samples from each compost pile were collected from the top, middle and bottom of the compost heap during turning the piles at 1st, 23rd, 45th, 59th, 80th, and 117th days of composting. Samples were air-dried for 3 days, oven-dried at 60 °C for 24 h, and then grounded to pass through 0.2 mm sieve screen to be analyzed.

For organic matter and organic carbon determination, Ash was determined in a muffle furnace at 550 °C for 5 h. Organic matter was calculated as the difference between ash and dry weight as a percentage (Tiquia and Tam, 1998). From values of organic matter, the percentage of organic carbon was calculated as described by Haug (1993).

The pH was directly measured in the water extracted sample 1:5 *w*/*v* using a glass electrode pH meter (Orion Expandable ion analyzer EA920). Electrical conductivity measurements were run in 1:5 *w*/*v* compost water extracts using EC meter (ICM model 71150). Total nitrogen was determined as described by Chapman and Parker (1963). *C*/*N* Ratio was calculated using values of the organic carbon and total nitrogen. Phosphorus (%) of samples was determined calorimetrically according to the methods described by Snell and Snell (1967). Available phosphorus was extracted using sodium bicarbonate 0.5 M and ascorbic acid method according to Watanabe and Olsen (1965). Available potassium was determined by ammonium acetate method, and measured by Flame-photometer as method described by Jackson (1967). Total potassium was determined in the digested solution by flame photometer (Jackson, 1967). Available N was extracted by 1 M K_2SO_4 and determined by MgO and Devarda alloy using Kjeldahl method (Jackson, 1967).

Toxicity analyses

A water extract of each compost was prepared by shaking the samples with distilled water at 1:10 *w*/*v* ratio for 1 h, and then filtered to be used for germination of Eruca sativa seeds using Petri dishes. Seed germination in distilled water was used as control. The percentage of seed germination was calculated using the following equation (Zucconi et al., 1981):

$$\text{Seed germination }(\%) = \frac{\text{NO. of seeds germinated in compost extract}}{\text{NO. of seeds germinated in control}} \times 100$$

Pathogenic bacterial count were determined using agar plates containing specific media for coliform group, *Escherichia coli*, *Salmonella* and *Shigella*.

Results

Temperature profiles and degree of composting

The temperature changes for the eight treatments (T_1–T_8) during composting were recorded for assessment of composting progress as shown in Fig. 1. The ambient temperature variations throughout the composting period were between 12 and 32 °C. The initial temperature in all treatments was 15 °C. An increase in piles temperatures was observed right after composting started.

In T_1, and T_2 (Uninoculated control piles, Fig. 1A), a gradual increase in the temperature was obtained that reached thermophilic phase at 55.3 °C and 57 °C, respectively after 18 days of composting. It then decreased gradually to reach second mesophilic phase (on the day 32) for both piles and remained almost constant near ambient temperature at days 96 and 89 for T_1 and T_2, respectively. Feldspar supplementation resulted in a slight increase in pile temperature compared to feldspar free pile.

In T_3, T_4, T_5, and T_6 (piles inoculated with single strain *B. licheniformis* 1-1v or *B. sonorensis* 7-1v with/without feldspar supplementation, Fig. 1B and C), the temperature was increased gradually and reached thermophilic phase at 46, 45.6, 45.6, and 53.7 °C, respectively after nine days of composting. The temperature was increased after the first turning to reach the peak of curve, while it decreased gradually after the second turning to mesophilic phase. After that, it stabilized near the ambient temperature at day 79 for T_3 and T_4, and day 72 for T_5 and T_6. Feldspar supplementation (T_3 and T_5) resulted in an increase in pile temperature in the early composting stages compared to feldspar free piles (T_4, T_6).

