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# Microbial decomposition of Longan leaf: II. screening of effective cellulolytic microorganisms and development of microbial product prototype for accelerating composting process

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## Abstract

Microorganisms are the main decomposer of cellulose, hemicellulose and lignin in composting process. In the present study, we screened bacteria, fungi and actinomycetes, which showed high cellulolytic activity from longan leaves + cow dung (LC). The results indicated that Comf3004 gave the highest clear zone ratio (3.5). Three isolates exhibiting the highest endo-1,4-beta-D-glucanase (CMCase) activity were bacterial isolate Comb3012 (0.596 unit/mL), fungal isolate Comf6012 (0.875 unit/mL) and actinomycetal isolate Comac9009 (0.525 unit/mL). The LC was inoculated with the prototype of three selected microbial inoculants; LC+Comb3012 (LCb), LC+Comf6012 (LCf), LC+Comac9009 (LCac) and the mixture of the three inocula (LCbfac). The LC+sterile carrier (LCC) was also applied for comparison. After 120 days, the maximum germination index (GI) value (95.13%) was recorded in LCf. The LCf and LCb compost exhibited a remarkably low organic matter (OM) content (23.0 and 26.3%, respectively). The results indicated that inoculation of microbial inoculants could effectively accelerate the decomposition of LC. The nutrient contents of all treatments with microbial inoculation were also higher than LC and LCC. The lowest cellulose and hemicellulose content were obtained with LCf (18.5 and 12.5%, respectively). Moreover, the lowest content of lignin was obtained with LCac (4.9%). All inoculated treatments exhibited higher decomposition of cellulose, hemicellulose and lignin than the control. Fungal isolate Comf6012 performed the best in breaking down cellulose and hemicellulose fractions while actinomycetal isolate Comac9009 exhibited the highest lignin decomposition. The results highlight the high possibility in the application of effective microbial inoculum for rapid composting of longan leaves.

**Keywords:** Compost, Cellulase, Cellulose, Hemicellulose, Lignin

## 1. Introduction

Composting is microbial decomposition of organic materials and it is governed by various factors including microbiological one. The importance of microbial communities (bacteria, actinomycetes and fungi) during composting is well established [1]. Mixed microbial population processing with various enzymes during composting is significantly vital for organic wastes decomposition. The major substrate for leaf litter composting is lignocellulose, which is difficult to decompose naturally, and it has led to screen effective microbes to accelerate the decomposition process. In general, these waste materials are composed of organic components such as cellulose (30-75%), lignin (15-40%) and hemicelluloses (7-25%) [2]. Microorganisms is capable of degrading cellulose as well as producing a battery of enzymes with different specificities to work together. Cellulolytic enzymes degrade cellulose, the polymer forming a significant portion in the plant cell walls and the major portion of agricultural wastes. Cellulase is an enzymes system that breaks down cellulose, the main component of the cell walls of plant residues. This enzymatic system is responsible for the hydrolysis of cellulose which is the most important step in organic matter (OM) degradation. Cellulases hydrolyze the β-1,4-glycosidic linkages of cellulose

[3]. In composting, there are various groups of cellulolytic microbes which are generally have a certain potential in producing cellulase enzymes. These microbes can also be developed as a microbial product and used to accelerate composting process. Microorganisms/microbial products which are added to materials to improve composting are referred to as inocula/inoculants. Applying inocula in the process has been suggested to accelerate composting or improve its efficiency by providing the proper set of organisms or enzymes [4].

In the northern part of Thailand, a huge waste of longan leaves (around 50 kg/tree) is obtained after heavy pruning following fruit harvest. Most of the leaf wastes are subject to open burning in each longan orchard. Composting is a good alternative for longan-leaves wastes management however they are difficult to degrade. Therefore, the application of cellulose degrading microorganisms is certainly essential. To apply effective cellulolytic microorganisms as microbial inoculant is an alternative for successful composting in hard degradable agricultural wastes. However, it must first be economically mass-produced first and then formulated into a form that is cost-effective, uniform and readily applicable by the end user. As mentioned above that cellulose is the most abundant component of plant biomass. Thus, screening of cellulolytic microbes should be done as an initial step toward development of microbial inoculant for accelerating composting process. Therefore, in the present study, we aimed at isolating and screening high cellulase producing microorganisms from longan leaves compost and other types of compost conducted in our previous experiments. Some effective isolates were selected to produce a prototype of microbial inoculum. An effectiveness evaluation of the microbial prototype was also tested with longan leaves compost. Promising microorganisms obtained from this study would be useful for accelerating the composting process of longan leaves and other materials which are hard to break down under traditional decomposition.

## 2. Materials and methods

Compost samples from three treatments conducted in our previous experiment including 1) Longan leaves + cow dung (LC), 2) Rice straw + cow dung (RC), and 3) Longan leaves + rain tree leaves + cow dung (LRC). All of them were collected at 30, 60 and 90 days after incubation. Three groups of microorganisms i.e. bacteria, fungi and actinomycetes, were isolated after counted and purified. The specific media for isolation of bacteria, fungi and actinomycetes were nutrient agar (NA), malt extract agar (MEA) and inhibitory mold agar-2 (IMA-2), respectively. All the purified isolates were tested for their potential and effectiveness in cellulase enzyme production. Promising isolates were selected and developed as prototype of microbial inoculants. The effectiveness of microbial inoculants was then tested in the composting of longan leaves. The method of each determination was described below.

### *2.1 Culture media*

Nutrient broth (NB): The medium contained (g/L), beef extract 1.0 g, yeast extract 2.0 g, peptone 5.0 g, and NaCl 5.0 g (pH 7.4). For NA, agar 15 g was added. Malt extract broth (MEB): The medium contained (g/L), malt extract 20 g and peptone 5 g. For MEA, agar 15 g was added. IMB-2: The medium contained (g/L), D-glucose 5.0 g, starch soluble 5.0 g, beef extract 1.0 g, yeast extract 1.0 g, Nz-case 2.0 g and $CaCO_3$ 1.0 g (pH 7.2). For IMA-2, agar 15 g was added. Antibiotics were added to warm agar medium prior to plating: trimethoprim 20 mg/L, naldixic 10 mg/L and heritage 10 mL/L. Carboxymethyl cellulose (CMC) medium: The medium contained (g/L), $(NH_4)_2SO_4$ 1.0 g, $K_2HPO_4$ 1.0 g, $KH_2PO_4$ 4.0 g, $Na_2HPO_4$ 6 g, yeast extract 1.0 g, CMC 5 g and $MgSO_4.7H_2O$ 0.2 g (pH 7.0). Agar 15 g was added for solid medium. The pH of each medium was adjusted before autoclaving at 121 ˚C for 15 min.

### *2.2 Compost sampling and isolation of compost microorganisms*

Compost samples from all the treatments were collected at 30, 60 and 90 days after incubation. Dilution technique was adopted to isolate bacteria, fungi and actinomycetes in the compost. Different morphological colony appearing on plate of each microbial group (bacteria, actinomycetes and fungi) were selected and purified in specific media, NA, MEA and IMA for bacteria, fungi and actinomycetes, respectively. The purified isolates were stored in sterile 25% glycerol (final concentration) and kept in -20 ˚C for further investigations.

