ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2019, 8(1), pp. 23 - 31, DOI:10.18036/aubtdc.411229

UTILIZATION OF FMN07 AND FGN37 *MICROMONOSPORA* SP. IN REGULATING THE NITROGEN LOSS DURING COMPOSTING

Fadime ÖZDEMİR KOÇAK [1, 3, *], Levent DEĞİRMENCİ [2, 3]

[1] Department of Nursing, School of Health, Bilecik Şeyh Edebali University, 11210, Bilecik, Turkey
[2] Department of Chemical and Process Engineering, Bilecik Şeyh Edebali University, 11210, Bilecik ,Turkey
[3] Biotechnology Application and Research Center, Bilecik Şeyh Edebali University, 11210, Bilecik, Turkey

ABSTRACT

Isolated and purified *Micromonospora* sp. FMN07 and FGN37 strains were utilized in composting experiments in a miniscule system in order to determine their effects on mesophilic stage. Results indicated deceleration of organic matter degradation in the presence of these microorganisms. This result also indicated that these microorganisms had no or negligible activity towards cellulose degradation which was the main constituent of compost mixture. Statistical analyses and measurements were also conducted to determine the effects of microorganisms on nitrogen content. Result indicated an increase in nitrogen amount in their presence. It was our belief that the activity of microorganisms effective in thermophilic stage of composting could have been increased by proper adjustment of FMN07 and FGN37 strains since nitrogen amount crucial for microorganism growth would have been higher in their presence.

Keywords: *Micromonospora*, Composting, Nitrogen amaount

ÖZET

İzole edilen ve saflaştırılan *Micromonospora* sp. FMN07 ve FGN37 suşları küçük ölçekli bir kompostlama sisteminde, kompostlaşmanın mezofilik aşamasına etkilerinin belirlenmesi amacıyla kullanılmışlardır. Yapılan ölçüm ve analizlerin sonunda sonuçlar mikroorganizma eklenmesine bağlı olarak organik madde kaybının azaldığını göstermektedir. Sonuçlar ayrıca eklenen mikroorganizmaların büyük ihtimalle, kompostun temel bileşeni olan selüloza karşı aktif olmadıklarını da ortaya koymuştur. Mikroorganizmaların kompost karışımındaki azot oranına etkilerinin belirlenmesi amacıyla yapılan ölçüm ve istatistiksel analizler sonucunda mikroorganizma eklenmesinin kompost karışımındaki azot miktarı üzerinde büyük ölçüde etkili olduğu görülmüştür. Söz konusu mikroorganizmaların miktarlarının ayarlanması ile kompostlaşmanın termofilik evresinde etkili olan mikroorganizmaların aktivitesinin arttırılacağı ve kompostlaşmanın mikroorganizma eklenmeyen karışımlara kıyasla daha kısa sürede tamamlanacağı öngörülmektedir.

Anahtar Kelimeler: *Micromonospora* sp., Kompostlaşma, Azot oranı

1. INTRODUCTION

Compositing has long been utilized as an efficient method to produce mineralized and humidified soil amendment from solid organic wastes. Nitrogen, which is crucial for plant growth, is generally lost in the form of ammonia emissions during composting [1, 2]. The fastest and easiest way of regulating nitrogen amount in soil is utilization of nitrogen fertilizers as supplement, which results in serious environmental contamination. Hence controlling the amount of nitrogen loss during composting emerged as a fine solution in reducing the apparent impact of chemical fertilizers on environment [2]. Nitrogen fixation via microorganisms during composting would decrease the amount of nitrogen supplied by chemical fertilizers. However, this is a difficult challenge as only few microorganisms are capable of achieving the task. In case of chemical fertilizer utilization an increase in emission of greenhouse gases would eventually occur which are harmful to the environment [3].

*Corresponding Author: fadime.ozdemirkocak@bilecik.edu.tr
Received: 30.03.2018 Accepted: 22.10.2018

Based on the facts demonstrated above it is our understanding that controlling nitrogen release in composting through microorganism utilization would be beneficial in reducing the amount of nitrogen that has to be supplied externally. This can either be achieved through the using of nitrogen fixing bacteria [4-6] or utilization of various carbon sources [7]. Zeolite addition to compost mixture [8] and vermicomposting [9] were also investigated as alternative pathways. An approach different from those in literature was followed in the present study by the use of FMN07 and FGN37 strains in compost mixture in order to regulate nitrogen release during composting.