Interestingly, T_7 and T_8 (piles inoculated with mixed culture with/without feldspar supplementation, Fig. 1D) reached to

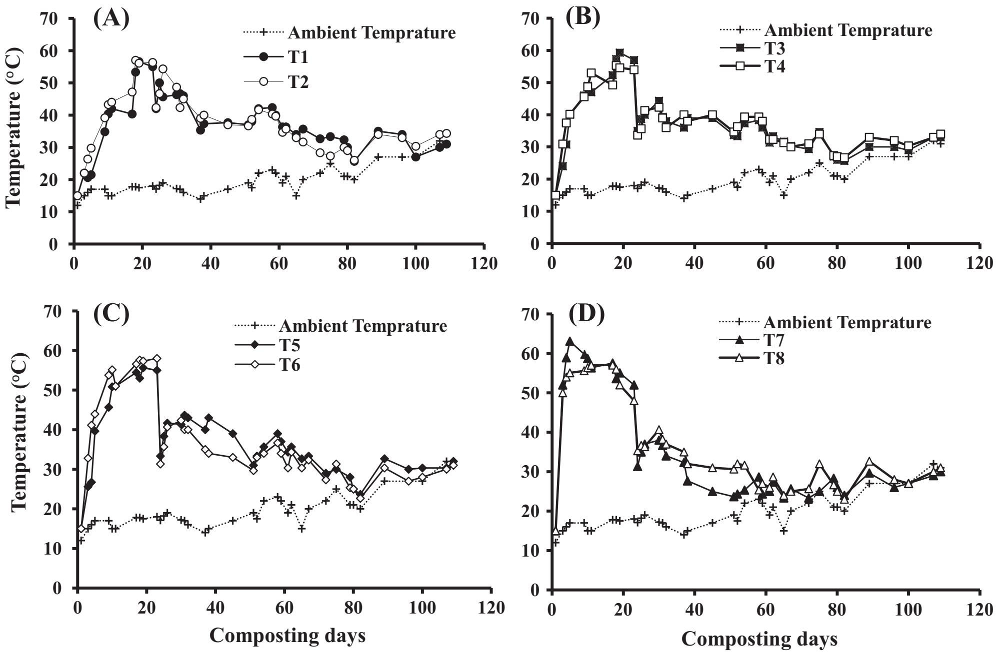

Fig. 1 Changes in temperature during the composting process for all piles in relation to the ambient temperature. (a) Uninoculated treatments with/without feldspar [T_1 & T_2]; (b) treatments inoculated with strain 1-1v with/without feldspar [T_3 & T_4]; (c) treatments inoculated with strain 7-1v with/without feldspar [T_5 & T_6]; (d) treatments inoculated with mixed strains, 1-1v + 7-1v, with/without feldspar [T_7 & T_8]. T_2, T_3, T_5, and T_7 were supplemented with feldspar.

Table 3 Influence of construction piles (T_1–T_8) treatments on organic carbon contents (%) throughout composting process.

Composting day	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	39.4 ± 0.0	39.4 ± 0.0	39.4 ± 0.0	39.4 ± 0.0	39.4 ± 0.0	39.4 ± 0.0	39.4 ± 0.0	39.4 ± 0.0
23	34.4 ± 0.54	33.6 ± 0.0	31.9 ± 0.52	32.1 ± 1.13	31.3 ± 0.0	30.2 ± 0.47	27.8 ± 0.33	30.1 ± 0.7
45	32.3 ± 0.0	30.4 ± 0.0	27.5 ± 0.0	27.8 ± 0.0	26.7 ± 0.0	26.3 ± 0.0	24.7 ± 0.0	25.9 ± 0.0
59	29.0 ± 0.66	28.0 ± 0.37	25.9 ± 1.2	26.1 ± 0.0	24.9 ± 0.0	24.4 ± 0.53	23.3 ± 0.49	24.9 ± 0.0
80	26.8 ± 0.82	26.5 ± 0.06	24.7 ± 0.0	25.3 ± 0.96	24.7 ± 0.0	23.9 ± 0.79	23.1 ± 0.42	24.4 ± 1.39
117	25.8 ± 1.03	25.8 ± 0.0	24.3 ± 0.0	24.7 ± 0.02	23.7 ± 0.29	23.5 ± 0.68	22.9 ± 0.0	24.1 ± 0.56

thermophilic phase at 52 °C and 50 °C, respectively on the third day of composting process. The temperature was decreased after the first turning but still in the thermophilic phase (> 45 °C). On the other hand, the temperature decreased gradually to mesophilic phase in both piles after the second turning and remained almost constant near the ambient temperature at day 51 for T_7 and day 58 for T_8. Feldspar supplementation (T_7) showed a significant effect on the composting efficiency compared to feldspar free pile (T_8).