### *2.3 Qualitative screening of cellulase production by isolates obtained from compost*

All the purified isolates of bacteria, fungi and actinomycetes obtained from the section 2.2, were multiplied in their specific media, NB, MEA and IMA2, respectively. An aliquot (0.1 mL) of the culture broth of bacteria was dropped on the center of CMC agar medium in Petri dish. Plugs of fungal and actinomycetal mycelium (7 mm in diameter) were cut with a sterile cork-borer and placed on the center-surface of CMC medium. All the cultures on CMC were then incubated for 3, 5 and 7 days for bacteria, fungi and actinomycetes, respectively.

After incubation, the microbial colonies on the plates were flooded with Congo red solution (0.1%) for 20 min. And after Congo red solution was poured off, the plates were washed by flooding with 1M NaCl for 10 min following by rinsing several times using NaCl. A clear zone formation around the microbial colonies indicates the CMC. Clear zone ratio is the ratio of the diameter of clearing zone and colony was calculated. Different ability in cellulase production (clear zone ratio) of just 10 isolates or below of each microbial group was selected for quantitative assay of cellulolytic activity by 3, 5-dinitro salicylic acid (DNS) reagent method (section 2.4).

*2.4 Quantitative analysis of endo-1,4-beta-D-glucanase (CMCase) enzyme production*

Cellulase activity was assayed using DNS reagent by estimating the quantity of reducing sugars released from CMC solubilized in a buffer solution (pH 6.0). All the isolates selected from the section 2.3 were enriched in basal medium until they reached the maximum growth (3, 5 and 7 days for bacteria, fungi and actinomycetes, respectively). After the maximum growth, the culture broth of each isolate was centrifuged at 10,000 rpm for 20 mins at 4 ˚C and supernatant was used as a source of crude enzyme. The crude enzyme solution was utilized for determination of enzyme activity.

Cellulase enzyme assay: Carboxymethyl cellulase activity was estimated by using a 1% solution of carboxymethyl cellulose in 0.05 M citrate buffer (pH 6.0) as a substrate. The reaction mixture contained 1 mL citrate buffer, 0.5 mL of substrate solution and 1 mL of crude enzyme solution. The reaction was carried out at 45 ˚C for 30 min. The amount of reducing sugar released in the hydrolysis was measured by DNS method, adapted from the method of Chaiyaso et al. [5]. The Enzyme unit (EU) was determined as the amount of CMCase required to release 1 μmole of reducing sugar per mL per minute under above assay condition as mentioned above.

Only one isolate of each microbial group (bacteria, fungi and actinomycetes) exhibited the highest CMCase activity, was selected for further experiments.

*2.5 Development of compost microbial inoculants*

Preparation of carriers: The carrier used to produce microbial inoculants was modified from Shutsrirung [6]. The dried, ground raw materials used to produce carrier were the mixture of matured coconut coir compost and sphagnum peat moss at the ratio of 6:4 (w/w). Then, the mixture was blended with 2.5% leonardite and 2.5% diatomite (w/w). After that the homogeneous mixture (mix-carrier), was packed into small plastic bags (600 g). Sterile mix-carrier was prepared by autoclaving twice (45 min at 120 ˚C, 15 lbs/in$^2$), which the second one would follow a 24 h room temperature incubation.

Production of inoculants: The starter broth culture of each selected isolate (3 isolates) was blended with sterile mix-carrier (60% water holding capacity of the carrier) to obtain the product prototype. The inoculants were incubated at room temperature for 7 days then they were kept in refrigerator until use. Aseptic conditions were maintained throughout the production period.

*2.6 Effectiveness evaluation of compost microbial inoculants*

LC (longan leaves: cow dung 8:2 (w/w)) was used to test the effectiveness of inoculants prototype prepared as described in the section 2.5. Each inoculum prototype was thoroughly mixed with the LC according to each treatment at 3 different times: at the beginning, 14, and 30 days, of composting. Completely randomized design (CRD) was applied in this experiment with three replications. The treatments were as following:

T1: Control (Longan leaves + cow dung) (LC);
T2: Longan leaves compost + sterile carrier (LCC);
T3: Longan leaves compost + selected bacterial-inoculant (LCb);
T4: Longan leaves compost + selected fungal-inoculant (LCf);
T5: Longan leaves compost + selected actinomycetal-inoculant (LCac);
T6: Longan leaves compost + 3 selected isolates-inoculants (LCbfac).

Eighteen plastic-net cabinets (80x80x100 cm) were used to conduct the above composting treatments. Ten kilograms of LC based on dry weight were mixed with microbial inoculants according to the above treatment. Water was added to each treatment until reach optimum moisture content (no water seeps out when the compost is squeezed; the water content was approximately under 40%), the moisture was maintained at this level throughout the composting period. After mixing the waste components were put in the plastic-net cabinet and allowed to decompose for 120 days. Samples were collected from composts of all the treatments at 0, 7, 14, 30, 60, 90, and 120 days after composting.

*2.7 Analysis of compost properties*

During composting process, the samples from each treatment were collected and analyzed for pH, electrical conductivity (EC), germination index (GI), OM, C:N ratio and nutrient contents including total nitrogen (TN), total phosphorus (TP), and total potassium (TK). In brief, the compost samples were analyzed for pH and EC in a water-soluble extract 1:10 (w/v). OM was done by Walkley-Black wet digestion. Total N was determined by the Kjeldahl method including acid digestion followed by distillation and titration. Total P and K were analyzed after acid digestion, P by the colorimetric method as a molybdovanadate phosphoric acid, K by flame photometer [7].

*2.8 Fiber analysis (cellulose, hemicellulose and lignin)*

The oven-dried compost sample (60 ˚C) of each treatment was analyzed for cellulose, hemicellulose and lignin contents using forage fiber analysis as described by Goering and Van Soest [8]. In brief, the compost samples were digested using the neutral detergent solutions and acid detergent solution to obtain neutral detergent fiber (NDF) and acid detergent fiber (ADF), respectively. Lignin was measured by gently oxidizing the ADF residue with potassium permanganate. Acid detergent lignin (ADL) content was calculated by subtracting permanganate residue from ADF residue. The NDF = hemicellulose + cellulose + lignin, and the ADF = cellulose + lignin. The amount of hemicellulose and cellulose was calculated by (NDF-ADF) and (ADF-ADL), respectively.

*2.9 Statistical analysis*

The data of nutrient composition of compost product at day 120, were subjected to analysis of variance by Statistix 8.0 (Tallahassee, FL, USA). The differences among various treatment means were analyzed by one-way analysis of variance (ANOVA) to determine if they were different from one another. Differences between means were tested by LSD at a significance level of $p < 0.05$.