2. MATERIALS AND METHODS

Soil samples were collected from Yuga Zapadnaya Southwest Forest Park in Moscow, Russia and from oak tree rhizosphere around Golcuk Crater Lake located in Kutahya, Turkey. FMN07 strains were isolated from Moscow, Russia and FGN37 strains were isolated from Kutahya, Turkey. FMN07 was isolated via sucrose-gradient centrifugation method. Tryptone-yeast glucose extract (TYG) with vitamin agar plates were utilized as selective isolation medium. Filter sterilised cycloheximide (50 μg ml^{-1}), nalidixic acid (10 μg ml^{-1}) and rifampicin (0,5 μg ml^{-1}) were added as supplements. Conventional method was applied in isolation of FGN37 strain. Glucose-yeast extract-malt extract agar (GYME) with $CaCO_3$ was used as selective medium [10, 11]. Phylogenetic line of these *Micromonospora* isolates was determined using 16S rRNA gene sequencing.

Initial step in 16S rRNA sequencing is chromosomal DNA isolation of isolates which was conducted through modification of a method previously utilized by Pitcher et al. (1989) [12]. PCR amplification of isolates was achieved by using universal primers 27f and 1525r [13] and 16S rRNA sequencing were conducted according to literature [14]. PCR conditions applied for 50 μl of reaction mixture were illustrated in Table 1, the primers used in 16S rRNA sequencing analyzes were given in Table 2. Sequences of 16S rRNA had FMN07 and FGN37 strains were 1510 and 1497 bp, respectively. These were combined and analyzed in Mega 6.0 programme in order to assess the identities of isolated strains. Phylogenetic tree was constructed by utilization of neighbor-joining tree algorithms [15, 16].

Table 1. The content of PCR solution and parameters used in thermal amplficiation

PCR soultions (50 μl)	Primers (20 μM, Invitrogen) Deoxynucleoside triphosphate mixture (25 μM, Promega) Taq polymerase tampon (HotstarTaq, QIAGEN) Taq polymerase (2.5 U, HotstarTaq, QIAGEN) Chromosomal DNA (50-300 ng)		
Amplification parameters	5 min, 95°C; 1cycle	1 min, 95°C; 2 min, 55°C; 3 min, 72°C; 35 cycles	10 min, 72°C; 1 cycle

Table 2. Primers used in PCR and sequencing for 16S rRNA.

Primer	Sequence (5' and 3')	Size (bp)	Utilization
800r	TACCAGGGTATCTAATCC	18	Sequence
MG3f	CAGCAGCCGCGGTAATAC	18	Sequence
MG5f	AAACTCAAAGGAATTGACGG	20	Sequence
27f	AGAGTTTGATCTGGCTCAG	20	Amplification
1525r	AAGGAGGTGWTCCARCC	17	Amplification

Mesophilic composting was achieved by activation of the strains inoculated in glucose yeast extract broth at 30°C for 7 days. Determination of *Micromonospora* sp. density by spectrophotometric analysis was not possible as these species had a tendency to form clusters during reproduction. The microorganisms were placed in 1 ml broths in eppendorf for determination of their amounts. The broths were centrifuged, microorganisms were removed and weighed following freeze drying. This

procedure was repeated thrice and average of measurements was used in composting experiments corresponding to a total of 0.88 mg/L for FMN07 and 0.60 mg/L for FGN37.

The composting system consisted of 85g soil and 25g cabbage (10% moisture). Eight systems with identical duplicates were utilized in the experiments (total of 16 systems). Four of them were sterilized under ultra violet radiation and the remaining four used without further treatment. Equal amounts of microorganisms (0.5 mg/L) were added in ringer solutions (50 ml) to prevent lysis. Based on the amount of nutrient (cabbage) utilized in composting systems, it was unlikely to experience a transition to thermophilic stage. Nevertheless, the systems were kept at laboratory for 30 days under constant control of temperature and moisture to prevent transition to thermophilic stage and drying. Systems were also turned in every three days to enable constant aeration. The systems were denoted as A and B based on the type of microorganisms used and 0 code was given to systems utilized as control to prevent confusion and maintain easy follow-up (Table 3)

Table 3. The codes used in experiments.

