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The efficiency of potential food waste-degrading bacteria under harsh conditions

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Abstract

Aims: Investigate the impact of highly adapted bacterial strains and their ability in waste degradation under a wide range of temperatures.

Methods and Results: Bacteria isolated from soil and food waste were grown in various media under fluctuated temperatures. After screening for organic compound degradation, the seven strongest bacterial strains have been selected for further experiments. Their enzyme activities were expressed in terms of the size of the hydrolysis zone in a wide temperature range of 2·5–70°C. The enzyme production assay was carried out for each protease, cellulase and amylase. The waste degradation was determined with a maximum 80% decrease in the volume of food waste in 21 days compared to the control in lab scale with enriched bacterial cultures and soil bacteria as additives at room temperature around 18–20°C.

Conclusion: These seven bacteria are promising candidates for food waste biodegradation in composting especially in the winter without heating expense for maintaining ambient temperature.

Significance and Impact of the Study: It is necessary to coax the uncultured bacteria from the various environments into the laboratory for investigating their valuable functions. Herein, using enrichment culture of consortium and additive of soil has illustrated the significant mean in food waste degradation.

KEYWORDS

organic waste decomposition, psychrophiles, soil bacteria, waste treatment bacteria

Introduction

Composting is considered an effective natural biological treatment because of its positive environmental impact, sustainability and cost-effectiveness compared to chemical and physical treatment methods (Coker 2006). However, poor quality and long periods required for composting are challenges. Co-composting of organic waste is globally recognized to be an effective method in composting. The chicken/cow manure has high nitrogen in co-composting could favour microorganisms to degrade different organic solid wastes into qualified compost.

Therefore, many researchers have used pig, cow or chicken manure as additives to supply cellulose-degrading bacteria and minimize the composting period (Park 2011; Haroon *et al*. 2020; Hwang *et al*. 2020).

In other study, the results showed that short-period composting of 40 days could be achieved by adding palm oil mill effluent sludge, including nutrients and indigenous microbes which are known as lignocelluloses-degrading bacteria (Zainudin *et al*. 2013, 2017). Another concept was developed with a bacterial consortium as well as the addition of 2% lime resulting in mature compost after 42 days (Awasthi *et al*. 2018).

Cellulose, protein and complex saccharides are well-known common components of food waste. Microbes utilize carbon and nitrogen from the organic waste as energy sources for their growth by a series of enzymatic activities to form water-soluble compounds. Proteins, polysaccharides, nucleic acids and starch are decomposed by oxidation and hydrolysis (Chandra and Rustgi 1998; Leja and Lewandowicz 2010).

On the other hand, in previous studies, the use of enriched bacterial matter in composting was not attractive because of the difficulty of controlling food waste content. Recently, however, this strategy has been used to investigate effective waste-degrading bacteria under mesophilic and thermophilic condition to evaluate the productivity of composting (Awasthi *et al*. 2018; Song *et al*. 2018).

In previous couple of decades, psychrophiles (organisms capable of growth at low temperatures) have been shown to have numerous economic and ecological advantages in enzyme production compared to their counterparts acting at high temperature (Margesin and Schinner 1994; Laurent *et al*. 2000; Mahadevan *et al*. 2008; Kasana 2010; Kasana and Gulati 2011). The strong activity of cold-adapted microorganisms contributes to a rapid warming from the initial temperature, allowing decomposing matter to enter a thermophilic stage quickly. However, few studies have focused on microorganisms that can survive in a wide range of temperatures including extremely low and high temperatures, as well as their ability to degrade waste under the same conditions. As composting is known to be self-heating, a candidate degradation that has strong activity at low temperatures in the initial days of composting process may save energy used for heating composting location to maintain process stability. Moreover, in the thermophilic phase, these bacteria are still active in degrading waste, which may inhibit harmful bacteria activity, improve the quality of compost and shorten the overall composting process because of an extension of the thermophilic phase. However, the temperature of composting reactors drops sharply from thermal to mesophilic conditions in winter. Therefore, this study introduces both of bacterial candidates working individually and in cooperation with waste and soil bacterial communities in the degradation of food waste in cold weather. The analysis of functional bacteria and the examination of enzymatic activities of microorganisms in lab scale is recommended as an important and promising approach for large-scale environmental engineering application.

Soil as an attractive source of functional bacteria

Besides waste materials, the soil has also attracted many researchers owing to its microbial diversity, and unknown functions more than 99% of uncultured bacteria have not yet been coaxed in the laboratory (Hayat *et al*. 2010; Pham and Kim 2012). Besides, the soil is an ideal habitat for a complex community of living microorganisms. Bacteria account for 10 billion cells g^{-1} in soil (Morris and Blackwood 2015). The high capacity to produce diverse extracellular enzymes such as amylase, protease, lipase, pectinase, cellulase and chitinase from bacteria has attracted much attention (Mishra and Behera 2008; Sasmita and Niranjan 2008; Alariya *et al*. 2013; Oseni and Ekperigin 2013). However, there is little research on soil micro-organisms as an additive in composting (Fathallh Eida *et al*. 2012; Dida *et al*. 2018). Therefore, in this study, we continue studying soil bacteria that may contribute significantly to organic waste degradation.

Materials and methods

Sampling and bacterial isolation

The sources for bacterial isolation in this study were from soil and a mixture of food waste and pig manure which were prepared for composting process.

The soil samples were collected from root surrounding in the mountain of Kyonggi University, Food waste was collected from the Jowon Industry. Acidic soil and waste were adjusted to pH 7·0 before the isolation step in order to explore the function of bacteria in waste treatment.

Five grams of each sample were added to 50 ml of R2A modified medium containing (g l^{-1}): casein acid hydrolysate 0·5; yeast extract 0·5; proteose peptone 0·5; dextrose 0·5; soluble starch 0·5; dipotassium phosphate 0·3; magnesium sulphate 0·024; sodium pyruvate 0·3 and 10 ml of trace element, 10 ml of vitamin solution and 10 ml of autoclaved soil extract (ASE). The final pH (at 25°C) was 7·0 ± 0·2. The trace element compositions (g l^{-1}) was $MnSO_4{\cdot}7H_2O$ 0·01; $ZnSO_4{\cdot}7H_2SO_4$ 0·05; H_3BO_3 0·01; $N(CH_2COOH)_3$ 4·5; $CaCl_2{\cdot}2H_2O$ 0·01; Na_2MoO_4 0·01; $CoCl_2.6H_2O$ 0·2 and $AlK(SO_4)_2$ 0·01. The vitamin solution contained (g l^{-1}) riboflavin 0·025; citric acid 0·02; folic acid 0·01 and para-aminobenzoic acid 0·01. ASE was prepared by adding 100 g of soil to 1000 ml of distilled water and was adjusted to pH 7 before autoclaving (ASE) (Pham and Kim 2016). Media were autoclaved for 20 min (121°C, 103 kPa) before their use for culture. These samples were incubated at 28°C for 5 days on a rotary shaker at 200 rev min^{-1}. During a 5-day period of incubation time, the samples were changed the growth conditions at different temperatures of 5, 10, 20, 40 and 45°C and pH values of 5, 7 and 9. The enrichment of well-adapted bacteria in growth fluctuated conditions was repeated with 1 ml of culture transferred to fresh medium and prepared for further isolation steps. After incubation,

well-separated colonies were subculture to separate agar plates, and the enzyme production and waste degradation ability were examined in the next step.