**3. Results and discussion**

*3.1 Cellulase enzyme production potential on CMC agar plates*

Cellulolytic microorganisms existing during composting process have a major role in recycling and turning organic wastes into valuable product (humified compost). Cellulolytic enzymes produced by these microorganisms are significantly important since cellulose is the major component of plant biomass (30-50%) therefore screening of microorganisms capable of cellulase production is necessary as well. A group of different enzymes collectively known as cellulase is produced during composting process. This enzyme can be assayed using soluble cellulose substrates, i.e. the CMCase assay [9]. In the present study, all the purified isolates (235 isolates) were evaluated for their cellulase production potential using CMC agar medium. Not all the isolates were able to produce clear zone around the colony. The percentage of bacterial, fungal and actinomycetal isolates producing clear zone on CMC agar was 23.9 (17/71), 56.8 (50/88) and 48.7% (37/76), respectively. High clear zone ratio (CR) indicated high cellulose hydrolysis capacity (HC). Bacterial isolates obtained from LC, RC and LRC could produce CR with the average ratio range of 2.40 to 2.65, 2.30 to 2.63 and 2.35 to 2.40, respectively (Figure 1). Actinomycetes showed similar ability of cellulase activity with bacteria with the average ratio range of 2.04 to 2.73, 2.16 to 2.50 and 2.10 to 2.43, for LC, RC and LRC, respectively. On the average, the clearing zone of actinomycetes and bacteria was similar while fungi seemed to produce the widest clear zone on CMC agar. The average CR values of fungal isolates were 2.75 to 2.83, 2.60 to 2.83 and 2.56 to 2.72, for LC, RC and LRC, respectively. Considering all types of microbes (bacteria, fungi and actinomycetes) isolates from the LRC treatment seemed to provide the lowest CR ratio (Figure 1). The ability of bacteria to produce CR in this study was moderate when compared with the CR of *Bacillus* sp. isolated from different types of compost (1.3 to 3.4) [9]. Ten isolates from each type of microbes (bacteria, fungi and actinomycetes) showing the highest clear zone were selected for further experiments (Table 1). Among bacterial isolates, the highest CR (3.2) was obtained from isolate Comb3012. Cellulose hydrolysis capacity of fungal isolates obtained in this study was found to be highest (2.57 to 3.5) among all the HC positive isolates (Table 1). According to this screening, fungal isolate Comf3004 had the highest activity with CR of 3.50 followed by bacterial isolate Comb3012 and Comb6005 with the CR of 3.20 and 3.10, respectively (Table 1). In other study, it was reported that the CR of fungal isolate was around 2.5 and above [10]. There seemed to be little information related to cellulase activity of actinomycetes. The CR of actinomycetal isolates ranged from 2.10 to 3.1 with the average value of 2.23. Actinomycetal isolates that exhibited the highest CR was Comac9009 with value of 3.1. A total of 30 isolates (Table 1) that generated large and medium clearing zone by Congo red assay on CMC agar were selected to examine their enzyme activity.

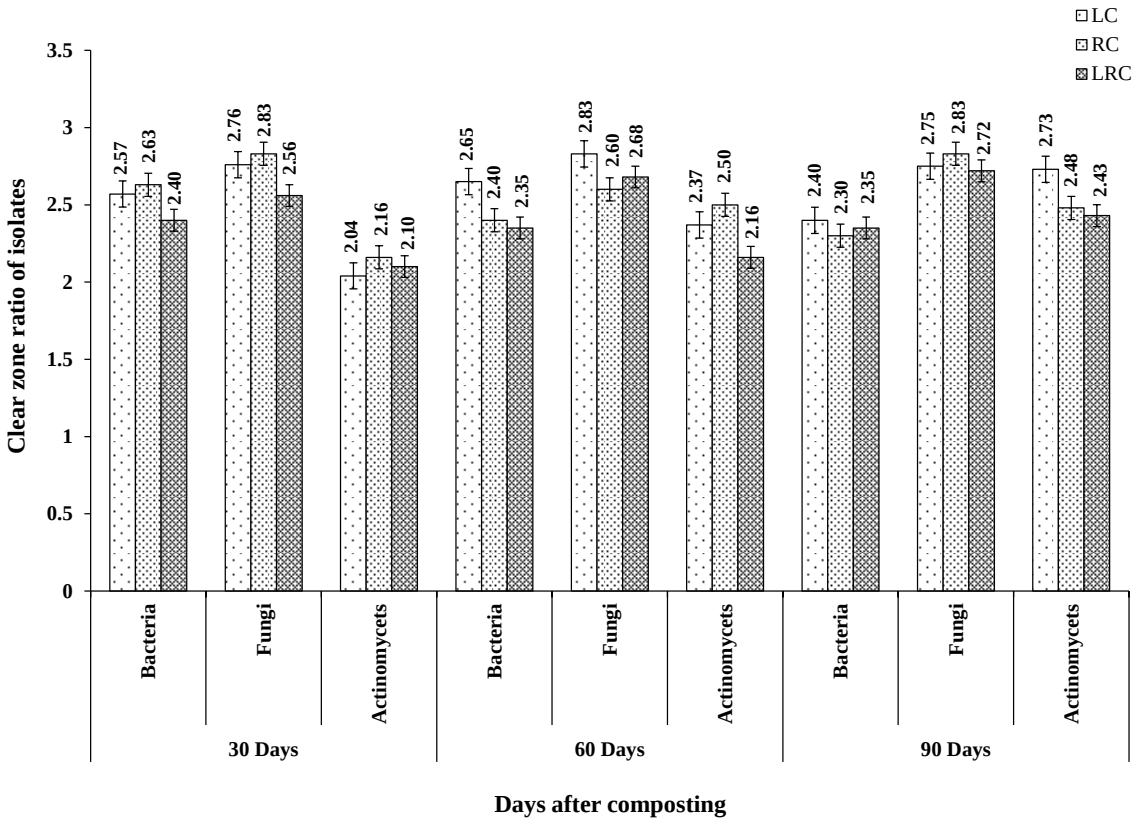


**Figure 1** The Average of clear zone ratio of bacteria, fungi and actinomycetes that showed clear zone ratio on CMC medium.

**Table 1** Selected bacterial, fungal and actinomycetal isolates based on clear zone ratio.

| Selected compost microorganisms | | | | | |
|---|---|---|---|---|---|
| Bacteria | | Fungi | | Actinomycetes | |
| isolate | CR[1] | isolate | CR[1] | isolate | CR[1] |
| 1. Comb3001 | 2.51 | 1. Comf3004 | 3.50 | 1. Comac3005 | 2.30 |
| 2. Comb3002 | 2.87 | 2. Comf3005 | 2.95 | 2. Comac6001 | 2.40 |
| 3. Comb3005 | 2.83 | 3. Comf3008 | 2.46 | 3. Comac6004 | 3.03 |
| 4. Comb3006 | 2.90 | 4. Comf6003 | 2.57 | 4. Comac6009 | 2.60 |
| 5. Comb3009 | 2.78 | 5. Comf6007 | 3.09 | 5. Comac6011 | 2.60 |
| 6. Comb3011 | 2.54 | 6. Comf6009 | 2.88 | 6. Comac6013 | 2.60 |
| 7. Comb3012 | 3.20 | 7. Comf6012 | 3.13 | 7. Comac9001 | 2.40 |
| 8. Comb6005 | 3.10 | 8. Comf9006 | 2.60 | 8. Comac9009 | 3.10 |
| 9. Comb6001 | 2.65 | 9. Comf9010 | 2.90 | 9. Comac9015 | 2.80 |
| 10. Comb9004 | 2.95 | 10.Comf9013 | 2.60 | 10. Comac9018 | 2.80 |

CR[1] = clear zone ratio

*3.2 Screening of endo-1,4-beta-D-glucanase (CMCase) cellulase enzyme activities production*

Among ten selected bacterial isolates, only one isolate could not produce enzyme (Figure 2A). The rest of bacterial isolates (9 isolates) showed high variation in producing enzyme. Among bacterial isolates, the highest CMCase activity was obtained by comb3012 (0.596 U/mL) followed by comb3011, comb3009 and comb3002 (0.265, 0.224 and 0.201 unit/mL, respectively). In other compost microbes studies, T1 and T2 were screened and selected as the most efficient enzyme producers, with values of 0.410 and 0.320 unit/mL, respectively [11] while C12 and EB6 showed a higher rate of glucose reduction with 1.29 and 1.64 unit/mL, respectively [9].