Total organic carbon (TOC, %)

The initial values of TOC were 39.4% for all piles. As shown in Table 3 and Fig. 2a, the TOC content of the composting mass was decreased during composting process. Higher reduction for all treatments occurred during the thermophilic phase, and lower decreases toward the end of composting period were observed after that. The TOC content was declined slightly in control treatments T_1 and T_2, in addition both piles showed similar pattern till 23 days of composting. On the other hand, a sharp decline in TOC content was obtained in inoculated treatments (T_3–T_8) in first weeks of composting. Among all treatments, the maximum TOC reduction was observed in inoculated piles with mixed cultures (T_7–T_8) followed by piles inoculated with single inoculant (T_3–T_6) followed by control treatments.

Total nitrogen content (%)

A decrease in total nitrogen content of the composting mass was observed during thermophilic phase as represented by the data obtained at 23rd day of composting (Table 4; Fig. 2b). Total nitrogen content was decreased from 1.1% to 1.01%, 0.95%, 1.05%, 0.98%, 1.02%, 0.99%, 0.94%, and 0.98% for T_1–T_8, respectively. Nitrogen content was then increased gradually during composting period in all piles and reached to 1.63, 1.54, 1.68, 1.64, 1.7, 1.84, 1.75, and 1.84 for T–T_8, respectively at the end of composting.

Organic carbon to total nitrogen (C/N ratio)

The C/N ratios were evaluated through composting process to assess the rate of decomposition of piles constituents as shown

Table 4 Influence of construction piles (T_1–T_8) treatments on total nitrogen contents (%) throughout composting process.

Composting day	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0
23	1.01 ± 0.0	0.95 ± 0.0	1.05 ± 0.0	0.98 ± 0.0	1.02 ± 0.0	0.99 ± 0.12	0.94 ± 0.1	0.98 ± 0.0
45	1.21 ± 0.17	1.17 ± 0.12	1.23 ± 0.1	1.16 ± 0.0	1.26 ± 0.0	1.24 ± 0.32	1.4 ± 0.1	1.35 ± 0.02
59	1.42 ± 0.25	1.49 ± 0.0	1.66 ± 0.0	1.54 ± 0.02	1.42 ± 0.0	1.44 ± 0.1	1.56 ± 0.1	1.54 ± 0.1
80	1.62 ± 0.12	1.52 ± 0.15	1.66 ± 0.2	1.63 ± 0.2	1.44 ± 0.0	1.50 ± 0.1	1.70 ± 0.2	1.63 ± 0.12
117	1.63 ± 0.12	1.54 ± 0.32	1.68 ± 0.12	1.64 ± 0.02	1.7 ± 0.17	1.84 ± 0.7	1.75 ± 0.12	1.84 ± 0.12

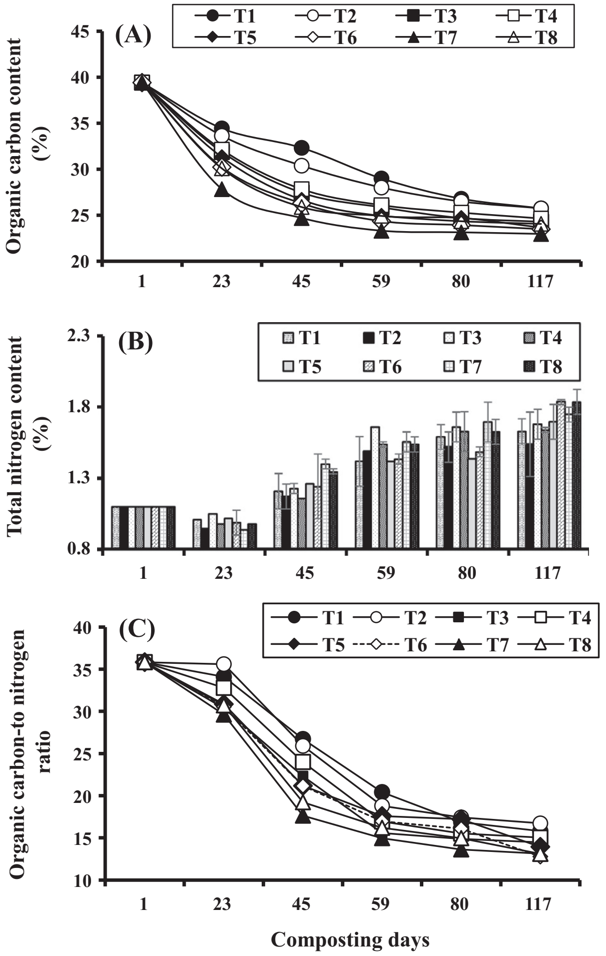

Fig. 2 Changes in (a) organic carbon, (b) total nitrogen, and (c) carbon to nitrogen ration during the composting process for all compost piles.