***Micromonospora* sp.**	**Code name (Non-sterilized)**	**Code name (Sterilized)**
FMN07	A	AS
FGN37	B	BS
Control	0	0S
FMN07+FGN37*	AB	ABS

*25ml FMN07 and 25 ml FGN37 was used in composting experiments

Total organic content [17] of the samples, collected at 15th and 30th day of experiment, total nitrogen content and soluble nitrogen content [18] of the samples, collected at the 15th day of experiment, were determined and their results were evaluated in accordance with the results of ATR-FT-IR analyses. Analyses of total organic, total nitrogen and soluble nitrogen contents were conducted at least twice until achieving an interval of confidence which was determined as 5%.

It was predicted that the added microorganisms would have affected the change in total organic content along with total and soluble nitrogen amounts. Hence the data acquired during experiments were divided into two parts. First part was the change of total organic content in 15th and 30th days of experiments. Second part was the change of total and soluble nitrogen amount for the samples obtained at 15th day of experiments. Both data sets (with replicates) were subjected to ANOVA in order to determine the relevance of microorganism addition on these parameters. Statistical data were obtained by Microsoft Excel 2010 version. Significance level was taken as α=0.1.

3. RESULTS AND DISCUSSION

Neighbor-joining tree indicated 100% resemblance of FMN07 strain to *Micromonospora aurantiaca*. The strain also formed a clade with *Micromonospora marina* (99.65%; 5 nt differences). FGN37 strain formed a clade with *Micromonospora chokoriensis* (99.3%; 10 nt differences) and *Micromonospora lupine* (99.0%; 13 nt differences) (Figure 1).

