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The Efficiency of Some Wild Strain Bacilli on Cellulase and Nitrogenase Activities During the Rice Straw Decomposition

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Abstract

This experiment aimed to select new effective bacteria with high ability for cellulose decomposition and nitrogen fixation from various natural materials in order to improve organic fertilizers. CMC, N-free medium were used as culture broth and agar. The procedure determined 60 isolates of bacteria from decomposing rice straw, decomposing wood, cow manure, bagasse organic fertilizers, termite nest and the LDD1 inoculum. After that process, the selected bacteria (6 isolates) were identified for species level. All isolates were rod-shaped, gram-positive and spore forming bacteria that belonged to a bacillus group in species of *Geobacillus stearothermophilus* and *Bacillus cereus* which some species showed thermophillic character. Then, three sub-experiments were conducted. By considering the testing of the bacterial ability on cellulase activity, CRD with 3 replications were employed. The result showed that 1) *G. stearothermophilus* DW3 had the highest potential of CMCase activity (37.50 mm diameter), 2) *G. stearothermophilus* RS6 had the highest potential of FPase activity (4.942 mU/ml), 3) *G. stearothermophilus* RS2 had the highest potential of Avicelase activity (12.680 mU/ml) and 4) *G. stearothermophilus* DW3 had the highest potential of α-cellulase activity (4.289 mU/ml). In addition, regarding the study of bacterial nitrogenase activity, CRD with 3 replications were employed. The result showed that *B. cereus* CM6 had the most outstanding nitrogenase activity (278.788 nmolC_2H_4/ml/hr**)**. In case of the study on the effectiveness of rice straw decomposition, the CRD with 3 replications were conducted. The study based on the standard index of organic fertilizer of Land Development Department (LDD), Thailand Ministry of Agriculture and Cooperatives. The result was found that the decayed rice straw in the inoculated treatment was qualified at 75 days, whereas the control treatment was not. The percentage of N, P and K in the inoculated treatments at 90 days was better than the previous period. In addition, C/N ratio decreased with times but the amounts of N, P and K increased. On the contrary, the percentages of N, P and K of the control treatment were satisfied while the C/N ratio was not in the standard level. The amounts of bacterial population among the inoculated treatments were not significantly different, but, they were significantly different between the inoculated and the control treatments.

Keywords: *Bacillus cereus***,** Cellulase enzyme, *Geobacillus stearothermophilus*, Nitrogenase enzyme

1. Introduction

Naturally, cellulose decomposition is caused from various microorganisms (Alexander, 1977; Couhlan & Ljungdahl, 1988). Some species of bacillus group have strong ability to produce cellulase enzyme. As a result, the effectiveness in cellulose decomposition is high (Mizukoshi *et al*., 1977). Cellulase enzyme consists of different types, react **β**-1,4-glycosidic linkage, including endoglucanase, cellobiohydrolase, and β-glucosidase. At the last stage of the activity, glucose is obtained which is useful to heterotrophs for their growth (Brexnak & Brune, 1994). Nitrogenase enzyme stimulates the nitrogen reduction and transforms N_2 to NH_3. This enzyme consists of two components, dinitrogenase (protein I) and dinitrogenase reductase (protein II). Dinitrogenase component is a large protein, 220-240 kDaltons. It fixes and reduces N_2 while dinitrogenase reductase component transmits electrons to dinitrogenase component (Tate, 2000). Nitrogen fixation, transforming N_2 to

NH_3, is a result from a group of nitrogen fixing bacteria (Burns & Hardy, 1975; and Postgate, 1982). Cellulose decomposition increases the nitrogen content of decaying organic material, which can be done in two ways. One is the utilization by both cellulolytic heterotrohs and nitrogen fixing microorganisms. The other way was the utilization by heterotrohs which have the potential of cellulose decomposition and nitrogen fixation in the natural environment (Halsall *et al.*,1993). The rice straw consists of cellulose as the main component and is mostly found at approximately 30-50 % **(**Soil science professor, Kasetsart University. 1998**)**. The rate of the decomposition process depends on the efficiency of bacteria. The environment factors, species and quantity of microorganisms and types of organic debris control the efficiency of the decomposition (Regen & Jeris, **1970)**. Nitrogen fixation by free living microorganism increases some parts of nitrogen in the environment, actually in the decayed organic materials. The total nitrogen in common organic fertilizers, particularly in the compost, is about 1 % **(**Land Development Department**,** 1997). High activity of bacteria on cellulose decomposition may reduce the time of compost production and release more plant nutrients through mineralization processes than low active one. While, biological nitrogen fixation of some free living bacteria and plant growth promoting rhizobacteria may increase the nitrogen content of the derived compost. Therefore, the time consuming problem of the compost production and low quality of the derived compost may be reduced by this experimental finding.