Out of ten fungal isolates, up to nine isolates showed cellulolytic enzyme activity. The highest of CMCase activity was obtained by comf6012 (0.857 unit/mL) followed by comf3004, comf9010, and comf6009 (0.602, 0.578 and 0.549 unit/mL, respectively) (Figure 2B). Rittiboon et al. [12] reported the highest CMCase activity of

the isolate FA68 followed by FA50 with the enzyme activity of 0.170 and 0.130 unit/mL, respectively. Very high cellulase producing activity was found with fungal isolates identified as *Aspergillus terrues* (CMCase 3.05 unit/mL) [13]. Therefore, it could be concluded that the fungal isolates tested in this study appeared to exhibit moderate CMCase enzyme activity.

The same results were obtained for bacteria and fungi. Nine out of ten selected actinomycetal isolates were able to exhibit CMCase activity. The highest of CMCase activity was obtained by isolates Comac9009 (0.525 unit/mL) followed by Comac6004, Comac6009 and Comac3005 (0.389, 0.378 and 0.342 unit/mL, respectively) (Figure 2C). These results were very similar to the findings reported by Mohanta [14] who tested nine isolates of cellulose-degrading actinomycetes. He found that the total cellulase activity ranges from 0.50 to 1.38 unit/mL. Actinomycetes in four genera (*Kibdelosporangium*, *Micromonospora*, *Streptomyces* and *Nocardioides*) generated cellulolytic enzyme and could efficiently degrade rice straw [15]. Getbuakhow et al. [16] isolated actinomycetes from animal waste and screened them for cellulase activity. Their result showed that maximum activity was obtained by JNN-1501 (0.217 unit/mL) followed by JJN-1255, JJN-1500 and JJN-1816 with values of 0.165, 0.156 and 0.151 unit/mL, respectively. Cellulase enzyme activity method applied in this study revealed that bacterial, fungal and actinomycetal isolates exhibited medium to high ability to produce cellulase in CMC broth. From the results of CMCase activity in this study, one isolate from each microbial group that show highest ability in exhibiting cellulase enzyme activity was selected to develop microbial inoculum for longan leaves composting. The three selected isolates were Comb3012, Comf6012 and Comac9009.

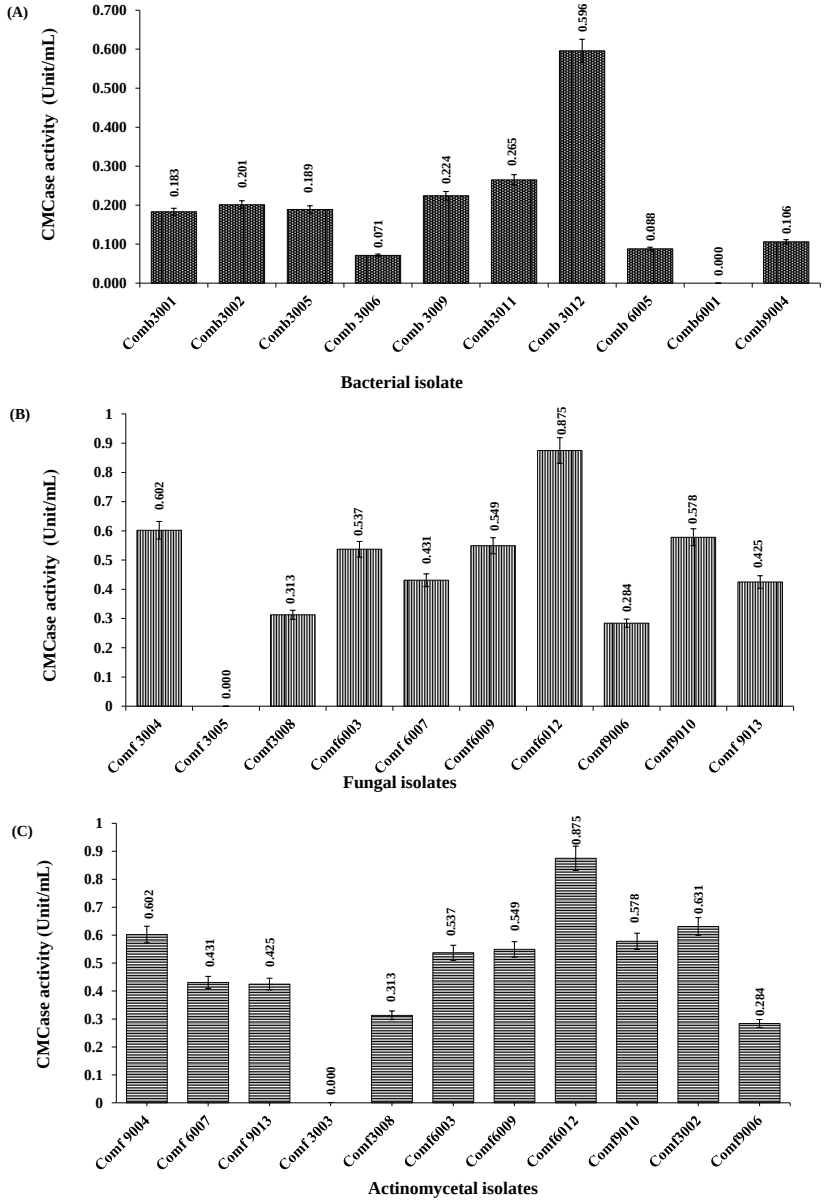


**Figure 2** Cellulase enzyme activity of the high potential (A) bacterial (B) fungal and (C) actinomycetal isolates.

*3.3 Production of microbial inoculants prototype*

The sterile mix-carrier was used to produce inoculants by blending with the starter culture (60% water holding capacity of the carrier) to obtain the product prototype of Comb3012-inoculant, Comf6012-inoculant and Comac9009-inoculant (Figure 3). The three inoculants were used to incorporate in the LC under each treatment.


**Figure 3** Prototype of microbial inoculum; cellulose decomposer (A) Comb3012, (B) Comf6012, (C) Comac9009 and (D) Comb3012+Comf6012+Comac9009.

*3.4 Physico-chemical properties changes in the LC inoculated with microbial inoculants*

The longan leaves + cow dung (LC) was inoculated with the prototype of three microbial inoculants: LCb, LC + Comf6012 (LCf), LC + Comac9009 (LCac) and LC + the mixture of Comb3012 + Comf6012 + Comac9009 (LCbfac). The LC without inoculation was used as a control while LC plus sterile carrier (LCC) was also applied for comparison. Changes in physico-chemical properties were monitored during 120 days of composting. The pH values of all the treatments decreased from 7 for 60 days. Afterwards, the values increased and ranged from 7.0 to 7.3 (Figure 4A). The compost microorganisms operate best under neutral to acidic conditions, with pH in the range of 5.5 to 8. Therefore, the pH values of the LC and LC plus inoculants(s) conducting in this study were suitable for microbial activity. In general, at the early stages of decomposition, various types of organic acids are formed and led to pH decrease. The acidic conditions are favorable for growth of fungi and breakdown of lignin and cellulose. As composting proceeds, the organic acids become neutralized, the pH increase, and mature compost generally has a pH between 6 and 8. The results of this experiment were in agreement with Selim et al. [17] who reported that the wastes were alkaloid of which pH values varied from 8.19 to 8.32. It was also found that during composting, the pH values ranged between 7.1-7.9 [18]. Therefore, in general, the pH of finished compost appeared to be alkali.