Screening the ability of isolates to degrade organic compounds

A series of dilutions was prepared from 10^{-1} to 10^{-6}, and then, 100 μl of each dilution was transferred to agar plate. Pure isolates were tested the degradation of organic components using starch, skim milk and carboxymethylcellulose (CMC) to represent amylose, protein and cellulose, respectively. Colonies, which formed a clear zone on each agar plate, were recorded as positive activities. Iodine was used as an indicator for starch and CMC after incubating at 30°C for 48 h. The strains were storage in glycerol stock (25% final concentration) and kept at −80°C for preservation.

Adaptation of pure enrichment culture in community

An examination of the adaptation of target candidates in the composting process by pure bacterial strains isolated from soil and waste is necessary. Therefore, a series of samples was set up as follows: each individual strain, a consortium of strains C3, C5, C7 and C8 and a consortium of seven effective strains in individual and in a mixture of (i) supplemented by soil or compost and (ii) both soil and compost added.

Optimal growth conditions for enzyme production by isolates

Growth media

Seven different modified media were prepared for each isolate: Reasoner's 2A (mR2A) broth (BD, Seoul, South Korea), Luria–Bertani broth (mLB; Oxoid Ltd, Basingstoke, UK), nutrient broth (mNB; Oxoid), trypic Soy Broth (mTSB; Oxoid Ltd), skim milk (mSK; BD, South Korea), starch (mST; BD), carboxymethylcellulose sodium salt (mCMC; Sigma, Seoul, South Korea) and a combination of skim milk, carboxymethylcellulose sodium salt and starch (mSCS) with ASE as mineral supplement to each medium (Pham and Kim 2016). Each original medium was used as a control without modified solution. Pure culture of each isolate was prepared in subculture before inoculation in various media and incubated at 30°C at 120 rev min^{-1} for 5 days. The results were recorded by visually decreasing the turbidity of skim milk containing protein and a colourless level of iodine as an indicator.

pH values

The degradation of seven bacterial strains was test in each medium CMC sodium salt agar, skim milk and starch included ASE (1 ml l^{-1} medium) medium at different pH values ranging from 5 to 9 (at intervals of one unit) measured by a pH metre (HI 2210). Size of clear zone on the agar was measured to indicate the degradation ability.

Temperature

Three screenings for each strain were carried out at various temperature values in a range of 2·5–75°C and incubated for 48 h. Strong activity of the bacterial strain was determined by the size of clear zone in which the substrates were degraded.

Determination of food waste degradation by bacterial enrichment

The capable strains were further selected for lab-scale trials with 500 g of small heaps of food waste. Each heap was inoculated with 5% of enriched individual bacterial culture and a mixture of seven strains. All trials included 5 g of soil and were compared to control samples without additives (inocula and soil) at no-initial pH control under ambient temperature ranging from 13 to 19°C. The heaps were mixed thoroughly to maintain aeration in the period. The volume of the waste was calculated accordingly on 3, 7, 10, 15 and 21 days following the equation below:

$$\text{Volume} = \pi \times r^2 \times h.$$

where h is the height of the small heap and r the radius of the bottom of the heap.

Enzyme assay

Six selected bacterial strains were enriched in suitable media at 30°C at 150 rev min^{-1} for 5 days. For cellulase fermentation, media contained (g l^{-1}): NaCl 5; peptone 10; yeast 5; carboxymethylcellulose 5 and KH_2PO_4 1. Amylase production media was composed of (g l^{-1}) peptone 10; beef 5; NaCl 5 and starch 2. Protease fermentation included (g l^{-1}): peptone 15; yeast 3; NaCl 5 and glucose 5.

To prepare for enzyme assay, enriched bacterial cultured were centrifuged at 10 000 rev min^{-1} for 10 min at 4°C. The supernatant was used for enzymatic assay. All trials were conducted in duplicate, and the standard error was then calculated.

Amylase examination was carried out following DNSA (3, 5-dinitro-salicylic acid) method. Enzyme samples from supernatant extracted from bacterial culture were prepared at various dilution factors. One millilitre of 1% starch and 1 ml of citrate-phosphate buffer (pH 6·0) were mixed with an enzyme sample and incubated at 50°C for 30 min. The mixture was then added to 2 ml of DNSA and boiled in a water bath for 10 min, and the absorbance was recorded at 540 nm using glucose as a standard. The enzyme activity was determined as the amount of enzyme that releases 1 μmol of reducing sugar as glucose per minute (U ml^{-1} min^{-1}; Bernfeld 1955).

The protease assay was started with a mixture of different dilutions of enzyme samples and 50 mmol l^{-1} glycine-NaOH buffer (pH 9) to make 1 ml volume. One millilitre of 1% casein as a substrate was added to the mixture and incubated at 60°C for 10 min. The reaction was stopped by adding 0·5 ml of 20% trichloroacetic acid (TCA) (w/v). The mixture was filtered after being kept at room temperature for 30 min. 5 ml of 0·5 mol l^{-1} Na_2CO_3 solution were added to 1 ml of filtrate. The sample was then kept in the dark after adding 0·5 ml of Folin and Ciocalteu's phenol reagent. The absorbance was measured spectrophotometrically at 660 nm against tyrosine as a standard. One unit of protease activity was determined as the amount of enzyme required to release 1 g of tyrosine per ml in 1 min (U ml^{-1} min^{-1}; Folin and Ciocalteau 1927; Anson 1938).