Table 6 Final values of available nitrogen, phosphorous, and potassium in the finished compost products.

Treatment No.	Nitrogen (ppm)	Phosphorus (ppm)	Potassium (ppm)
T_1	106.4 ± 3.96	699.0 ± 3.73	558.9 ± 7.58
T_2	124.6 ± 17.8	635.1 ± 19.5	514.3 ± 20.2
T_3	120.4 ± 7.92	675.2 ± 4.37	535.7 ± 0.0
T_4	123.2 ± 0.0	746.7 ± 22.8	532.1 ± 7.14
T_5	116.9 ± 16.8	736.0 ± 18.5	513.0 ± 32.8
T_6	124.6 ± 17.8	751.5 ± 9.40	544.1 ± 58.9
T_7	117.6 ± 15.3	760.2 ± 18.4	558.9 ± 27.7
T_8	124.6 ± 9.9	754.8 ± 3.36	607.1 ± 10.1

in Table 5 and Fig. 2C. The data showed T_1 and T_2 required 18 days to reach active phase. On the other hand, the presence of active inocula had fastened the composting process that required short time to enter active phase as indicated in T_3–T_8. T_7 and T_8 treatments that are inoculated with bacterial consortium have reached maturity (C/N ratio $\leqslant 20$) faster than other piles where the C/N ratio reached to 17.6 and 19.2, respectively after 45 days of composting, followed by piles inoculated with *B. sonorensis* 7-1v (T_5–T_6), followed by piles inoculated with *B. licheniformis* 1-1v (T_3–T_4).

Available Nitrogen, phosphorus, and potassium

As shown in Table 6, it is observed that all available nitrogen, phosphorus and potassium contents for all compost piles were ranged 106.4–124.6, 635.1–760.5, and 513.0–607.1 ppm, respectively.

Moisture content, electrical conductivity, pH values and bulk density

Moisture content, electrical conductivity (The sum of soluble ions in water extracts), pH values, and bulk density at the

Table 5 Organic carbon to total nitrogen (C/N ratio) changes during composting process.

Composting day	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	35.8 ± 0.0	35.8 ± 0.0	35.8 ± 0.0	35.8 ± 0.0	35.8 ± 0.0	35.8 ± 3.31	35.8 ± 0.0	35.8 ± 0.0
23	34.9 ± 0.53	35.6 ± 0.0	30.4 ± 0.49	32.8 ± 1.16	30.9 ± 0.0	30.5 ± 0.0	29.6 ± 0.35	30.7 ± 0.71
45	26.7 ± 0.0	25.9 ± 0.0	22.3 ± 0.0	24.0 ± 0.0	21.1 ± 0.0	21.1 ± 0.0	17.6 ± 0.0	19.2 ± 0.0
59	20.4 ± 0.0	18.8 ± 0.44	15.6 ± 0.22	16.9 ± 0.39	17.6 ± 0.0	16.9 ± 0.18	14.9 ± 1.27	16.19 ± 0.0
80	16.9 ± 1.13	17.4 ± 1.71	14.8 ± 1.49	15.4 ± 1.28	17.2 ± 0.0	16.0 ± 1.13	13.6 ± 1.27	14.9 ± 2.0
117	15.8 ± 1.84	16.7 ± 3.66	14.6 ± 1.1	15.1 ± 0.24	13.9 ± 1.50	12.8 ± 0.89	13.1 ± 0.0	13.4 ± 0.56