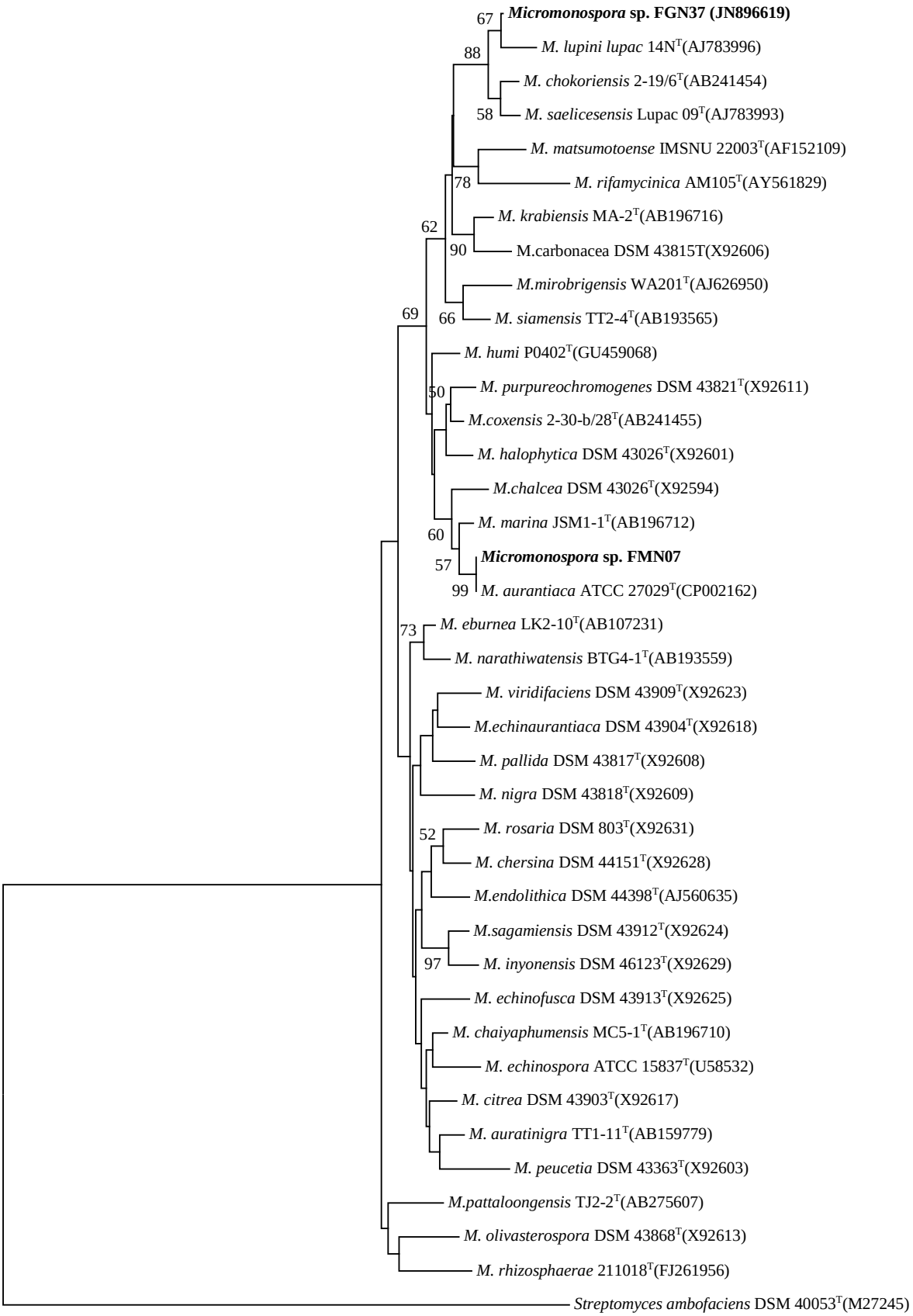

Figure 1. Neighbour-joining tree based on 16S rRNA sequences.

As previously stated cabbage was used in composting experiments to increase nitrogen content of soil. Since the microorganisms were active in mesophilic stage, the amounts were kept in minimum to prevent transition to thermophilic stage. Samples at the 15th and 30th days of experiments were taken with the intention of observing the change in organic and nitrogen content by microorganism addition (Table 4). Results indicated a higher decrease in organic content of "0" sample compared to A, B and AB. In other words, the activity of original microbiota was higher in the absence of these strains. As previously mentioned, a group of samples were sterilized prior to use in composting and it would be logical to assume lower amounts of microorganism reproduction inside these samples within 15 day period (Table 4).

Table 4. Total organic content (15 and 30th days), total and soluble nitrogen contents of the samples obtained at the 15th day of experiments

Sample	Total organic content % (15th day)	Total organic content % (30th day)	Total nitrogen content %	Soluble nitrogen content %
0	3.39	3.74	5.03	3.72
A	7.38	3.29	5.91	5.22
B	8.41	3.40	5.72	4.86
AB	6.35	3.39	6.07	5.24
0S	6.45	3.18	5.56	4.23
AS	9.68	3.08	5.84	4.91
BS	6.16	3.46	4.18	3.49
ABS	6.90	3.04	5.06	4.25

Higher organic content obtained in the presence of A, B and AB microorganisms was expected as their amounts either separate or combined would be higher than any other microorganism present in the original microbiota. On the other hand, inhibition of degradation in the presence of these strains implied that these could have a little or no activity towards cellulose which was thought to be the main constituent of compost mixture. The total and soluble nitrogen amount (compared to "0" sample) was higher in these samples which should be expected due to inhibition of organic matter degradation (Table 2). The change trend of organic content was also validated by FT-IR analyses by comparing intensities of the peaks at 1000, 1420, 1640 cm^{-1} revealing the presence of organic content in compost systems [19, 20]. The increase in peak values of samples A, B and AB showed the presence of higher organic content. (Figure 2).

The deceleration in decomposition of organic content also resulted in deceleration of nitrogen release during mesophilic stage. It was our belief that compost mixtures containing A, B and AB microorganisms would experience lower losses in total and soluble nitrogen during thermophilic stage. In other words mature composts prepared with microorganisms (A, B and/or AB) addition should have higher nitrogen amount at the end. Since nitrogen was also crucial for microorganism development, higher activity should be expected in thermophilic stage in the presence of these microorganisms. Transition to thermophilic stage could easily be regulated by adjusting the amounts utilized during composting and effects of these microorganisms on total and soluble nitrogen content at the end of thermophilic stage would be investigated in nearest future.