The cellulase assay was conducted according to a standard method (Ghose 1987). The total cellulose activity was determined by measuring the amount of reducing sugar formed from filter paper. Endoglucanase (β1-4 endoglucanase) activity was examined by measuring the reducing sugar from amorphous cellulose. This examination was performed by adding 0·5 ml of supernatant with 0·5 ml of 2% amorphous cellulose in 0·05 mol l^{-1} Na-citrate buffer (pH 4·8) for 30 min at 50°C. Filter paper cellulase (FPCase) activity was carried out by adding 0·05 ml of supernatant to 1 ml of 0·05 mol l^{-1} Na-citrate buffer (pH 4·8) containing Whatman No. 1 filter paper strip (50 mg 20 ml^{-1}). The reaction was stopped at the end of an hour incubation by adding 3 ml of DNSA reagent to 1 ml of the mixture. The reagent blank was 1·5 ml of citrate buffer. The substrate control was 1·5 ml of citrate buffer and filter paper strip. Enzyme controls were prepared by adding 1 ml of citrate buffer to 0·5 ml of enzyme each dilution. A stock of anhydrous glucose was made at 10 mg ml^{-1} and stored frozen. A series of dilution was prepared. Glucose standards were prepared by adding 0·5 ml of each glucose dilution to 1 ml of citrate buffer. Blank, controls and glucose standards were incubated at 50°C for 1 h along with the enzyme assay tubes and was stopped by addition of 3 ml of DNSA reagent. The reduction of sugar was estimated spectrophotometrically at 540 nm. One unit of enzymatic activity is defined as the amount of enzyme that releases 1 μmol of reducing sugars (measured as 2 mg of glucose) per ml per minute.

Estimation of cellulose degradation was carried out by a gravimetric determination of cellulose digestion. Selected bacterial strains were cultured at the optimal temperature and pH value for each strain at 150 rev min^{-1} in enzyme production media containing KH_2PO_4 0·5; $MgSO_4$ 0·25; gelatin 2 and Whatman filter paper (50 mg 20 ml^{-1}) in 1 l of distilled water. Three-day-incubated cultures were centrifuged at 6000 rev min^{-1} for 15 min at 4°C. Supernatants were later used as crude enzymes for further test. Pellets collected by centrifugation were used to estimate the weight loss of trials with and without bacterial inocula at constant weight after drying. All experiments were carried out in triplicate. Data are presented as the mean ± standard error of the triplicate results.

16S rRNA phylogeny of isolated bacteria

The strongest positive degradation bacterial strains were identified using 16S rRNA gene by PCR.

The InstaGene Matrix kit (Bio-Rad, Korea) was used to extract genomic DNA from strains according to the manufacturer's instructions. Amplification of the 16S rRNA gene was performed by PCR using primers 27F and 1492R (Frank *et al*. 2008). A multiscreen filter plate (Millipore Corp, Bedford, MA) was used to purify the PCR product, which was then sequenced using primers 518F (5′-CCA GCA GCC GCG GTA ATA CG-3′) and 800R (5′-TAC CAG GGT ATC TAA TCC-3′) with a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). This process was conducted at 95°C for 5 min. The product was cooled on ice for 5 min and analysed using an ABI Prism 3730XL DNA analyser (Applied Biosystems). Finally, SeqMan software (DNASTAR Inc., Madison, WI) was used to assemble the nearly full-length 16S rRNA sequence. Sequence similarity was determined by comparing with the sequence available in the gen bank database using EZBioCloud server (Yoon *et al*. 2017).

Results

Isolation and identification of isolated bacteria

Screening for bacterial enzyme production was detected by the hydrolytic zone on culture plates as evidence of the hydrolytic capacities of potential isolates. Twenty-five strains were able to degrade all cellulose, protein and saccharides. Among of them, seven strains showed the strongest activity. Phylogenetically, they belong three

genera and closest to the strains are *Exiguobacterium acetylicum* DSM 20416^T (99·73%), *Burkholderia contaminans* LMG 23361^T (99·86%), *Bacillus paranthrasis* Mn5^T (100%), *Bacillus siamenis* KCTC 13613^T (99·86%), *Bacillus tequilensis* KCTC 13622^T (99·93%), *Bacillus cereus* ATCC 14579^T (99·39%) and *Bacillus velezensis* CR-502^T (99·86%; Table 1).

Optimal growth conditions for the degradation of organic compounds of isolates

Optimal media

A modified solution including trace elements, vitamins and soil extract solutions showed an effect on enzyme production of all strains compared to control trials. A combination of skim milk, starch and cellulose, R2A, TSB and LB agar containing modified a modified solution at pH 7·5, was identified as the optimal medium for acceleration of the enzyme production test in bacterial culture enrichment. The agar plates were incubated at 30°C for 48 h.

Almost all bacterial strains grew well in a modified medium of mTSB, mLB, mR2A and a mixture of skim-cellulose-starch (mSCS) after 12 h. A medium containing mTSB and mR2A was best for strains S4 and C3, while a medium with mTSB and mSCS was found to be the most effective for strains C2 and C7. Stronger activity in protein degradation of strain C5 and C8 was found for media mLB and mR2A, respectively. Finally, mLB and mSCS were found to be optimal media for strain C15, with the highest degradation rates of protein, cellulose and starch, respectively.

Optimal pH value

All strains decomposed protein at all pH values and performed optimally at pH 9 and 8. In particular, strains C3, C5, C7, C8 and C15 showed higher degradation ability compared to strains S4 and C2 at pH values of 5 and 6. The results are shown in Table 2.

The efficiency of cellulose is considered to depend on pH rather than protein or starch degradation. Cellulose was degraded strongly by strains S4, C3, C5, C7, C8 and C15. Among them, only three strains (C5, C8 and C15) showed high effective activity over a wide range of pH values from acidic to alkaline, whereas strains S4 and C3 were not able to produce cellulase enzyme at a pH lower than 6.

As a result, starch was degraded significantly by strains C5, C7 and C15 at all pH values tested, whereas lower pHs were not ideal for amylase production in strains S4, C3 and C8. Therefore, adjustment of the pH value of compost to the range 7·0–8 is usually necessary because of acidic pH of the original food waste.

Optimal temperature

All strains showed ability to degrade organic compounds in a wide range of temperature from 15 to 45°C, except strain C2, which was only weakly active at 15°C. In protein degradation, almost all strains were capable degrading in the range 2·5–10°C except strains C2 and C3, which produced protease weakly at 2·5°C. In the range 2·5–15°C only, strain C2 did not degrade cellulose. At 10°C, all of isolates were active in amylase degradation. However, four of the seven strains, C5, C7, C8 and C15 degraded well at 5 and 2·5°C, and only strain C15 was unable to produce amylase enzyme at 2·5°C.