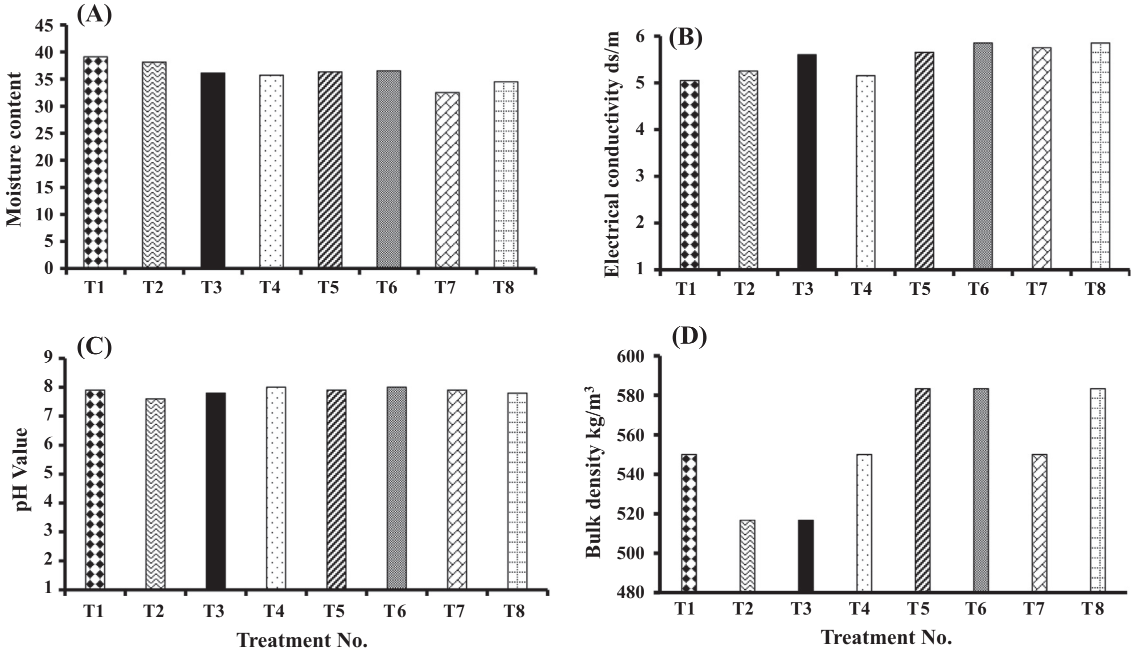

Fig. 3 Physicochemical characteristics of finished compost samples produced from for T_1–T_8 piles (a) moisture content, %; (b) electrical conductivity, ds/m; (c) pH values, and (d) bulk density, kg/m^3.

end of composting period were presented in Fig. 3. As indicated in Fig. 3a, the moisture content (%) in the finished compost was ranged 32.5–39.1 for all piles. The EC values were ranged 5.05–5.85 ds/m (Fig. 3b) while pH values for all products showed the slightly alkaline in the range 7.6–8.1 (Fig. 3c). As shown in Fig. 3d, the bulk density was in the range of 516–583 kg/m^3 for finished compost that is almost double-fold of the initial bulk density at 271.3 kg/m^3.

Toxicity analysis

The responses of rocket salad plant (*Eruca sativa*) to the toxicity of the compost water extracts for all treatments in terms of seed germination (%) were illustrated in Fig. 4. The data reveal that the phytotoxicity in all piles was eliminated as the germination ratios was exceeded 86.0% in all piles. In addition, no pathogenic bacteria were detected in the final compost products.

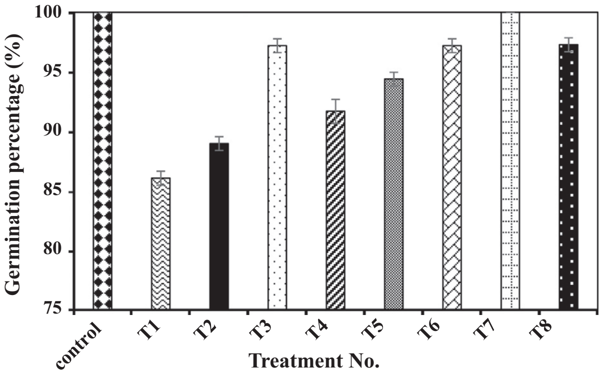

Fig. 4 Germination percentage of rocket salad seeds using the finished compost.

Analysis of color and odor

A gradual darkening of the compost material was noticed by naked eye during composting process. The color of the final product after sufficient period of maturation was dark brown. The unpleasant odor of compost materials was decreased with composting time. Finally, the odor was similar to the odor of earth at the end of the process. The physical appearance of the best piles (T_7, and T_8) at different composting stages is shown in Fig. 5.