2. Materials and Method

2.1 The Selection of High Ability Bacteria on Cellulose Decomposition and Nitrogen Fixation

Decomposing materials, such as rice straw, wood, cow manure, bagasse organic fertilizer, termites with termite nest and LDD_1 inoculum were collected (table 1).The isolation of bacteria from various sources were conducted in CMC, N-free agar (in 1L: 20 g of CMC, 1.0 g of K_2HPO_4, 0.1 g of $CaCl_2$, 0.3 g of $MgSO_4.7H_2O$, 0.1 g of NaCl, 0.01 g of $FeCl_3$, 0.001 g of $Na_2MoO_4.2H_2O$,15 g of agar). The selection of high efficiency of bacteria was done from culturing the bacteria from various sources with the dilution at 10^{-3} and 10^{-4} intensity with pour plate method in CMC, N-free agar of replica plating technique (Teather & Wood, 1982). Sixty isolates of bacteria were selected. These isolates were screened for the second step by the method of Suyama *et al*. (1977). The bacterial culturing was incubated on CMC, N-free agar for one month. The diameter of clear zone of relevant isolates was determined. Six isolates with high efficiency on cellulose decomposition and nitrogen fixation were selected for further experiment.

Table 1. Types of materials, codes, isolated location and of bacterial isolates

Types of materials	Codes	Isolated location	Bacterial isolates
decomposing rice straw	RS	Banglen, Nakhon Pathom	RS1 – RS10
decomposing wood	DW	Bangbuathong, Nonthaburi	DW1 – DW10
cow manure	CM	Muang, Nonthaburi	CM1 – CM10
bagasse organic fertilizer	DF	Tha Muang, Kanchanaburi	DF1 – DF10
termites with termite nest	TM	Bangbuathong, Nonthaburi	TM1 – TM10
LDD_1 inoculum	LD	Chatuchak, Bangkok	LD1 – LD10

2.2 The Identification of Bacterial Isolates Based on Their Morphological Characteristics and DNA Sequence

Six isolates of bacteria were examined by Gram staining and their characteristics were determined under light microscope. Later, these isolates were identified for the genus and species levels by the 16S rDNA sequence (Jangaim, 2009). The amount of DNA was increased with PCR technique by using the primer, 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and primer, 1488R (5′-CGGTTACCTTGTTACGACTTCACC-3′) were used for Master Mix. PCR product, comparing with 1kb DNA ladder from 0.9 % agarose gel, was determined, dyed with ethidium bromide, checked with nucleotide sequence at Macro-gen co., Ltd., Korea, and analyzed nucleotide sequence with Vector NTI9. The result was sent to analyze the genetic information with GenBank at http://www.ncbi.nlm.nih.gov for the order of nucleotide BLAST and select highly similar sequences (megablast**)** to study the genus and species.

2.3 The Determination on Various Bacilli on Cellulase Activity

The CRD experiment was operated with 3 replications of 6 bacilli and non-inoculation (control). This procedure consisted of 4 types of cellulase activity of various bacilli; 1) CMCase activity on CMC, N-free agar, (Hankin & Anagnostakis, 1977), 2) FPase activity in N-free broth (Wood & MacCrae,1977), 3) avicelase activity in N-free broth (Nelson,1944) and 4) **α-**cellulase activity in N-free broth (Somogyi, 1952). The activities of various cellulose types were determined at 21 days after incubation.

2.4 The Determination on Various Bacilli on Nitrogenase Activity

The CRD experiment consisted of 7 treatments (6 bacilli and control with non inoculation) and 3 replications was done. The nitrogenase activity was measured by acetylene reduction assay **(**Hardy *et al*., 1973**)**. The each bacteria was cultured in 10 ml CMC, N-free broth contained in 50 ml vial at room temperature for 72 hours. Then, 10 ml of air inside the bottle was sucked out and replaced by 10 ml of acetylene and incubated for 12 hours. Ten ml of incubated gas inside the vial was sucked out and kept in the sample vial. Then, the nitrogenase activity of various bacilli was measured by Gas chromatography.

2.5 The Determination on the Efficiency of Various Bacilli in Rice Straw Decomposition and Nitrogen Fixation

The experiment was employed with 3 replications of completely randomized design (CRD). The treatments were 1) control (non inoculation), 2) *G. stearothermophilus* RS2, 3) *G. stearothermophilus* RS6, 4) *G. stearothermophilus* DW3, 5) *G. stearothermophilus* DW4, 6) *B. cereus* CM2 and 7) *B. cereus* CM6. The dry rice straw was cut into 1 cm pieces and soaked in the sterilized water for a night. After removing the water and kept the remaining humidity at 80 % by weight, the 100 g of rice straw (dry weight) was filled into 2 l sterilized plastic bottle. Then, 10 ml of bacilli broth was added. For the control treatment, distilled water was added instead. The experimental set were incubated at 28±2 ^{o}C. The humidity was adjusted at 80 % until 60 days. The decayed rice straw in every bottle was agitated every 2 weeks for three months. The properties and the quality of the decayed rice straw were studied (Attanan & Janjarernsook, 1999) at 15, 30, 45, 60, 75 and 90 days after incubation (organic carbon, organic matter, C/N ratio, and the contents of N, P and K). In addition, the microbial population was examined every two weeks by direct dilution technique in CMC, N-free agar (Mala *et al*., 2008)

The analysis of variance was utilized and the comparison of data according to Duncan's New Multiple Range Test was used at the level of confidence of 95 %.