Organic contents of the samples collected at 30th day of experiment were also determined to better demonstrate the effect of strains in organic matter decomposition. 30 days was an estimated time of decrease in the activity of strains which was shown to be exact based on the results. A stable organic content between 3-4% was obtained at the end of 30 days, as seen from the table. This result implied stable decomposition rate in the absence of these strains (Table 4).

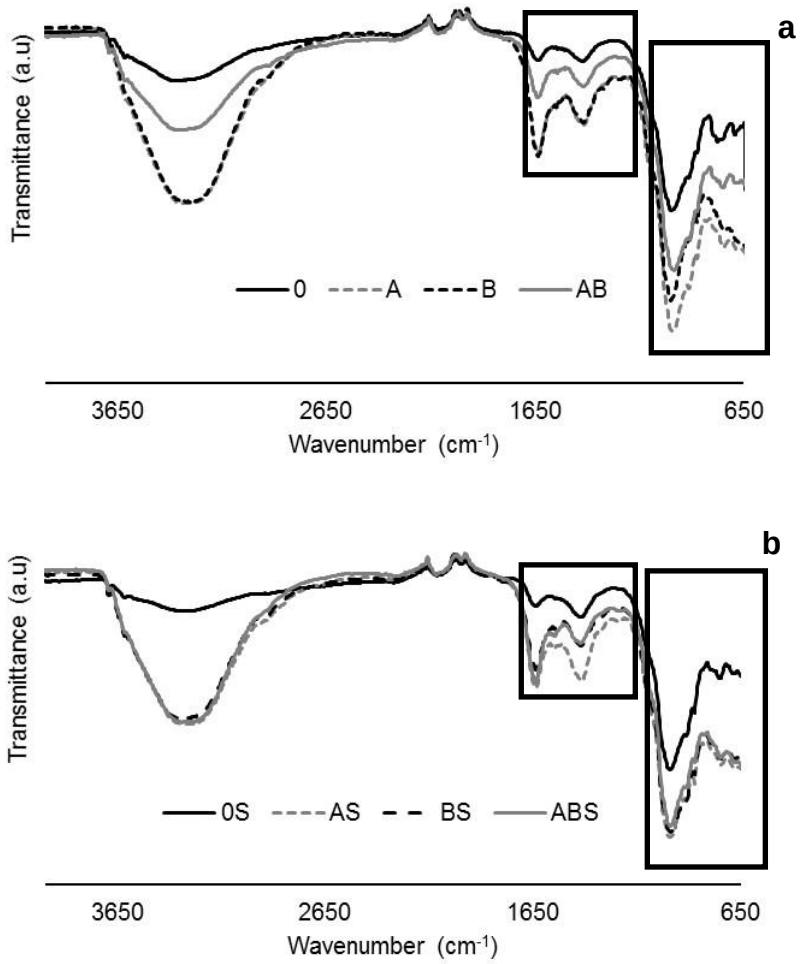

Figure 2. FT-IR analyses of a) Non-sterilized samples b) Sterilized samples obtained at the 15th day of experiment.

Statistical data obtained for total organic matter and nitrogen amounts (total and soluble) were illustrated in Table 5. Results clearly indicated relevance between microorganism addition and nitrogen amount. In other words, microorganism addition on compost mixture had an irrefutable effect on nitrogen amount. On the other hand, their effect on organic content was insignificant as previously stated.

Table 5. Effect of microorganisms on total organic content and nitrogen amount

	Organic content	**Nitrogen amount**
X	4.888	3.3038
SST	74.038	53.022
SSW	63.577	3.693
SSB	10.461	49.329
F (statistic)	0.188	15.264
F (critical)	3.4381	3.4381

It would be logical to state that organic matter decomposition during composting be mainly governed by cellulose degradation and the negligible effect of microorganisms on decomposition of organic matter implied weaker or no cellulose activity compared to other constituents such as pectin and xylan. This assumption was proven to be accurate in the work of Martinez-Hidalgo et.al.[21],in which a group of *Micromonospora sp.* were tested for their ability to degrade organic matter. Results indicated weak activity of the strains towards cellulose [21]. The activity of *Micromonospora sp.* towards cellulose might as well be negative as in the work of Lin et.al. [22].