Discussion

The global production of plant biomass amounts to ~200 × 10^9 tons per year. Of this biomass, over 90% is lignocellulosic material that represents the most abundant global source of largely unutilized material (Lin and Tanaka, 2006). Of these, rice straw is the main agricultural wastes in Egypt. Such biomass has received much attention due to their feasibility, abundance, low price, and high sugar content as a feedstock for the production of valuable products (Abdel-Rahman et al., 2011). To avoid the overuse of chemical fertilizers that largely contribute to environmental deterioration, compost production has received a great attention as a source of humus and nutrients to increase the fertility of soil (Gabhane et al., 2012). Composting is normally time-consuming and a labor intensive process that mainly depends on the abilities of microflora to produce and excrete specific degradative enzymes (Mishra and Nain, 2013). In a trial to fasten compost maturation process and to improve the quality of compost, we applied newly isolated cellulase-producing thermotolerant bacterial isolates (*B. licheniformis* 1-1v and *B. sonorensis* 7-1v) as additional microbial source for composting of rice straw materials.

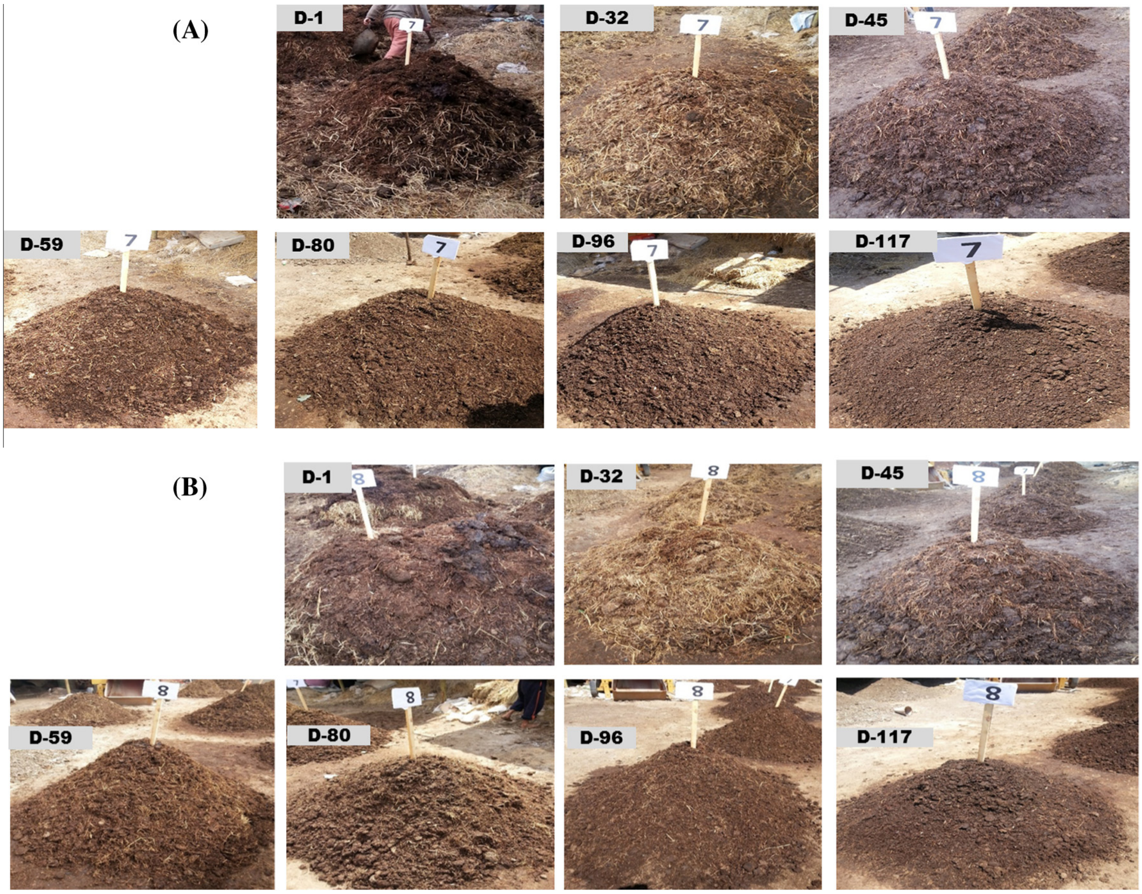

Fig. 5 Physical changes in compost pile appearance and structure at different stages of composting process (days 1, 32, 45, 59, 80, 96, and 117) for treatments T_7 and T_8 inoculated with mixed culture. T_7 was supplemented with feldspar at the pile construction. (a) T_7 and (b) T_8.