3. Results

3.1 The Selection of High Ability Bacteria on Cellulose Decomposition and Nitrogen Fixation

Regarding the results of the first selection of bacteria by replica plating technique, the diameter of clear zone on the CMC, N-free agar caused from the various isolates were highly significant (p=0.001). Then, 60 isolates those tended to promote high activity on cellulose decomposition and nitrogen fixation were selected for the second screening. After the second screening (incubation for 30 days), it showed clearly that 6 bacterial isolates had a significantly different ability on cellulose decomposition (Figure 1). These outstanding bacterial isolates were RS2, RS6, DW3, DW4, CM2, and CM6.

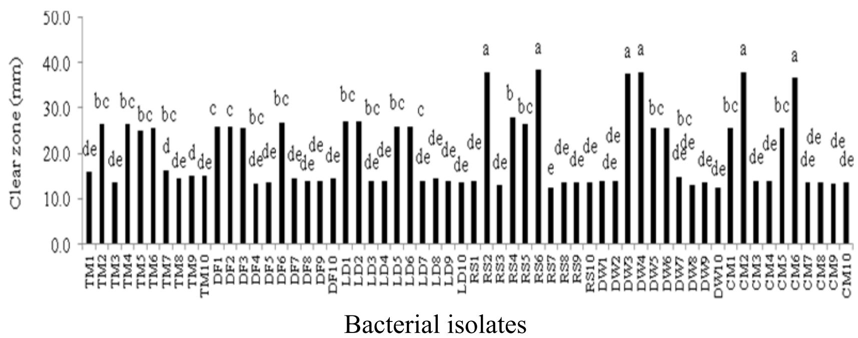


Figure 1. The clear zone diameter of CMC hydrolysis caused by various bacterial isolates at 30 day of incubation

3.2 The Identification of Bacterial Isolates Based on Their Morphological Characteristics and DNA Sequence

The results of the morphological and molecular information, the bacilli were identified as followed:

1)RS2 cell was a rod-shaped, Gram-positive and spore forming bacteria. A chain consisted of 2-3 rods. Based on partial 16S rDNA sequence, RS2 isolate was closely similar to *G. stearothermophilus* at 99 %. RS2 was identified as *G. stearothermophilus* RS2.

2) RS6 cell was rod-shaped, Gram-positive and spore forming bacteria. A chain consisted of 3-5 rods. Based on partial 16S rDNA sequence, RS6 isolate was closely similar to *G. stearothermophilus* at 98 %. Then, this isolate was identified as *G. stearothermophilus* RS6.

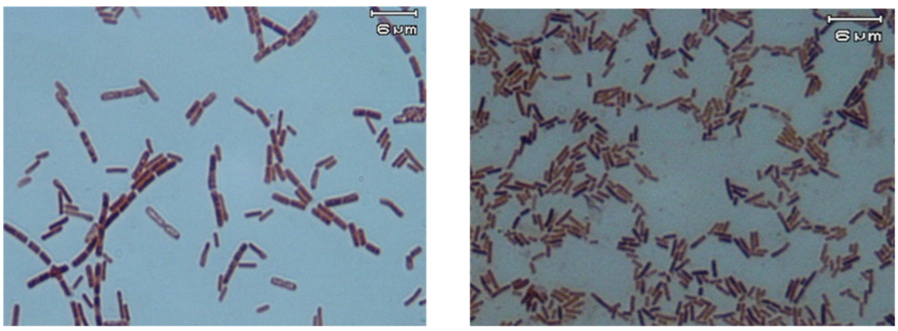
3) DW3 cell was rod-shaped, Gram-positive and spore forming bacteria. A chain consisted of 5-10 rods or more. Based on partial 16S rDNA sequence, DW3 isolate was closely similar to *G. stearothermophilus* at 98 %. DW3 isolate was identified as *G. stearothermophilus* DW3.

4) DW4 cell was rod-shaped, Gram-positive and spore forming bacteria. A chain consisted of 5-10 rods. Based on partial 16S rDNA sequence, this DW4 isolate was closely similar to *G. stearothermophilus* at 99 % and was identified as *G. stearothermophilus* DW4.

5) CM2 cell was rod-shaped, Gram-positive and spore forming bacteria. A chain consisted 3-5 rods. Based on partial 16S rDNA sequence, CM2 isolate was closely similar to *Bacillus cereus* at 94 % and identified as *B. cereus* CM2.

6) CM6 cell was rod-shaped, Gram-positive and spore forming bacteria. A chain consisted of 10 rods or more. Based on partial 16S rDNA sequence, this isolate was closely similar to *Bacillus cereus* at 96 % and was identified as *B. cereus* CM6.

This study agrees with GenBank that the *G. stearothermophilus* is a kind of bacteria belonging to thermophillic species. Its cell is a rod-shaped, Gram-positive bacteria. It spreads into the soil, hot springs, and marine sediments. It grows very well in a broad range of temperature around 30-75 ^{o}C. Meanwhile, *B. cereus*, another species, grows in the soil. It is Gram positive bacteria. The shape looks like a rod and mobile. Some species are dangerous in food contamination. Some are beneficial for promoting the growth of animals because it is probiotics. Since it is facultative anaerobe, it can adjust itself for growing in both aerobic and anaerobic conditions and produce the endospores as face to the unsuitable environment.