All strains were able to degrade the three components more strongly at temperatures of 20–40°C than at higher temperatures of 45–70°C. Strains S4, C2 and C5 have been shown to have an optimal temperature ranging from 30–40°C. However, strains S4 and C2 had weak protease and amylase activity at 45°C and no activity at 50°C, and strain C3 was not able to degrade amylase at 50°C. Strain C5 was active until 70°C and stopped at 75°C. A range of 30–45°C

TABLE 1 Organic compound degrading bacteria and the closest bacterial strains

Strain name	Isolated source	Closest strains	Similarity (%)
S4	Forest soil	*Exiguobacterium acetylicum* DSM 20416^T	99·73
A2	Food waste	*Burkholderia contaminans* LMG 23361^T	99·86
C3	Food waste	*Bacillus paranthrasis* Mn5^T	100
C5	Forest soil	*Bacillus siamenis* KCTC 13613^T	99·86
C7	Food waste	*Bacillus tequilensis* KCTC 13622^T	99·93
C8	Food waste	*Bacillus cereus* ATCC 14579^T	99·39
C15	Forest soil	*Bacillus velezensis* CR-502^T	99·86

TABLE 2 The summary of optimal conditions of bacterial growth and organic degradation rate by a measurement of clear zone around the colony

	Optimal growth condition			Degradation ability (measured in diameter of clear zone in mm)			
Strain name	Media	Temperature (°C)	pH	Protein	Cellulose	Starch	Tolerant condition
S4	mR2A, mTSB	30–40	8–9	25	35	25	In a range of pH 5–9 and 2·5–45°C
C2	mTSB, mSCS	30–40	8–9	24	30	20	In a range of pH 5–9 and 2·5–45°C
C3	mR2A, mTSB	30–45	8–9	30	40	25	In a range of pH 5–9 and 2·5–55°C
C5	mR2A, mLB	30–40	6–9	35	30	28	In a range of pH 5–9 and 2·5–70°C
C7	mSCS, mTSB	45–55	5–7	35	32	25	In a range of pH 5–9 and 2·5–65°C
C8	mR2A, mLB	45–55	7–9	40	40	28	In a range of pH 5–9 and 2·5–70°C
C15	mLB, mSCS	45–55	7–9	40	32	25	In a range of pH 5–9 and 2·5–70°C

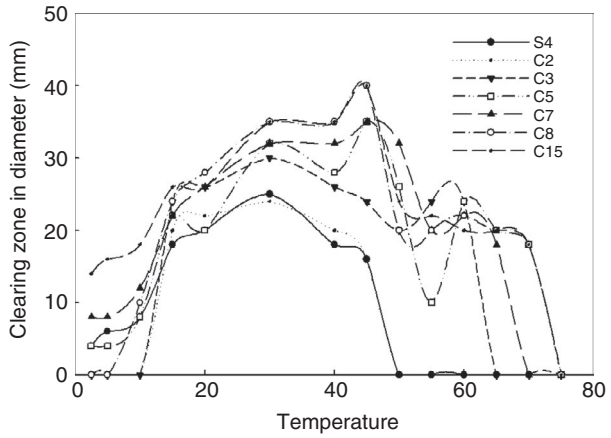

FIGURE 1 Cellulose degradation of seven isolates at various temperatures was measured in diameter of clearing zone on an agar plate containing carboxymethylcellulose as a substrate.

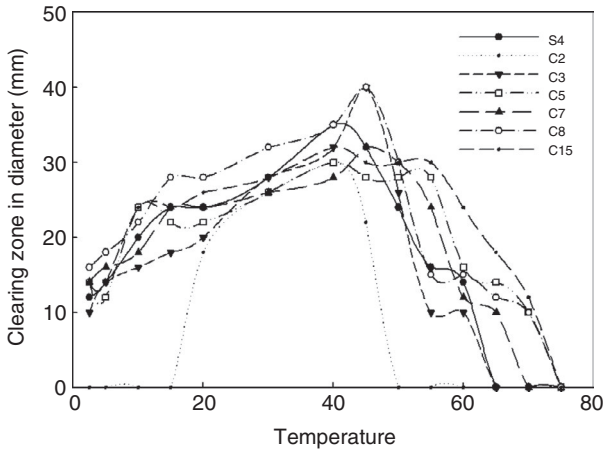

FIGURE 2 Amylose degradation of seven isolates at various temperatures was measured in diameter of clearing zone on an agar plate containing starch as a substrate.

is the optimal temperature for strain C3, even though the activity of this strain is limited at a temperature higher than 50°C. Strains C7, C8 and C15 had a highest organic compound degradation at approximately 40–45°C with a limitation at 70°C (Table 2).

Maximum amylase activity of *Bacillus* sp. BCC 021-50^T was found at a hyperthemophilic temperature of 70°C (Simair *et al*. 2017). The effect of temperature on enzyme production is described in Figs 1–3.

Enzyme production and cellulose degradation rate

The initial screening of seven isolates revealed zones of hydrolysis for all starch, protein and cellulose agar plates after 24 h (Fig. 4). The largest clear zone of 40 mm was measured for strains C8 and C15, followed by a clear zone of 35 mm for strains C5 and C7 in protein degradation test. In the screening of cellulose degradation, strains C3 and C7 were found to have a clear zone diameter of 40 mm, followed by 35 mm for strain S4 and 32 mm for both strains C7 and C15, and the weakest ability measured was 30 mm for strains C2 and C5. The largest size of clear zone of 28 mm for starch degradation was found for strains C5 and C8, followed by strains S4, C3, C7 and C15 with a size of 25 mm. The smallest size of degradation zone was 20 mm for strain C2 (Table 2).

A protease assay was used, examined to determine the enzyme activity of each isolate. The highest enzyme activity was found in strain C15 (803 U ml^{-1}), followed by strains C8, C7 and C5 at 726, 655 and 625 U ml^{-1}, respectively. Lower amounts were calculated for strains C3, S4 and C2 at 557, 484 and 466 U ml^{-1}, respectively (Fig. 5).

An amylase assay was carried out to assess the amylase activity of each isolate. Strain C15 exhibited the highest enzyme production of 218 U ml^{-1}, followed by 183, 182 and 179 U ml^{-1}, for strains C5, C8 and C7, respectively (Fig. 5).