On the average, the EC value of all the treatments slightly increased after around 30 days of incubation. It appeared that the EC values at 120 days of LCC (2.2 dS/m) and LC plus microbial inoculants (1.9 to 2.3 dS/m) exhibited higher EC values than the control without microbial inoculation (1.7 dS/m) (Figure 4B). The higher EC values might indicate higher mineralization of organic materials. However, from a cropland point of view, the EC value of the final compost is important because it can become a limiting factor for plant growth and seed germination. Too high or too low of EC value is not conducive to the growth of plants. The EC value reflected the degree of salinity in the compost, indicating its possible phytotoxic/phyto-inhibitory effects on the growth of plant if applied to soil [19]. EC is important parameter to determine the compost quality as high salt concentration can inhibit the seed germination. So, it is essential to measure the EC of compost before its application as a soil conditioner. Three different composts in another study showed an initial EC of 8.3, 4.82, and 7.19 dS/m [20] which were much higher than found in the present study. In general, the value of 4.0 dS/m for EC is tolerable by plants whereas values from 6 to 12 dS/m indicating toxicity level of salts for most plants up to the Greek standard [21]. Therefore, the EC values obtained from various compost under this study would not be harmful to the plant. The results revealed that the GI value of all the treatments increased with composting time. It appeared that the GI value more than 80% was achieved shortly before 90 days of composting for all the treatments (Figure 4C). GI indicates the maturity of a compost in general. Immature compost produces phytotoxins which restrain the growth of plant. When GI value is greater than 80%, the compost is considered to be matured and non-toxic to plant. After 120 days after composting, the maximum GI value (95.13%) was recorded in LCf followed by LCbfac (93.3%) and LCac (93.2%), respectively. While the lowest GI value (91.2%) was found in LC (control) and LCb. In another study, it was reported that a maximum of GI was observed in six easily available bio-wastes compost (102%) and a lower one was in six modes of co-compost (58%) [22].

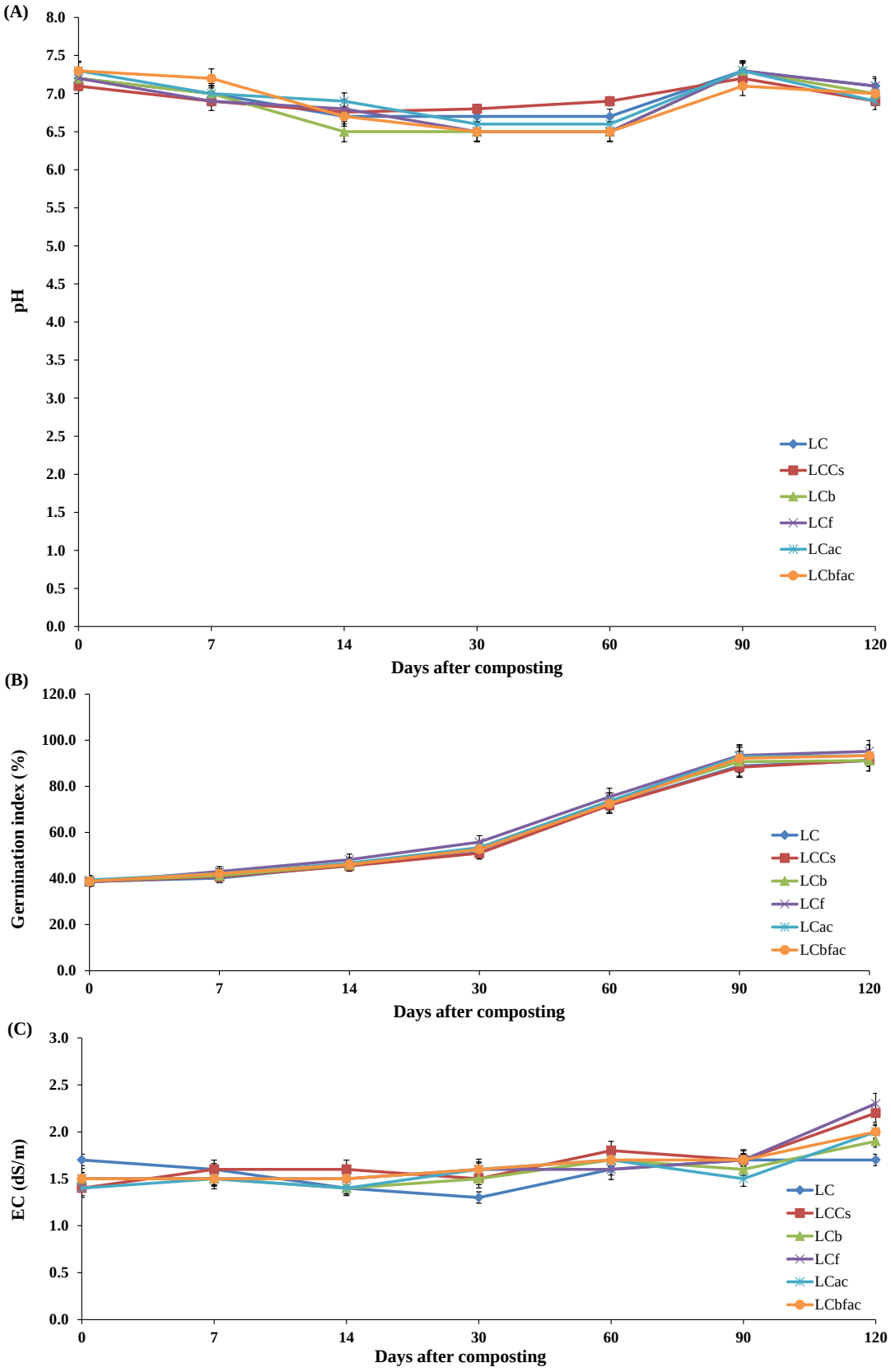


**Figure 4** Changes in (A) pH, (B) EC and (C) germination index (GI), during composting with microbial inoculants. Longan leaves + cow dung (LC), LC + sterile carrier (LCCs), LC + Comb3012 (LCb), LC + Comf6012 (LCf), LC + Comac9009 (LCac), LC + mixture of three inocula (LCbfac).

The percentage of OM at the beginning of composting varied between 61.1 to 62.5%. At 120 days, %OM of all treatments was reduced and ranged from 23.00 to 42.10%. The LCf and LCb compost exhibited a remarkably low OM content (23.0 and 26.3%, respectively). The results indicated that inoculation of microbial inoculants could effectively accelerate the decomposition of longan leaves compared with LC (control) and LCC (Figure 5A). The similar results were also obtained for C:N ratio. The inoculation of fungal inoculum in LCf gave the lowest C:N ratio (8.1) and the highest C:N ratio was observed in the control (12.9) (Figure 5B). Kausar et al. [23] reported that during composting of rice straw C:N ratio was reduced to 18.1 from an initial value of 29.3 in 6 weeks of composting. In this study, due to the GI and C:N ratio, the mature compost from longan leaves can be achieved and used without harmful to plant after 90 days of incubation. Saidi et al. [24] reported that a stable C:N ratio could be achieved after 95 days of decomposition. Changes in the C:N ratio reflect OM decomposition and stabilization during composting process because microorganisms used carbon as source of energy and N for building cell structure [25]. Carbon and nitrogen are the two most important elements among those required for microbial decomposition of OM to produce compost. Low initial C:N ratios caused a fast degradation of fibers during the first three months of composting (hemicellulose: 50-80%, cellulose: 40-60%) with high initial C:N ratios resulted in 10-20% degradation of both hemicelluloses and cellulose [26].