A B

Figure 2. (A) Geobacillus stearothermophilus, (B) Bacillus cereus

4. The Determination on Cellulase Activity of Various Bacilli

4.1 CMCase Activity

Regarding the ability in CMCase activity of 6 bacilli and the control treatment, the average diameter of the clear zone, dued to the their activities on carboxymethyl cellulose decomposition through the experiment, was highly significantly different (Figure 3). Inoculation of various bacilli showed the higher effectiveness of CMCase activity than non-inoculated one. The maximum diameter of clear zone was exposed in the treatment of *G. stearothermophilus* DW3 and followed by that of *B. cereus* CM2.

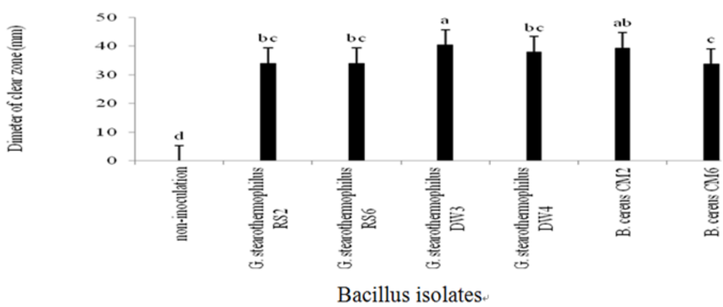


Figure 3. The clear zone diameter of CMC hydrolysis dued to the activity of various bacilli at 21 days

4.2 FPase Activity

Considering the ability of various 6 bacilli in FPase activity, the quantity of the FPase activity among treatments was highly significantly which was higher than that of control (Figure 4). The maximum activity was found in the treatment that inoculated with *G. stearothermophilus* RS6 and the second was in the order of *G. stearothermophilus* RS2, *G. stearothermophilus* DW3 and *B. cereus* CM2, *G. stearothermophilus* DW4 and *B. cereus* CM6.

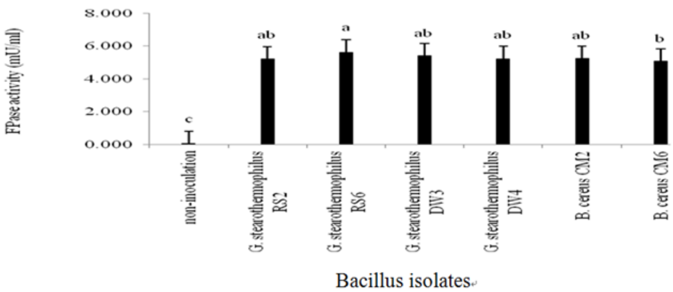


Figure 4. The FPase activity of various bacilli at 21 days

4.3 Avicelase Activity

The ability of various bacilli on avicelase activity at 21 days was shown in fig 5. The results revealed that the activity among treatments was highly significant. The highest activity of avicelase was found in the treatment that inoculated with *G. stearothermophilus* RS2. The others had lower activity of avicelase.

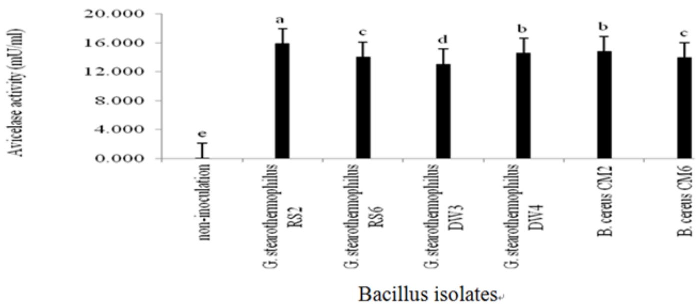


Figure 5. The avicelase activity of various bacilli at 21 days

4.4 The Ability in **α-***Cellulase Activity*

The ability in **α-**cellulase activity of various treatments at 21 days was highly significantly different (Figure 6). The lowest activity was found in the control, while, the highest one was appeared in the treatment that inoculated with G. stearothermophilus DW3, G. stearothermophilus DW4 and B. cereus CM6. The second level of the activity was found in 3 treatments which consisted of G. stearothermophilus RS2, G. stearothermophilus RS6 and B. cereus CM2 inoculations.

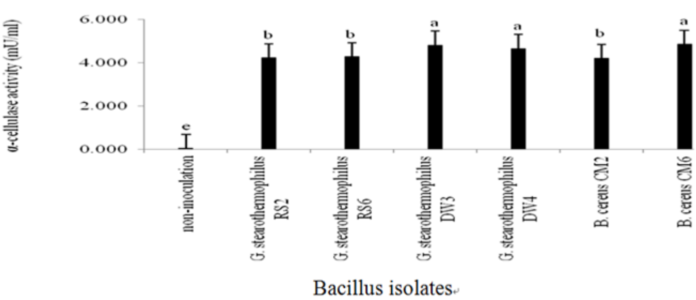


Figure 6. The **α-**cellulase activity of various bacilli at 21 days

5. The Determination on Nitrogenase Activity of Various Bacilli

The nitrogenase activities among the inoculated treatments were not different after 72 hr of the incubation (Figure 7). They showed the activity at 222.870-278.788 nmol C_2H_4/ml/hr. On the contrary, the control treatment has the least nitrogenase activity **(**8.774 nmole C_2H_4/g cell/hr.) and it was significantly different from those of the inoculated treatments.