The cellulolytic potential of seven isolates was determined in two tests to determine enzyme activity on filter paper and on endoglucanase. Strain C8 was found to have the strongest cellulase activity on filter paper as well as in endoglucanase assay, estimated at 0·74 and 3·7 U ml^{-1}, respectively. The activity of other strains ranged from 1·1 to 1·62 U ml^{-1} for endoglucanase and 0·43 to 0·622 U ml^{-1} for FPCase (Figs 6 and 7).

Gravimetric analysis demonstrated that the maximum and minimum degradation rates of the filter paper were 79 and 62%, respectively, examined after the 4 days of incubation.

Lab trials of food waste degradation in volume

Degradation of each isolate and their performance in the mixture of seven strains were indicated by a gradual decrease in the volume over 28 days. All seven trials inoculated with bacterial cultures show a high degradation rate in the 21-day time incubation period, with at least 62% reduction in volume compared to only 50% reduction for the control sample without inoculation at the same

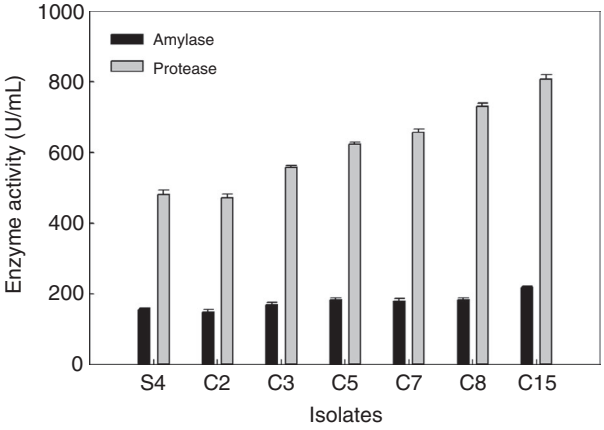

FIGURE 5 Proteas production investigated by turbidity reduction with medium containing protein. 0, skim milk medium containing protein as a control. Tested strains in a combination of soil and compost bacteria: 1–7, S1; C2–C7, respectively.

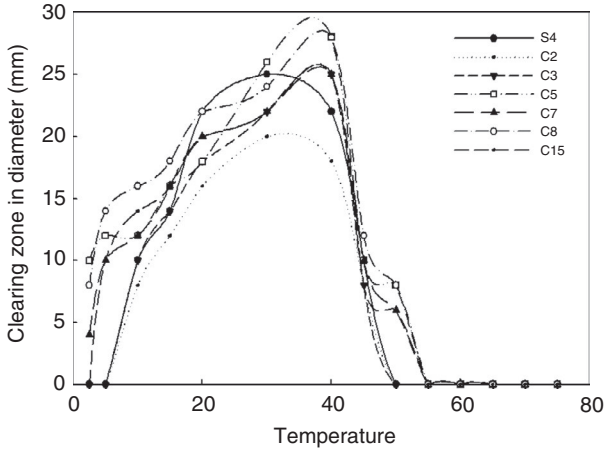

FIGURE 3 Protein, starch, and cellulose degradation tested on media containing skim milk, starch, and carboxymethyl cellulose as substrates.

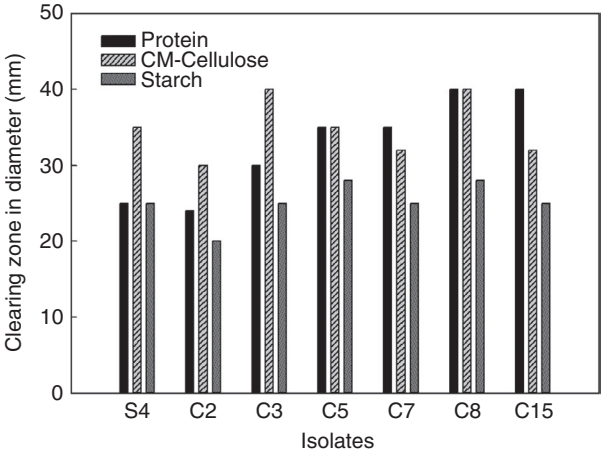

FIGURE 6 Protein, cellulose, and starch degradation of each isolate measured in diameter of the clear zone at 30°C for 72 h.

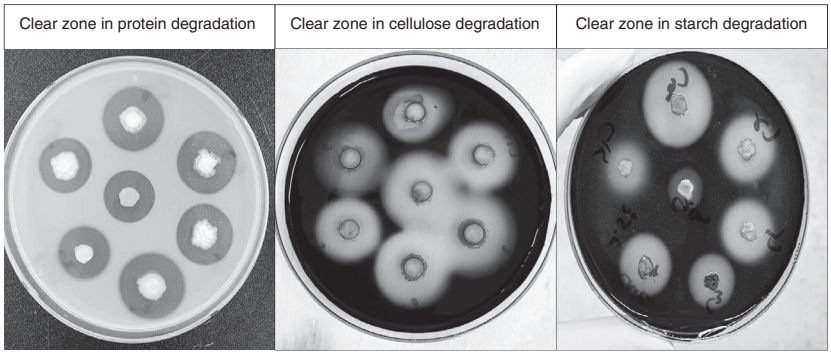

FIGURE 4 Cellulose degradation test illustrated in discolored iodine. 1, control without inoculum; 2, compost bacteria only; 3, soil and compost bacteria inoculated seven bacterial strains.

time. The maximum reduction was observed in the trial using the consortium of seven strains at 80%, followed by 73 and 70% for strains C15 and C8, respectively. The other strains exhibited the degradation rate of 62–68% (Fig. 8).

Pretesting adaption of target strains before application in compost

Previous studies have investigated the effect of different organic matter on the microbial biomass of soil and microbial community structure (Pérez-Piqueres *et al.* 2006; Saison *et al.* 2006; Toyota and Kuninaga 2006; Carrera *et al.* 2007). However, the efficiency of composting was demonstrated to vary depending on the type of compost because of different waste source and the type of soil in each area. Conversely, the purpose of this study was to introduce beneficial organic degrading bacterial strains isolated from soil and wastes in composting process. Therefore, examining their degradation in laboratory in a combination of compost microorganism and in a community where they are isolated from is necessary to confirm their efficiency before applying them in the composting process on a large scale. The results of our study show that all seven strains demonstrate good degradation of all cellulose, protein and starch in community, including soil, and compost bacteria and their combination performed better than the individual strains (Figs 9–12).