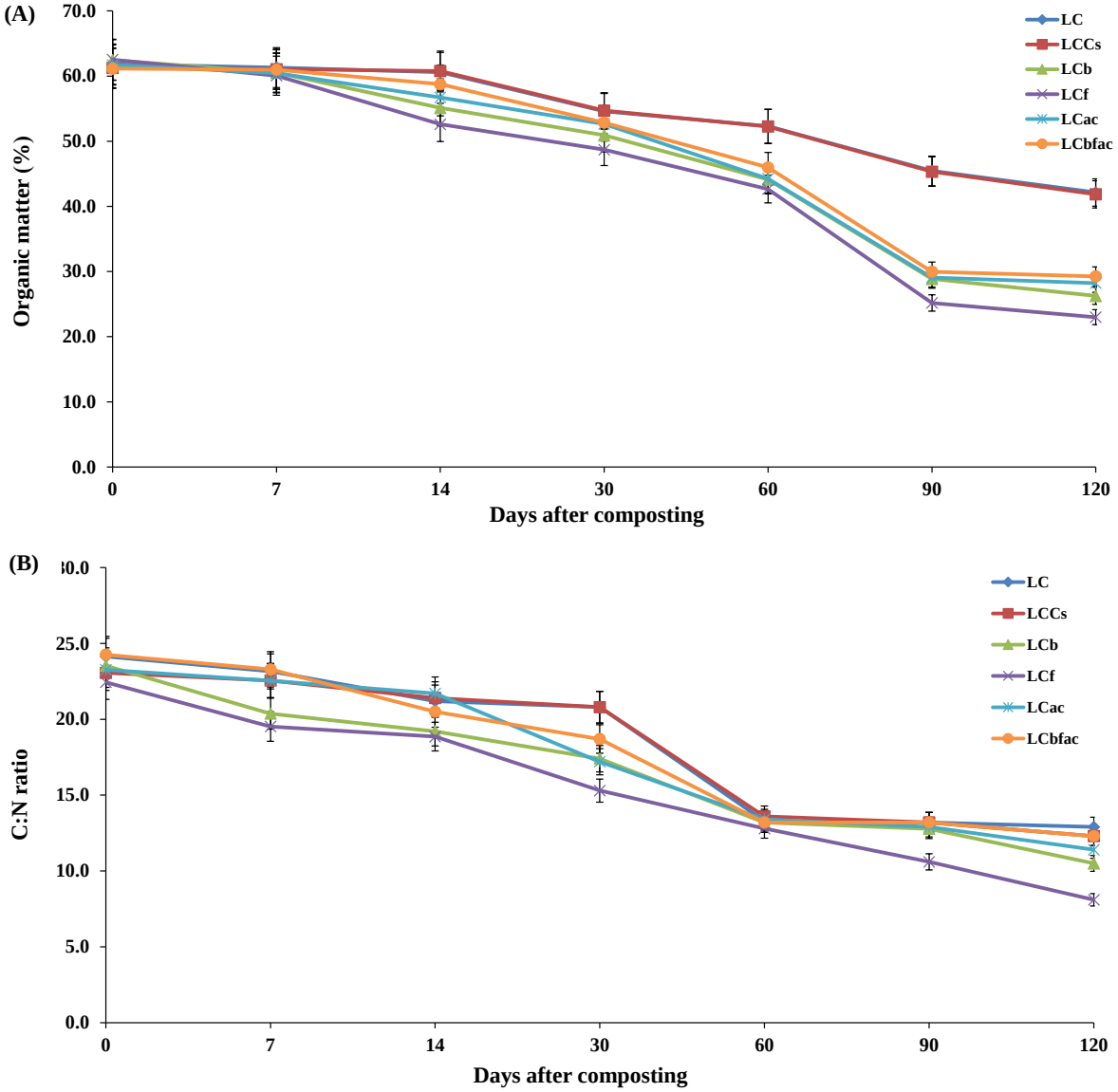


**Figure 5** Changes in (A) organic matter (OM) and (B) C:N ratio, during composting with microbial inoculants. Longan leaves + cow dung (LC), LC + sterile carrier (LCCs), LC + Comb3012 (LCb), LC + Comf6012 (LCf), LC + Comac9009 (LCac), LC + mixture of three inocula (LCbfac).

*3.5 Nutrient concentration in the LC inoculated with microbial inoculants*

The nutrient content (TN, TP and TK) of compost product for all the treatments are shown in Table 2. The maximum TN was obtained in LCf, LCb and LCac with the values of 2.5, 2.3 and 2.2%, respectively, at 120 days after composting. The high TP value at 120 days was obtained in LCf, LCac, LCbfac and LCb with values of 0.59, 0.44, 0.37% and 0.32%, respectively (Table 2). While the TP value of compost without inoculation was only around 0.3%. The highest TK content of 1.33% was recorded in LCf followed by 1.17% in LCC, 1.13% in LCb, and 1.10% in LCbfac. The application of microbial inoculum Comf6012 gave the highest decomposition rate (Figure 5A) and the highest TN, TP and TK with values of 2.50, 0.59 and 1.33%, respectively (Table 2). All the treatments with microbial inoculant(s) gave significantly higher TN, TP and TK than those of the control treatment. It was found in another study that TP content of coir pith composted in 12 weeks varied between 0.39 and 0.47% while the TK content had a great variation between 0.77 and 1.28% when compared to the control [27]. In general, good compost properties should contained OM >20%, C:N <20:1, Total N >1%, P > 0.22%, and K> 0.41%.

**Table 2** Changes in nutrient values at 120 days after longan leaves composting.

| Treatments | Nutrients concentration (%) | | |
|---|---|---|---|
| | N | P | K |
| Control (Longan leaves) | 2.17bc | 0.13d | 0.93d |
| Longan leaves + sterile carrier | 2.10c | 0.15d | 1.13b |
| Longan leaves + Comb3012-inoculant | 2.27b | 0.32c | 1.17b |
| Longan leaves + Comf6012-inoculant | 2.50a | 0.59a | 1.33a |
| Longan leaves + Comac9009-inoculant | 2.17bc | 0.44b | 1.10bc |
| Longan leaves + (Comb3012-Comf 6012-Comac 9009-inoculant) | 2.20bc | 0.37bc | 1.00cd |
| CV (%) | 4.09 | 17.03 | 6.71 |

*3.6 Changes in cellulose, hemicellulose and lignin content*

In general, composting of agricultural wastes is driven by microorganisms under aerobic conditions. The numbers and diversity of microbial population can affect the rate of composting process, the physical and chemical properties, as well as the carbohydrate content (e.g. cellulose, hemicellulose and lignin) of the product [28]. Several techniques have been explored to improve compost quality including the addition of microbial inoculants. Microorganisms in the compost produce cellulolytic enzymes to degrade cellulose which is the main component of plant cell walls. In the present study, microbial inoculum prototypes were developed using highest efficient isolates of bacteria Comb3012, fungi Comf6012 and actinomycetes Comac9009. The microbial inoculum prototypes of the three isolates were incorporated into the longan leaves compost as single and the combination of the three. The proportion of three main components including cellulose, hemicellulose and lignin in the initial longan leaves before composting was 33.9, 19.9 and 8.7%, respectively.