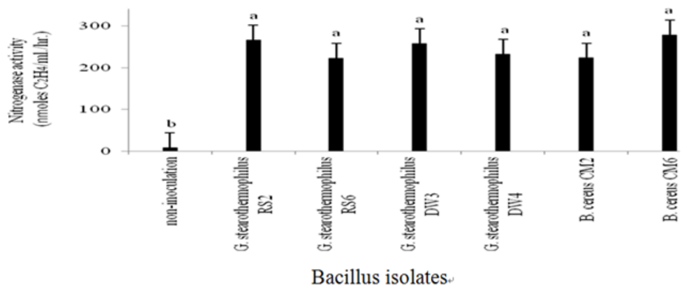


Figure 7. The nitrogenase activity of various bacilli at 72 hr of incubation

6. The Determination on the Efficiency of Various Bacilli in Rice Straw Decomposition and Nitrogen Fixation

The amounts of the organic carbon in decayed rice straw during the decomposition were shown in table 2. They were significant in 15 days of incubation and were highly significant in 30, 45 and 60 days of incubation. The organic carbon in those four stages of all inoculated treatments was lower than that of control. However, the organic carbon of the decayed rice straw at 75 and 90 days among treatments was not significant. They showed 16.90-17.37 and 16.30-16.76 % of organic carbon at 75 and 90 days, respectively.

Table 2. The amount of organic carbon (%C) of decayed rice straw at various periods of decomposition.

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	18.73a	18.24a	18.02a	17.68a	17.37	16.76
G. stearothermophilus RS2	18.09b	17.74bc	17.40b	17.09b	16.97	16.47
G. stearothermophilus RS6	18.16b	17.80b	17.36b	17.19b	16.90	16.46
G. stearothermophilus DW3	18.01b	17.52c	17.33b	17.25b	17.16	16.42
G. stearothermophilus DW4	18.16b	17.50c	17.35b	17.12b	17.02	16.30
B. cereus CM2	18.11b	17.80b	17.36b	17.25b	17.08	16.47
B. cereus CM6	18.11b	17.86b	17.35b	17.22b	17.18	16.46
F-test :	*	**	**	**	ns	ns
P-value	0.049	0.001	0.001	0.003	0.065	0.791
CV(%)	1.70	1.49	1.56	1.26	1.19	1.85

Note. ns=non-signifigantly different, ($P<0.05$)

* signifigantly different, (level of 95% based on DMRT)

** signifigantly different, (level of 99% based on DMRT)

The amount of organic matter (table 3) at 15 days in various treatments was not significantly different. But, those at 30, 45 and 60 days, were highly significantly different. The organic matter in the decayed rice straw of inoculated treatments at 15 days was in the same level (30.97-31.24 %), but, lower than that of control. Harmony with those amounts at 30, 45 and 60 days, the highest amount of organic matter was appeared in the non-inoculated treatment. Contrary, those amounts at 75 and 90 days were not significantly different. The amount of organic matter in the decayed rice straw at 75 and 90 days were between 29.08-29.87 and 28.04-28.82 %, respectively.

Table 3. The amount of organic matter (%) of decayed rice straw at various periods of decomposition.

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	32.22a	31.37a	30.99a	30.40a	29.87	28.82
G. stearothermophilus RS2	31.12b	30.51bc	29.93b	29.40b	29.19	28.32
G. stearothermophilus RS6	31.24b	30.61b	29.86b	29.57b	29.08	28.32
G. stearothermophilus DW3	30.97b	30.13c	29.81b	29.67b	29.51	28.24
G. stearothermophilus DW4	31.24b	30.11c	29.84b	29.45b	29.28	28.04
B. cereus CM2	31.14b	30.62b	29.86b	29.67b	29.39	28.34
B. cereus CM6	31.15b	30.72b	29.85b	29.62b	29.56	28.31
F-test :	*	**	**	**	ns	ns
P-value	0.049	0.001	0.001	0.002	0.068	0.791
CV(%)	1.69	1.49	1.56	1.26	1.18	1.85

Note. ns=non-signifigantly different, (P<0.05)

* signifigantly different, (level of 95% based on DMRT)

** signifigantly different, (level of 99% based on DMRT)

The C/N ratios (table 4) at all periods of the incubation were highly significantly different. The control treatment of all periods had the highest C/N ratio at 73.93, 62.89, 48.69, 34.43, 24.07 and 21.01 of 15, 30, 45, 60, 75 and 90 days, respectively. The C/N ratio of all treatments decreased with the increasing time of incubation periods. At 75 days, the C/N of the inoculated treatment was lower than 20. On the other hand, that of control was still higher than 20.