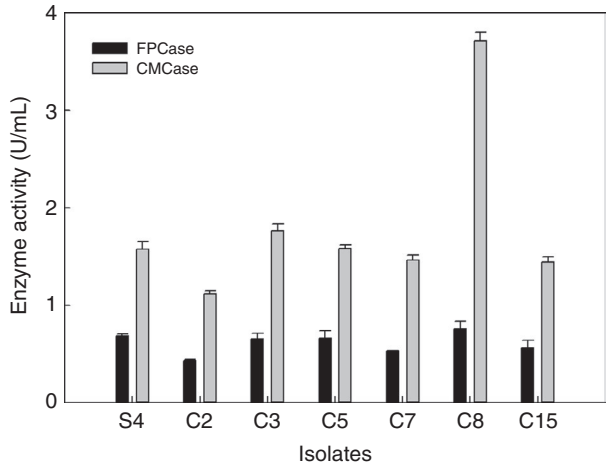

FIGURE 7 Extracellular cellulose activity including FPCase and Endoglucanase of seven isolates. Values in the figure are means of three replicates with standard deviations.

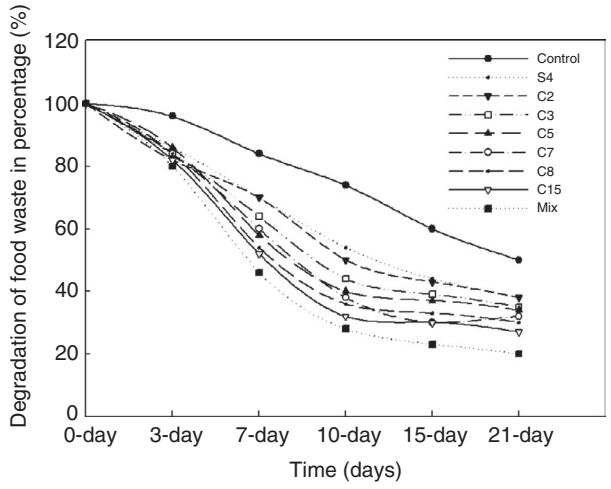

FIGURE 8 Amylase and protease activity of seven isolates was carried out using starch as the substrate. Values in the figure are means of three replicates with standard deviation.

Discussion

Phylogenetically, seven bacterial strains in this study are closed to the bacterial strains available in GenBank over 99% of similarity. Nevertheless, some of them are capable to degrade organic compounds which are absent or have not found yet in previous studies. In the comparison, the strain *Exiguobacterium acetylicum* DSM 20416^T (99·73%), close to strain S4, was found to be a bacterium unable to degrade cellulose (Chaturvedi and Shivaji 2006).

While strain S4 in this study can degrade cellulose even at low temperature of 2·5°C, strains C3 and C7 were capable to degrade complex saccharides as starch, but *Bacillus paranthracis* Mn5^T, close to strain C3, and *B. tequilensis* KCTC 13622^T, close to strain C7, were confirmed to be unable to degrade starch (Gatson *et al.* 2006; Liu *et al.* 2017).

Modified medium components-based traditional broth containing ASE as culture supplements showed more efficient in bacterial growth and enhanced the enzyme production of all isolates. Since the composition of

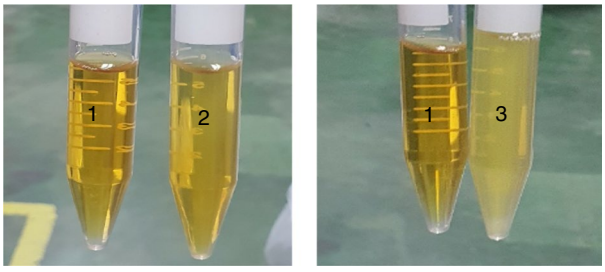

FIGURE 9 Bacterial growth test at 30°C after 24 h measured by turbidity of bacterial biomass. 0, starch medium containing complex saccharides as the control without inoculum. Tested strains: 1, soil bacteria; 2, compost bacteria only; 3, 7 strains with soil bacteria only; 4, 7 strains with compost bacteria only; 5–7, S4, C3+C7+C8, and seven strains with soil and compost bacteria, respectively.

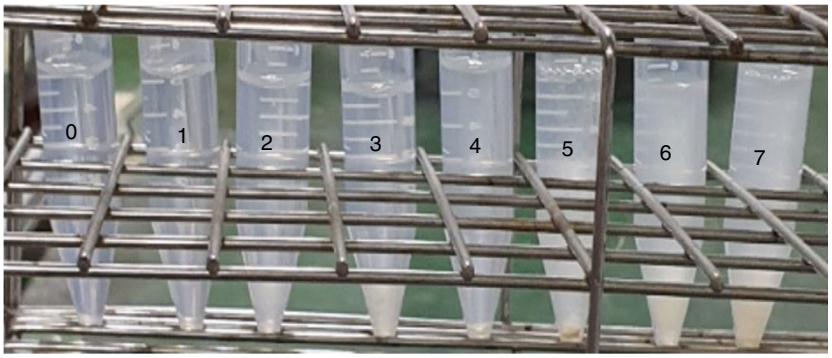

FIGURE 10 Amylase production in starch illustrated by discolored iodine. 1, starch media as a control; 2–8, each strain in individuals; 9, a mixture of seven strains (containing soil and food waste bacteria).

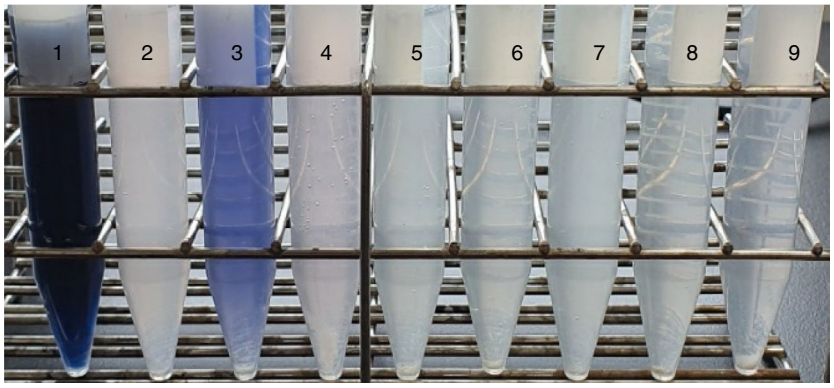

FIGURE 11 Protein degradation of seven isolates at various temperatures was measured in diameter of clearing zone on an agar plate containing protein as a substrate.