To evaluate the effect of microbial inoculum on decomposition rate, the cellulose, hemicellulose and lignin contents of the compost samples were analyzed after inoculation with high cellulase enzyme producing isolate (s) at 0, 7, 14, 30, 60, 90 and 120 days of composting. The cellulose content at the beginning of composting was between 33.3-34.3% (Table 3). The cellulose content in the compost pile obviously decreased in the inoculated treatments than that of the control and the sterile carrier treatment (Figure 6). Among the inoculated treatments, the use of fungal Comf6012-inoculum (LCf) exhibited the highest decrease in cellulose content with value of ten percent lower (20.4%) than the control (30.0%) at 90 days of composting. After 120 days of composting, the highest cellulose degrading activity was also obtained with LCf (18.5%) followed by LCb (21.4%), LCac (25.1%) and LCbfac (27.1%).The results indicated that the microorganisms could effectively degrade cellulose contained in longan leaves, when compared with the control one (Table 3). Additionally, microbial inoculation in LCf, LCb, LCac and LCbfac, resulted in a sharp and higher decrease in the total organic carbon (Figure 5A) as well as a rise in compost quality by an increase in total nitrogen, phosphorus, and potassium content, as compared to the control and the sterile carrier treatments (Table 2). Dubey and Maheshwan [29] concluded that the cellulolytic fungi, such as *Aspergillus*, *Trichoderma*, *Penicillium* and *Trichurus* effectively accelerated composting of dry crop wastes with high C:N ratio and reduced its period to be about one month. The fungal isolate Comf6012 obtained and tested in this study showed a very high potential to speed up the composting process of high C:N ratio agricultural wastes particularly longan leaves.

**Table 3** Changes in cellulose content during composting period.

| Treatments | Days after composting | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 30 | 60 | 90 | 120 |
| LC | 33.4 ±0.3 | 33.3±0.2 | 33.0±0.3 | 32.4±0.1 | 31.7±0.2 | 30.0±0.2 | 29.4±0.2 |
| LCCs | 33.7±0.2 | 33.7±0.3 | 33.5±0.2 | 32.7±0.3 | 31.3±0.3 | 29.8±0.1 | 28.3±0.2 |
| LCb | 33.4±0.2 | 32.0±0.2 | 31.0±0.2 | 28.8±0.2 | 26.9±0.3 | 24.3±0.2 | 21.4±0.3 |
| LCf | 33.3±0.3 | 31.2±0.2 | 28.8±0.3 | 25.8±0.1 | 23.7±0.3 | 20.4±0.3 | 18.5±0.2 |
| LCac | 34.3±0.2 | 33.3±0.3 | 31.9±0.1 | 30.1±0.2 | 28.8±0.1 | 27.1±0.2 | 25.1±0.3 |
| LCbfac | 33.7±0.2 | 33.6±0.3 | 32.8±0.2 | 31.8±0.1 | 30.4±0.3 | 29.1±0.3 | 27.1±0.3 |
| Mean | 33.6 | 32.8 | 31.9 | 30.3 | 28.8 | 26.8 | 25.0 |

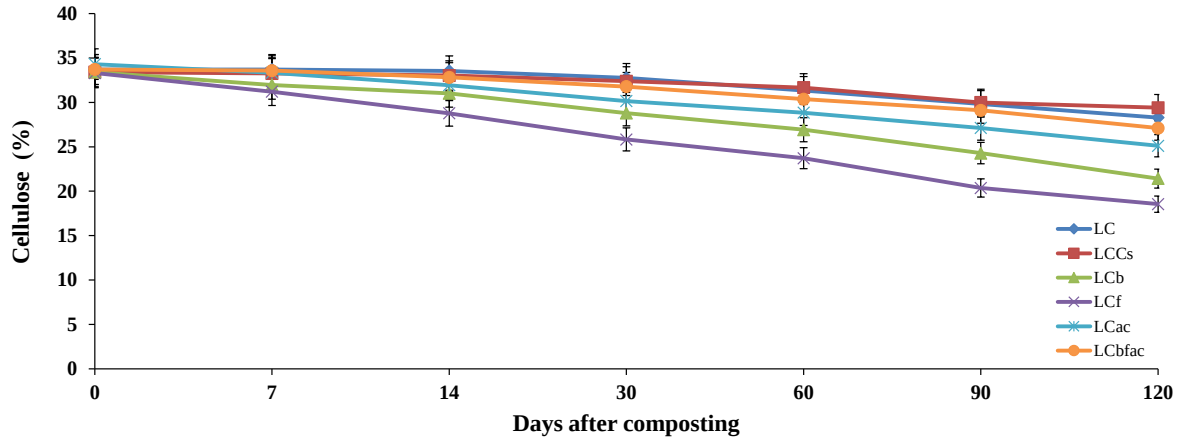


**Figure 6** Changes in cellulose content during composting with microbial inoculants. Longan leaves + cow dung (LC), LC + sterile carrier (LCCs), LC + Comb3012 (LCb), LC + Comf6012 (LCf), LC + Comac9009 (LCac), LC + mixture of three inocula (LCbfac).

The initial values of hemicellulose varied from 18.1-19.6% (Table 4). Changes of hemicellulose fractions during composting showed similar pattern as cellulose in that fungal inoculum (LCf) gave the maximum reduction of hemicellulose throughout the composting period. On the average, the hemicellulose content in the compost pile decreased more rapidly in the inoculated treatment than that of the control (LC) and the sterile carrier treatment (LCC) (Figure 7). However, the reduction of hemicellulose in LCb and LCac showed similar values at all incubation periods. Due to high cellulase activity of fungal isolate Comf6012, active degradation and maximum reduction of hemicellulose and cellulose were observed in LCf.

**Table 4** Changes in hemicellulose content during composting period.

| Treatments | Days after composting | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 30 | 60 | 90 | 120 |
| LC | 19.2±0.3 | 19.0±0.2 | 18.9±0.3 | 18.7±0.2 | 17.1±0.2 | 16.6±0.3 | 14.1±0.2 |
| LCCs | 19.4±0.3 | 19.2±0.2 | 18.9±0.3 | 18.2±0.2 | 17.2±0.3 | 15.5±0.4 | 14.1±0.1 |
| LCb | 18.8±0.2 | 18.1±0.3 | 17.4±0.2 | 16.9±0.2 | 16.0±0.2 | 14.5±0.3 | 13.3±0.2 |
| LCf | 18.1±0.3 | 17.2±0.2 | 16.8±0.1 | 15.4±0.2 | 14.7±0.4 | 13.7±0.3 | 12.5±0.1 |
| LCac | 18.4±0.2 | 18.4±0.2 | 17.5±0.3 | 16.8±0.2 | 15.6±0.3 | 14.6±0.2 | 13.6±0.1 |
| LCbfac | 19.6±0.2 | 19.1±0.2 | 18.5±0.2 | 18.0±0.2 | 17.4±0.3 | 17.1±0.3 | 15.1±0.2 |
| Mean | 18.9 | 18.5 | 18.0 | 17.3 | 16.3 | 15.3 | 13.8 |