Accordingly, we constructed eight piles with different compositions that are inoculated or not-inoculated with *B. licheniformis* 1-1v and *B. sonorensis* 7-1v using single strain or mixed cultures at 1:1 as shown in Table 2. Previously it was indicated that these bacterial treatment at 1:1 caused the highest cellulase productivity using rice straw under solid state fermentation (Abdel-Rahman et al., 2015b). These strains have the ability to utilize rice straw efficiently via production of multiple extracellular enzymes. Feldspar (0.75%, *w*/*w*) was added to some piles (T_3, T_5, and T_7) because it achieved a strong activation for cellulase productivities by strains under study (Abdel-Rahman et al., 2015a,b). It is noteworthy to focus that composting process was conducted in winter-spring season (January to May, i.e. cold season) for 117 days at the end of complete maturation of control treatments. Various parameters were evaluated to check the compost maturity and quality including changes in temperature, organic carbon, organic nitrogen, *C*/*N* ratio, color, odor, moisture content, available nitrogen, phosphorus and potassium contents, electric conductivity, pH values, bulk density, and toxicity analysis.

The temperature pattern showed a rapid increase from the initial mesophilic phase to the thermophilic phase for piles that were treated by mixed cultures (T_7, T_8) where it reached up to 50–52 °C after three days of composting process, followed by piles inoculated with single strain that reached thermophilic phase after nine days. On the other hand, control treatments (T_1–T_2) were required 18 days to reach thermophilic phase. Hassen et al. (2001) indicated that temperature profile determines the success of an aerobic composting. The increase in compost temperature is caused by the heat generated from decomposition of organic matters by the activity of aerobic microorganisms that is important at the beginning of composting process (Makan et al., 2012). Higher temperature denotes greater microbial activities. Based on temperature achieved, treatment with inoculated bacteria has higher microbial activities and consequently higher enzymatic activities and lignocellulose decomposition compared to uninoculated piles. The higher activities achieved by bacterial consortium of mixed cultures inoculum might be attributed to broader substrate utilizations by both isolates.

Interestingly, the piles inoculated with mixed strains have longer time above 55 °C of about 15 consecutive days, than control piles that achieved only 5 consecutive days. On the other hand the pile inoculated with strain 7-1v supplemented with feldspar has achieved about 13 consecutive days at temperature above 55 °C. Haug (1980) mentioned that high temperature (> 55 °C) is necessary for three consecutive days to kill the indigenous pathogens. Our result was superior to that obtained by Lim et al. (2014) who reported a thermophilic phase for only few hours during composting of oil palm empty fruit bunches inoculated with commercial microbial inoculant product. Jusoh et al. (2013) reported a longest thermophilic time above 55 °C for 6 consecutive days in rice straw piles inoculated with effective microorganisms.

The fluctuation of pile temperatures immediately after each turning operation is due to the reactivation of the composting process by the incorporation of external materials into the pile. In addition, the stability of temperature near the ambient temperature took shorter time in piles treated with mixed culture (51–58 days) followed by that inoculated with single culture (72–79 days) compared to untreated piles (89–96 days).

The decrease in organic carbon content during composting process exhibited a great difference in the carbon loss between treatments. In particular, piles with mixed culture showed a sharp decrease in organic carbon compared to other piles. The carbon loss is due to the emitting of carbon dioxide as a result of microbial metabolic activities and decomposition progress (Benito et al., 2003; Bernal et al., 1996; Vuorinen and Saharinen, 1997).

Total nitrogen content was reduced in the early stages of composting (during thermophilic phase). Then a gradual increase was achieved till the end of process in all compost piles under study. Huang et al. (2004) also reported loss of total nitrogen during 7 days and then slight increase after 63 days of composting. The late increase in nitrogen content may be due to the loss of dry mass in terms of carbon loss, or due to the contribution of nitrogen-fixing bacteria in the later stage of composting (Bishop and Godfrey, 1983; Jusoh et al., 2013; Wong and Fang, 2001).