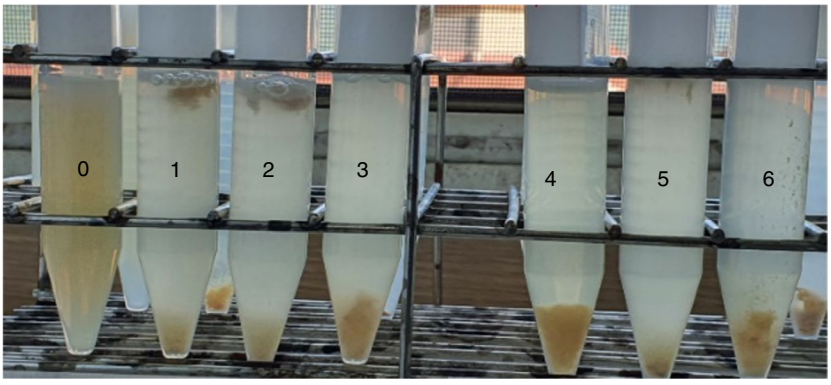

FIGURE 12 Laboratory scale degradation of food waste by best seven strains in individuals and in the mixture.

soil-extracted solubilized organic and inorganic matter investigated, soil extract was used as an additive to improve uncultured bacteria in many previous studies (Liebeke *et al*. 2009; Pham and Kim 2014, 2016). In this study, all bacterial strains were found the strong enzymatic activities in the modified medium rather than theirs in the traditional medium.

The effect of pH on cellulase production was found with a significant increase from 4 to 8 and decrease at pH 9 and showed the similar profile to the previous study (Sreedevi *et al*. 2013).

Strain C15 grew and be able to degrade protein and cellulose at both 5°C and 2·5°C, but only amylose was not decomposed by this strain at 2·5°C. In contrast to the previous study, the closest strain (*Bacillus velezensis* CR-502^T) to strain C15 did not grow at temperatures below 15°C or higher than 45°C (Ruiz-García *et al*. 2005). In other studies, most bacteria in Antarctica grew well between 20 and 40°C, but it was later confirmed that these bacteria were unable to degrade cellulose at high temperatures (Margesin and Feller 2010; Soares *et al*. 2012). In this study, strain C5 grew well at the maximum temperature of 70°C while its closest strain *Bacillus siamensis* KCTC 13613^T was confirmed to be unable to grow at temperatures higher than 60°C (Sumpavapol *et al*. 2010). The closest related strain *Bacillus cereus* ATCC 14579^T to strain C8 was found not to grow above 60°C (Liu *et al*. 2017). However, strain

C8 showed the survival and the activity at higher 60°C but limited at 70°C.

In general, bacteria at temperatures ranging from 30 to 40°C have been demonstrated to capable of enzyme production. However, thermophilic, psychrophilic, acidophilic, alkalophilic and halophilic bacteria exist in a wide variety of environments that are extremely resistant to environmental stress and even produce enzymes under exhaust conditions (Sreedevi *et al*. 2013).

This study, carrying out basic experiments of preapplication of bacteria at the lab scale, illustrated the potential of organic waste-degrading bacteria isolated from soil and waste. Examination of each strain individually and in combination with soil and composting bacteria showed high adaptation of these strains in these communities under a wide range of temperatures and pHs, which could not only save energy but also may result in improvements in efficiency and stability for large-scale composting in the winter. Partially, at moderate temperature, strains C5, C7, C8 and C15 exhibited the degradation of organic compounds more strongly compared to strains S4, C2 and C3. These promising strains may have a significant contribution to research focusing on enzyme production at low temperatures in the future.

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AUTHOR CONTRIBUTIONS

V.H.T.P. contributed in methodology, formal analysis, investigation, collect data, ideas, design, writing–original draft preparation, final edit and revision of the manuscript. S.W.C. and J.S.K. were in charge of conceptualization, ideas, design and review. J.Y.A. and Y.H.R. contributed to sampling and formal analysis. B.R. contributed to review and ideas. J.H.S. was in charge of resources, review and funding acquisition. W.J.C. was in charge of ideas, design, review, funding acquisition and supervision.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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REFERENCES

Alariya, S.S., Sethi, S., Gupta, S. and Lal, G.B. (2013) Amylase activity of a starch degrading bacteria isolated from soil. *Arch Appl Sci Res* 5, 15–24.

Anson, M.L. (1938) The estimation of pepsin, trypsin, papain, and cathepsin with haemoglobin. *J Gen Physiol* 22, 79–89.

Awasthi, M.K., Wang, Q., Wang, M., Chen, H., Ren, X., Zhao, J. and Zhang, Z. (2018) In-vessel co-composting of food waste employing enriched bacterial consortium. *Food Technol Biotech* 56, 83–89.

Bernfeld, P. (1955) Amylase α and β. *Methods Enzymol* 1, 149–158.

Carrera, L.M., Buyer, J.S., Vinyard, B., Abdul-Baki, A.A., Sikora, L.J. and Teasdale, J.R. (2007) Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. *Appl Soil Ecol* 37, 247–255.

Chandra, R. and Rustgi, R. (1998) Biodegrable polymers. *Prog Polym Sci* 23, 1273–1335.

Chaturvedi, P. and Shivaji, S. (2006) *Exiguobacterium indicum* sp. nov., a psychrophilic bacterium from the Hamta glacier of the Himalayan mountain ranges of India. *Int J Syst Evol* 56, 2765–2770.

Coker (2006) Environmental remediation by composting. *Biocycle* 47, 18–23.

Dida, G., Etisa, D. and Gizawu, B. (2018) Isolation and characterization of starch degrading rhizobacteria. *J Microbiol Biotechnol* 3, 2576–7771.

Fathallh Eida, M., Nagaoka, T., Wasaki, J. and Kouno, K. (2012) Isolation and characterization of cellulose-decomposing bacteria inhabiting sawdust and coffee residue composts. *Microbes Environ* 27, 226–233.

Folin, O. and Ciocalteu, V. (1927) On tyrosine and tryptophane determinations in proteins. *J Biol Chem* 73, 627–650.

Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A. and Olsen, G.J. (2008) Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 74, 2461–2470.

Gatson, J.W., Benz, B.F., Chandrasekaran, C., Satomi, M., Venkateswaran, K. and Hart, M.E. (2006) *Bacillus tequilensis* sp. nov., isolated from a 2000-year-old Mexican shaft-tomb, is closely related to *Bacillus subtilis*. *Int J Syst Evol Microbiol* 56, 1475–1484.

Ghose, T.K. (1987) Measurement of cellulase activity. *Pure Appl Chem* 59, 257–268.

Haroon, B., Hassan, A., Abbasi, A.M., Ping, A., Yang, S.H. and Irshad, M. (2020) Effects of co-composted cow manure and poultry litter on the extractability and bioavailability of trace metals from the contaminated soil irrigated with wastewater. *J Water Reuse Desal* 10, 17–29.

Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol* 60, 579–598.

Hwang, H.Y., Kim, S.H., Kim, M.S., Park, S.J. and Lee, C.H. (2020) Co-composting of chicken manure with organic wastes: characterization of gases emissions and compost quality. *Appl Biol Chem* 63, 3.

Kasana, R.C. (2010) Proteases from psychrotrophs: an overview. *Crit Rev Microbiol* 36, 134–145.

Kasana, R.C. and Gulati, A. (2011) Cellulases from psychrophilic microorganisms: a review. *J Basic Microbiol* 61, 572–579.

Laurent, P., Buchon, L., Guespin-Michel, J.F. and Orange, N. (2000) Production of pectate lyases and cellulases by *Chryseomonas luteola* strain MFCL0 depends on the growth temperature and the nature of the culture medium: evidence for two critical temperatures. *Appl Environ Microbiol* 66, 153–1543.

Leja, K. and Lewandowicz, G. (2010) Polymer biodegradation and biodegradable polymers. *Polish J Environ Stud* 19, 256–266.

Liebeke, M., Brözel, V.S., Hecker, M. and Lalk, M. (2009) Chemical characterization of soil extract as growth media for the ecophysiological study of bacteria. *Appl Microbiol Biotechnol* 83, 161–173.

Liu, Y., Du, J., Lai, Q., Zeng, R., Ye, D., Xu, J. and Shao, Z. (2017) Proposal of nine novel species of the Bacillus cereus group. *Int J Syst Evol Microbiol* 67, 2499–2508.Epub (2017 Aug 10) Erratum. Int J Syst Evol Microbiol. 2018, 2706.

Mahadevan, S.A., Wi, S.G., Lee, D.S. and Bae, H.J. (2008) Site-directed mutagenesis and CBM engineering of Cel5A (*Thermotoga maritima*). *FEMS Microbiol Lett* 287, 205–211.

Margesin, R. and Feller, G. (2010) Biotechnological applications of psychrophiles. *Environ Technol* 31, 835–844.

Margesin, R. and Schinner, F. (1994) Properties of cold-adapted microorganism and their potential role in biotechnology. *J Biotechnol* 33, 1–14.

Mishra, S. and Behera, N. (2008) Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *African J Biotechnol* 7, 18.

Morris, S.J. and Blackwood, C.B. (2015) *Soil Microbiology, Ecology and Biochemistry* ed. Eldor, P.A., 4th edn, pp. 273–309.Academic Press.

Oseni, A.O. and Ekperigin, M. (2013) Isolation and activity of alpha amylase from selected bacteria strains in the forest soil. *GJBB* 2, 17–20.

Park, K. (2011) Composting of food waste and mixed poultry manure inoculated with effective microorganisms. *Eng Agric Environ Food* 4, 106–111.

Pérez-Piqueres, A., Edel-Hermann, V., Alabouvette, C. and Steinberg, C. (2006) Response of soil microbial communities to compost amendments. *Soil Biol Biochem* 38, 460–470.

Pham, V.H.T. and Kim, J. (2012) Cultivation of unculturable soil bacteria. *Trends Biotechnol* 30, 475–484.

Pham, V.H.T. and Kim, J. (2014) *Bacillus thaonhiensis* sp. nov., a new species, was isolated from the forest soil of Kyonggi University by using a modified culture method. *Curr Microbiol* 68, 88–95. https://doi.org/10.1007/s00284-013-0443-1. Erratum. In: Curr Microbiol 2014, 69, 225.

Pham, V.H.T. and Kim, J. (2016) Improvement for isolation of soil bacteria by using common culture media. *J Pure Appl Microbiol* 10, 49–59.

Ruiz-García, C., Béjar, V., Martínez-Checa, F., Llamas, I. and Quesada, E. (2005) *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Vélez in Málaga, southern Spain. *Int J Syst Evol Microbiol* 55, 191–195.

Saison, C., Degrange, V., Oliver, R., Millard, P., Commeaux, C., Montange, D. and Le Roux, X. (2006) Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. *Environ Microbiol* 8, 247–257.

Sasmita, M. and Niranjan, B. (2008) Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *Afr J Biotechnol* 7, 3326–3331.

Simair, A.A., Khushk, I., Qureshi, A.S., Bhutto, M.A., Chaudhry, H.A., Ansari, K.A. and Lu, C. (2017) Amylase production from Thermophilic *Bacillus* sp. BCC 021–50 isolated from a marine environment. *Fermentation* 3, 25.

Soares, F.L., Melo, I.S., Dias, A.C.F. and Andreote, F.D. (2012) Cellulolytic bacteria from soils in harsh environments. *World J Microbiol Biotechnol* 28, 2195–2203.

Song, C., Zhang, Y., Xia, X., Hui, Q., Li, M., Hongwei Pan, H. and Xi, B. (2018) Effect of inoculation with a microbial consortium that degrades organic acids on the composting efficiency of food waste. *Microbial Biotechnol* 11, 1124–1136.

Sreedevi, S., Sajith, S., and Benjamin, S. (2013) Cellulase producing bacteria from the wood-yards on Kallai river bank. *Adv Appl Microbiol* 3, 326–332.

Sumpavapol, P., Tongyonk, L., Tanasupawat, S., Chokesajjawatee, N., Luxananil, P. and Visessanguan, W. (2010) *Bacillus siamensis* sp. nov., isolated from salted crab (poo-khem) in Thailand. *Int J Syst Evol Microbiol* 60, 2364–2370.

Toyota, K. and Kuniaga, S. (2006) Comparison of soil microbial community between soils amended with or without farmyard manure. *Appl Soil Ecol* 33, 39–48.

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H. and Chun, J. (2017) Introducing EzBioCloud: a taxonomically United database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67, 1613–1617.

Zainudin, M.H.M., Hassan, M.A., Tokura, M. and Shirai, Y. (2013) Indigenous cellulolytic and hemicellulolytic bacteria enhanced rapid co-composting of lignocellulose oil palm empty fruit bunch with palm oil mill effluent anaerobic sludge. *Bioresour Technol* 147, 632–635.

Zainudin, M.H.M., Ramli, N., Hassan, M.A., Shirai, Y., Tashiro, K., Sakai, K. and Tashiro, Y. (2017) Bacterial community shift for monitoring the co-composting of oil palm empty fruit bunch and palm oil mill effluent anaerobic sludge. *J Ind Microbiol Biotechnol* 44, 869–877.

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