Table 4. The C/N ratio of decayed rice straw at various periods of decomposition

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	73.93a	62.89a	48.69a	34.43a	24.07a	21.01a
G. stearothermophilus RS2	59.65b	47.09b	33.25b	23.96b	17.45b	13.96b
G. stearothermophilus RS6	61.22b	46.43b	32.75b	23.88bc	16.83b	13.59b
G. stearothermophilus DW3	59.36b	46.10b	31.71b	22.90bc	17.27b	13.33b
G. stearothermophilus DW4	59.23b	46.47b	31.35b	22.73c	16.62b	11.76c
B. cereus CM2	61.03b	49.00b	32.35b	23.11bc	17.00b	13.44b
B. cereus CM6	61.74b	48.27b	32.54b	23.27bc	16.80b	13.11b
F-test :	**	**	**	**	**	**
P-value	0.002	0.000	0.000	0.000	0.000	0.000
CV(%)	9.13	12.09	17.38	16.24	14.70	20.48

Note: ** signifigantly different, (level of 99% based on DMRT)

The amount of nitrogen content at 15 days in the decayed material was significantly different. But, those at 30, 45, 60, 75 and 90 days, were highly significantly different (table 5). The nitrogen contents of decayed organic material of control in all periods were lower than those of inoculated ones. The nitrogen content of the inoculated treatment in each period (15, 30, 45, 60 and 75 days) was appeared in the same level. Anyhow, the nitrogen content among inoculated treatment in 90 days of incubation was pronounced. The maximum nitrogen content was found in the treatment that inoculated with *G. stearothermophilus* DW4 (1.39 %) and followed by the treatment of *B. cereus* CM6, *B. cereus* CM2, *G. stearothermophilus* DW3, *G. stearothermophilus* RS6 and *G. stearothermophilus* RS2 with the nitrogen content of 1.26, 1.23, 1231.21 and 1.18 %, respectively.

Table 5. The total nitrogen (%N) of decayed rice straw at various periods of decomposition

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	0.25	0.29b	0.37b	0.51b	0.72b	0.80d
G. stearothermophilus RS2	0.30	0.38a	0.52a	0.71a	0.97a	1.18c
G. stearothermophilus RS6	0.30	0.38a	0.53a	0.72a	1.00a	1.21bc
G. stearothermophilus DW3	0.30	0.38a	0.55a	0.75a	0.99a	1.23bc
G. stearothermophilus DW4	0.31	0.38a	0.55a	0.75a	1.02a	1.39a
B. cereus CM2	0.30	0.36a	0.54a	0.75a	1.01a	1.23bc
B. cereus CM6	0.29	0.37a	0.53a	0.74a	1.02a	1.26b
F-test :	*	**	**	**	**	**
P-value	0.012	0.000	0.000	0.000	0.000	0.000
CV(%)	7.34	9.19	12.43	11.86	11.07	14.84

Note. * signifigantly different, (level of 95% based on DMRT)

** signifigantly different, (level of 99% based on DMRT)

The phosphorus content in the decayed rice straw was shown in table 6. The content at 15 days was not significant, while that of 30 days was significant, and the later periods at 45, 60, 75 and 90 days were highly significant. In all periods of the incubation, the phosphorus content in decayed rice straw of the control was the least. However, the phosphorus content seemed to be increased with increasing time of incubation. At 30 days, the maximum of phosphorus content was found in *G. stearothermophilus* DW4 (0.26 %), while the another treatments gave the content from 0.22 % of *G. stearothermophilus* RS2 to 0.25 % of both *B. cereus* CM2 and *B. cereus* CM6. The phosphorus contents of various inoculated treatment at 45 days were in the same level, but, higher than that of the control. The maximum content of phosphorus at 60 days of the incubation was found in 3 treatments which inoculated with *G. stearothermophilus* DW4, *G. stearothermophilus* DW3 and *B. cereus* CM2 at 0.33 0.30 and 0.32 %, respectively. The remaining treatments gave the lower phosphorus contents. In case of the results at 75 days of incubation, the phosphorus content seemed to be increased from the previous periods. The amount of every inoculated treatment was in the same level (above 0.50 %), but, higher than that of the control (0.42 %). Later, the phosphorus content of the inoculated treatment increased gradually. Those contents were higher than 0.60 % at 90 days. In this periods, the maximum content was exposed in 3 inoculated treatmemts, *G. stearothermophilus* DW4, *G. stearothermophilus* RS6 and *G. stearothermophilus* DW3 at 0.68, 0.66 and 0.65 %, respectively. The remaining 3 inoculated treatments gave the lower phosphorus content, but, higher than that of the control (0.50%).