Carbon to nitrogen ratio of the eight piles were decreased gradually during composting process and became comparatively stable. The inoculated compost piles, especially that with mixed cultures, yielded a more rapid decrease in C/N ratio than uninoculated ones. This might be due to the increased gaseous loss of carbon as CO_2 while the nitrogen remained more tightly bounded in organic combination with slight changes. Similar findings were obtained by Makan and Mountadar (2012) and Jusoh et al. (2013) who reported that C/N ratio tended to be decreased with time of composting. The decrease in carbon to nitrogen (C/N) ratio to less than or equal to 25 indicates the increase in the degree of humification of organic matter and considered one of the most important parameters to evaluate the maturity of compost product (Mathur et al., 1993; Tripetchkul et al., 2012).

In our study, available NPK were ranged 106.4–124.6, 635.1–760.5, and 513.0–607.1 ppm for N, P, and K, respectively. These data are in recommended amount ranges indicated by the European Standard as indicated by Brinton (2000). In addition, all piles showed increase in total potassium and phosphorus at the end of the process from 1.14% to 1.52–1.91% and from 0.22% to 0.64–0.79%, respectively. The treatments inoculated with mixed cultures exhibited the highest values compared to others.

Degree of compost maturity can be also assessed by physical parameters such as color and odor (Alberta Environment, 1999; Xue et al., 2013). In this study, the unpleasant odor of composting materials decreased with composting time to be as same as the odor of earth. The earthy smell of compost is due to the production of microbial volatile secondary metabolites such as geosmin (Li et al., 2004). The color of the final product after sufficient period of maturation was dark brown that is in accordance with optimal compost (Alberta Environment, 1999).

The moisture content (%) and bulk density in the finished compost of all treatments were ranged 32.5–39.1 and 516–583 kg/m^3, respectively. The piles inoculated by strain 7-1v and that inoculated by mixed culture showed higher bulk density than others. This indicates the higher activities of inoculated strains in the decomposition of organic materials to break down the loosely combined raw materials into smaller pieces. Compost with moisture content less than 35% achieves long storage period (Sullivan and Miller, 2001).

The electrical conductivity is a good indicator of the safety and suitability of compost (Lazcano et al., 2008). In this study the EC of the finished compost in all treatments was found to be in the optimal range of good compost (5.05–5.85 ds/m for all piles) as reported by Brinton (2000) where the recommended EC value is 2.0–6.0 ds/m. Similarly, pH of the finished compost by all piles was also in recommended range of good compost (pH 7.6–8.1). Makan et al. (2012) reported that, the pH of the finished compost should range from 7.5 to 8.5. Therefore, EC and pH values for these samples were within the ranges that normally do not adversely affect on plant growth or seed germination.

Phytotoxicity is also one of the most important parameters used for evaluating the compost usable for agricultural purposes (Selim et al., 2012). The germination rate greater than 80% is required to prove the absence of phytotoxicity (Alberta Environment, 1999). In this study, the germination rate at the end of composting process exceeds 91.7% in all piles that inoculated with our tested strains while ranged 86.1–89% for uninoculated control piles. Very interestingly, compost piles that inoculated with bacterial consortium of mixed cultures exhibited the highest germination ratio at 100% and 97.2%, respectively. This indicates that the finished compost is mature and free from phytotoxins. In addition, no pathogenic bacteria were isolated from all compost piles indicating its biosafety and availability for easily handling and use.

Conclusion

The application of cellulose degrading bacteria, *B. licheniformis* 1-1v and *B. sonorensis* 7-1v (separately or as mixed-cultures) in composting technology exhibited an improvement of composting maturation time and compost quality. Interestingly, the piles inoculated with mixed-culture were matured within 51–58 days while the maturation of piles inoculated with single strain was completed within 72–79 days compared to control piles that have been completed within 89–96 days. Higher decrease in TOC values and C/N ratio were achieved in inoculated piles. Furthermore, the application of effective inoculum in rice straw compost has increased the nutrient content (total NPK), indicating an increase in compost quality. The phytotoxicity analysis and physicochemical properties of the final compost products indicated that it was in the range of the matured level and can be used without any limitations. These data indicate that our isolated strains, especially when used as bacterial consortium, have the potentiality to be applied in large scale composting industry for reduction of manufacturing cost.

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