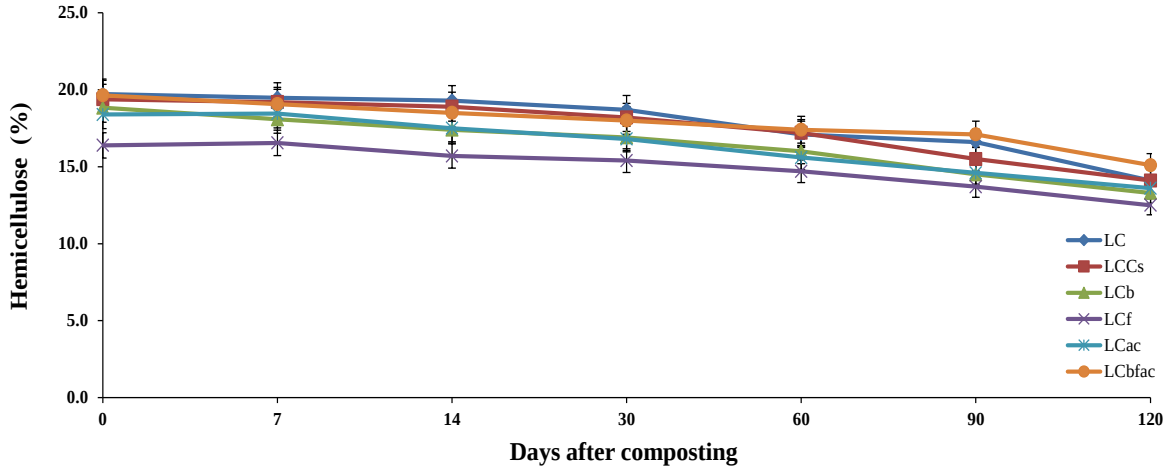


**Figure 7** Changes in hemicellulose content during composting with microbial inoculants. The error bars represent the standard deviation of measurements for 3 compost samples.

Actinomycetes are known to be one of the most efficient microbes of secondary metabolite producers as well as variety of enzymes which can be applied in biotechnological industry and biodegradation of agricultural waste. However, little investigation has been made in biodegradation processes driven by actinomycetes [30]. Therefore, it is worthwhile to investigate and apply composting actinomycete for biodegradation and humification of organic wastes, particularly high lignin materials. In the present study, at the beginning of composting the content of lignin varied between 7.2-8.7% (Table 5). In contrast to cellulose and hemicellulose, the application of actinomycetal isolate Comac9009 (LCac) exhibited the highest rate of lignin decomposition at all time of incubation period as compared to fungal isolate in LCf (Figure 8). This phenomenon indicated that actinomycetes can effectively decomposed hardly degradable substance i.e. lignin. After the end of composting at 120 days. The lowest content of lignin was obtained with LCac with value of 4.9% followed by LCf (5.0%), LCbfac (5.1%) and LCb (5.6%). All the inoculated treatments exhibited higher lignin degradation than that of the control and sterile carrier treatments. To date, microbial inoculants for rapid composting, are mainly bacteria and fungi, while there is rare investigation on the effect of actinomycetes inoculation in the degradation of lignocellulose polymers (i.e., cellulose, hemicellulose, and lignin) during composting. The inoculation of actinomycete has shown to accelerate the cellulose degradation and humification in composting [31].

Application of microbial inoculants (bacteria, fungi and actinomycetes) to longan leaves in this experiment could effectively promote the decomposition of all components of lignocellulose (cellulose, hemicellulose and lignin).

**Table 5** Changes in lignin content during composting period.

| Treatments | Days after composting | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 30 | 60 | 90 | 120 |
| LC | 8.2±0.3 | 8.2±0.2 | 8.0±0.1 | 7.9±0.3 | 7.5±0.1 | 6.9±0.3 | 6.4±0.1 |
| LCCs | 7.6±0.2 | 7.6±0.2 | 7.6±0.3 | 7.4±0.2 | 7.2±0.4 | 6.5±0.2 | 6.2±0.1 |
| LCb | 8.7±0.4 | 8.1±0.1 | 7.6±0.3 | 6.8±0.3 | 6.5±0.1 | 6.0±0.1 | 5.6±0.1 |
| LCf | 8.6±0.3 | 8.5±0.2 | 8.1±0.1 | 7.5±0.2 | 7.2±0.1 | 5.2±0.4 | 5.0±0.1 |
| LCac | 7.2±0.3 | 6.6±0.1 | 6.3±0.4 | 5.9±0.3 | 5.7±0.3 | 5.1±0.1 | 4.9±0.1 |
| LCbfac | 7.6±0.3 | 7.4±0.1 | 7.2±0.3 | 6.9±0.1 | 6.7±0.1 | 5.1±0.4 | 5.1±0.1 |
| Mean | 8.0 | 7.7 | 7.5 | 7.1 | 6.8 | 5.8 | 5.5 |

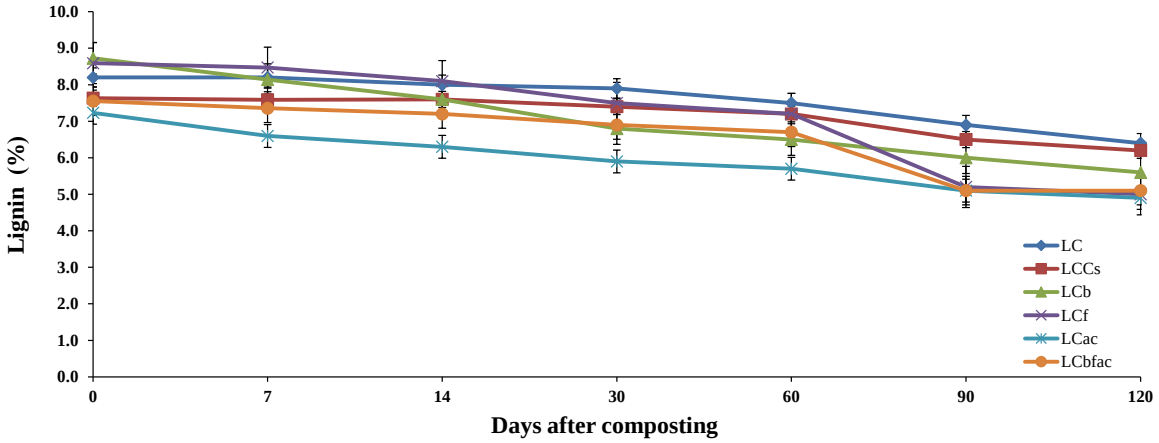


**Figure 8** Changes in lignin content during composting with microbial inoculants. Longan leaves + cow dung (LC), LC + sterile carrier (LCCs), LC + Comb3012 (LCb), LC + Comf6012 (LCf), LC + Comac9009 (LCac), LC + mixture of three inocula (LCbfac).

## 4. Conclusion

Among all the positive isolates that exhibited cellulose hydrolysis capacity, the fungal isolates produced the widest clear zone on CMC agar (2.56 to 3.5). Quantitative cellulase enzyme activity showed that the highest CMCase activity from each microbial group were bacterial isolate Comb3012 (0.596 U/mL), fungal isolate Comf6012 (0.875 U/mL) and actinomycetal isolate Comac9009 (0.525 U/mL). Microbial inoculum was successfully developed from these high potential isolates. The application of microbial inoculum in the present study aimed at accelerating the composting process of longan leaves and improving the quality of the final compost product. The results indicated that adding of the microbial inoculum (single or mix) could result in increasing the decomposition rate (rapid decrease in OM, C:N ratio) and faster maturity (reach the GI of >80% slightly faster than the control treatment). At the end of the composting process, the nutrient contents including TN, TP, and TK of all the treatments with microbial inoculation were also higher than the control treatment. The microbial inoculation of single or mix-inocula exhibited positive impact on the decomposition degree of the total lignocelluloses (cellulose, hemicellulose and lignin), particularly cellulose. Fungal isolate Comf6012 performed the best in breaking down cellulose and hemicellulose fractions while actinomycetal isolate exhibited the highest lignin degradation. The results highlight the high possibility in the application of effective microbial inoculum for rapid composting process of longan leaves.

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