Table 6. The total phosphorus (%P) of decayed rice straw at various periods of decomposition

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	0.20	0.20c	0.20b	0.22d	0.42b	0.50c
G. stearothermophilus RS2	0.21	0.22bc	0.25a	0.25cd	0.52a	0.62b
G. stearothermophilus RS6	0.21	0.24ab	0.26a	0.27bc	0.53a	0.66a
G. stearothermophilus DW3	0.20	0.24ab	0.26a	0.32a	0.53a	0.65a
G. stearothermophilus DW4	0.20	0.26a	0.28a	0.33a	0.55a	0.68a
B. cereus CM2	0.21	0.25ab	0.28a	0.32a	0.52a	0.61b
B. cereus CM6	0.20	0.25ab	0.26a	0.31ab	0.54a	0.61b
F-test :	ns	*	**	**	**	**
P-value	0.186	0.011	0.005	0.000	0.000	0.000
CV(%)	2.66	10.04	12.19	14.85	8.48	9.08

Note. ns=non-signifigantly different, (P<0.05)

* signifigantly different, (level of 95% based on DMRT)

** signifigantly different, (level of 99% based on DMRT)

The amount of potassium contents of various treatments at 15 days was not significantly different (table7), but, those of 30 and 45 days were significant. Later periods, the potassium contents in the decayed rice straw of various treatments were highly significant. It seemed to suggest that the potassium contents of the inoculated treatments in every periods of the incubation were higher than those of control, meanwhile those amount tended to increase with times of incubation. The difference of potassium contents between the control and the inoculated treatments were cleared since 30 days of incubation and the interval between them increased gradually. At the period of 75 days, the inoculated treatments gave the potassium contents of the decayed rice straw from 0.68 % of *G. stearothermophilus* RS2 to 0.79 % of *G. stearothermophilus* DW4 which contrasted with that in the control (0.48 %). At the latest periods, the determined potassium contents of the inoculated treatments were higher than 0.90 %.The maximum potassium content of this period was 0.98 % whicht belonged to the treatment that inoculated with *G. stearothermophilus* DW3. The remaining inoculated treatments gave the amount of potassium content from 0.91 % of *B. cereus* CM2 to 0.95 % of both *G. stearothermophilus* RS2 and *G. stearothermophilus* DW4.

Table 7. The total potassium (%K) of decayed rice straw at various periods of decomposition

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	0.27	0.28b	0.33	0.38b	0.48d	0.67c
G. stearothermophilus RS2	0.30	0.33a	0.35	0.52a	0.68c	0.95ab
G. stearothermophilus RS6	0.31	0.33a	0.38	0.53a	0.69c	0.94ab
G. stearothermophilus DW3	0.30	0.34a	0.35	0.52a	0.77ab	0.98a
G. stearothermophilus DW4	0.30	0.35a	0.36	0.48a	0.79a	0.95ab
B. cereus CM2	0.31	0.33a	0.41	0.54a	0.75b	0.91b
B. cereus CM6	0.30	0.33a	0.41	0.52a	0.74b	0.93ab
F-test :	ns	*	*	**	**	**
P-value	0.149	0.017	0.016	0.009	0.000	0.000
CV(%)	6.51	7.28	10.84	12.81	14.18	11.38

Note. ns=non-signifigantly different, ($P<0.05$)

* signifigantly different, (level of 95% based on DMRT)

** signifigantly different, (level of 99% based on DMRT)

The amounts of bacterial population in the decayed rice straw in every periods of the study were highly significant (table 8). The log numbers of the bacilli of the inoculated treatments in all periods were more than 6. It meant that the population of bacilli in the decayed rice straw was higher than 10^6 cfu/g. While, that of control had the lower amount of the population at 10^4 cfu/g, approximately.

Table 8. The population of bacilli in the decayed rice straw at various periods of decoposition (log number of population)

Inoculum	Days					
	15	30	45	60	75	90
Control (non-inoculation)	4.16^{b}	4.16^{d}	4.16^{c}	4.18^{c}	4.18^{b}	4.18^{c}
G. stearothermophilus RS2	6.26^{a}	6.24^{bc}	6.25^{b}	6.25^{a}	6.25^{a}	6.27^{a}
G. stearothermophilus RS6	6.25^{a}	6.25^{bc}	6.26^{ab}	6.25^{a}	6.24^{a}	6.25^{ab}
G. stearothermophilus DW3	6.25^{a}	6.23^{c}	6.28^{a}	6.26^{a}	6.26^{a}	6.24^{b}
G. stearothermophilus DW4	6.25^{a}	6.27^{a}	6.26^{ab}	6.22^{b}	6.27^{a}	6.26^{ab}
B. cereus CM2	6.26^{a}	6.26^{ab}	6.25^{ab}	6.26^{a}	6.26^{a}	6.25^{ab}
B. cereus CM6	6.27^{a}	6.26^{ab}	6.24^{b}	6.25^{a}	6.26^{a}	6.25^{ab}
F-test :	**	**	**	**	**	**
P-value	0.000	0.000	0.000	0.000	0.000	0.000
CV(%)	18.02	12.61	12.58	12.46	12.45	12.76

Note: ** signifigantly different, (level of 99% based on DMRT)

7. Discussions

7.1 The Effect of Various Bacilli on Cellulose Activity

The output from this experiment is 6 species from 60 isolates of free living bacilli with high efficiency on cellulose decomposition. Those compose of *G. stearothermophilus* RS, 2 *G. stearothermophilus* RS6, *G. stearothermophilus* DW3, *G. stearothermophilus* DW4, *B. cereus* CM2 and *B. cereus* CM6. The study of Bhat (1997), similarly, attributed that the cellulolytic microorganisms excreted extracellular, hydrolytic enzyme to break the cellulose molecule. They concluded that those were some fungi such as *Aspergilus* spp., *Chaetomium* spp., *Penicillium* spp., *Fusarium* spp., some bacteria such as *Cellulomonas* spp., *Bacillus* spp., *Pseudomonas* spp., and some actinomycetes such as *Streptomyces* spp., *Thermonospora* spp. Six species of bacilli from this study show the significant efficiency on the degradation of carboxymethyl-cellulose, filter paper cellulose, avicel cellulose and **α-**cellulose. This finding shows the advantage of the selected bacilli. Since the cellulose compounds found in natural environment are diversity in both kind and molecular size. The appropriated cellulose enzyme must be doubled. The microorganisms which created various types of cellulase will be the effective species. Generally, cellulase enzyme consist of, a group of enzyme, endo 1,4-β-glucanases, exo 1,4-β-glucanases and 1,4-β-glucosidase. Therefore, the microorganisms which released all 3 types of the enzymes may have the high efficiency on cellulose decomposition (Eriksson *et al.* 1990)**.** It may be suggested from this study that all 6 species produce and excrete better and higher all 3 types of the enzymes than the others.

7.2 The Effect of Various Bacilli on Nitrogenase Activity

The avtivities of nitrogenase of 6 free living bacilii was not significant, but, higher than those of the others. These activities were between 222.870-278.788 nmol C_2H_4/ml/hr. However, Tran Van *et al*. (2000) found the nitrogen fixing bacteria in the genera Burkholderia and Bacillus from decayed rice straw and rice root zone. The nitrogenase activity was around 0.1-0.2 µmol C_2H_4/ml/hr. Burd *et al.* (2000) and Leonardo *et al.* (2012) attributed that the nitrogen fixing bacteria in the genus of Bacillus was found from decaying food and organic waste. Anyhow, the amount of the activity was very little. As compared to this experiment, it indicates clearly that the bacilli with higher nitrogenase activity were found. This is because these bacilli grow and proliferate very well in the environment with a higher competition. They gain a lot of available nutrient from their mineralization process during the composition. The diversity of alternative degradable organic carbon, energy and electron sources are available profusely. These cases may enhance the efficiency on the synthesis of nitrogenase enzyme including with the mechanisms for enzyme protection from the damages (Mala, 2014). In general, Azotobacter, Beijerinckia and Azospirillium, free living nitrogen fixing bacteria in common soil and the rhizosphere of rice, sorghum and corn, have the nitrogenase activity around 3.46-236.91, 0.76-22.44 and 2.02-13.34 µmol C_2H_4/ml/hr, respectively. Meanwhile, that of the bacillus group is 0.76-14.44 µmol C_2H_4/ml/hr. (Jatoopornpipat *et al*., 2011)

7.3 The Quality of the Compost

The quality index of the general compost, in general, is the content of nitrogen, phosphorus, potassium including with the quantity of organic matter and C/N ration. According to Land Development Department, the standard quality of organic fertilizers should be 1.0, 0.5 and 0.5% of N, P and K with 20 % of the and lower than 20:1 of C/N ratio. Comparing between the two treatments, control and inoculated treatment at 75 days, the quality of the inoculated treatments is qualified, while that of the control treatment was not. It indicates that these bacilli are the effective species for the composing. This indicated that good quality of organic fertilizers without bacillus inoculants may takes a longer time (more than three months). It may suggest that the selected bacilli, due to the higher amounts of total N, P, K, organic matter content and lower C/N ratio, have higher efficiency on rice straw decomposition and nitrogen fixation. Compared to the experiment of Sawangpanyangkoon (2008), he studied the decomposition of plant debris that mixed with cow manure, chemical N fertilizer. The temperature of the compile increased gradually and began to decline at 60 days of incubation. The qualified compost was gained at least 90 days. He suggested that the factor affecting the degradation of plant debris composed not only carbon source but also the available nutrients such as nitrogen that essential for the synthesis of diverse bio-molecules. The C/N ratio of the cellulose is about 15, then, for every 15 g C for microbial growth need 1 g of the available nitrogen. Naturally, the C/N of plant debris is higher than 15. This causes the N deficiency for organic decomposition by concerning microorganisms. This problem may reduce by the adding of appropriated amount of animal manure or chemical N fertilizer. **(**Juntharaniyom and Pengnoo, 2012). On the other hand, this research found that the selected bacilli with high efficiency on cellulase and nitrogenase activities reduced the times for the rice straw composing. The qualified compost that inoculated with these bacilli will be gained within 75 days without adding any nitrogen source.

8. Conclusions

Six species of bacilli, *G. stearothermophilus* RS, 2 *G. stearothermophilus* RS6, *G. stearothermophilus* DW3, *G. stearothermophilus* DW4, *B. cereus* CM2 and *B. cereus* CM6 were selected. From the study, these species had high ability to produce not only various types of cellulase, CMCase, FPase, Avicelase, **α-**cellulase but also the nitrogenase enzymes. The rice straw that inoculated with the individual species of these bacilli would be decayed gradually in the rate which faster than that of non-inoculation. The problem on low quality and time consuming of compost production could be reduced by the application of these inoculants. The qualified compost that inoculated with appropriated bacilli could be gained within 75 days without adding any combined nitrogen.

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