Based on given examples from the literature a weak or negligible activity should be the reason of insignificancy in organic matter decomposition in the presence of FMN07 and FGN37 strains. Cellulose degradation is in fact a challenge for microorganisms due to its complex structure. Nevertheless, its degradation could be enhanced by changing certain parameters of composting; time of microorganism addition is among the most important parameters. In a study conducted by Zhao et.al. [23], effect of inoculation time on composting was studied in the presence of actinobacteria agent containing *Streptomyces sp.* and *Micromonospora sp.* Results indicated higher activity towards cellulose when agent was inoculated at the end of thermophilic stage of composting [23]. In the present study inoculation of the strains was conducted in mesophilic stage which might be the reason of their low activity.

The inhibition of organic matter degradation was in fact a distinctive property of *Micromonopsora sp*. among others as the amount of nitrogen crucial for microorganism development would always be higher in their presence. Nitrogen is vital for the development of microorganisms that were involved in thermophilic stage. Higher nitrogen amount was mostly responsible for prolonged thermophilic stage which would enable elimination of pathogens and higher metabolic activity during composting [24]. Hence, higher activity by means of composting should be expected in the presence of these microorganisms in long term.

4. CONCLUSION

FMN07 and FGN37 strains were isolated from soil in different regions and identified by 16S rRNA gene analyses. These microorganisms were utilized in compost mixtures to determine their effects on organic matter degradation and nitrogen amount. Analyses and statistical data clearly indicated an increasing effect on nitrogen amount in the presence of these microorganisms which was the highlight of present study. On the other hand, an inhibition on organic matter degradation was observed which had probably been due to high amount of microorganism introduced to original microbiota. Results also implied that these microorganisms had no or negligible activity towards cellulose degradation which would be determined by future studies. Regulated amounts of these microorganisms could have been successfully utilized in composting as their presence would enable higher nitrogen amounts in compost mixture during transition to thermophilic stage. Although further studies were required, it was our belief that higher nitrogen amounts would have increased the activity of microorganisms effective in thermophilic stage. There also had been a strong chance of obtaining higher nitrogen amounts in the presence of these strains at the end of composting.

REFERENCES

[1] Pepe O, Ventorino V, Blaiotta G. Dynamic of functional microbial groups during mesophilic composting of agro-industrial wastes and free-living (N2)-fixing bacteria application. Waste Manage 2013; 33: 1616-1625.

[2] Zheng Y, Zhao Y, Chen Y, Lu Q, Li M, Wang X, Wei Y, Xie X, Wei Z. A regulating method for reducing nitrogen loss based on enriched ammonia-oxidizing bacteria during composting. Bioresource Technol 2016;221: 276-283.

[3] Iwata K, Azlan A, Yamakawa H, Omori T. Ammonia accumulation in culture broth by the novel nitrogen-fixing bacterium, Lysobacter sp. E4. J Biosci Bioeng 2010; 110(4): 415-418.

[4] Yates RJ, Howieson JG, Reeve WG, Nandasena KG, law IJ, Brau L, Ardley JK, Nistelberger HM, Real D, O'Hara GW. Lotononis angolensis forms nitrogen fixing, lupinoid nodules with phylogenetically unique, fast-growing, pink-pigmented bacteria, which do not nodulate L. bainesii or L. listii. Soil Biol Biochem 2007; 39: 1680-1688.

[5] Zhang Y, Cao C, Peng M, Xu X, Zhang P, Yu Q, Sun T. Diversity of nitrogen-fixing, ammonia-oxidizing, and denitrifying bacteria in biological soil crusts of a revegetation area in Horqin Sandy Land, Northeast China. Ecol Eng 2014; 71: 71-79.

[6] Yan Z, Wang G, Gao Y, Wang Y, Gu JD, Wang W. Diversity of ammonia-oxidizing bacteria and archaea in response to different aeration rates during cattle manure composting. Ecol Eng 2016; 93: 46-54.

[7] Meng L, li W, Zhang S, Wu C, Jiang W, Sha C. Effect of different extra carbon sources on nitrogen loss control and the change of bacterial populations in sewage sludge composting. Ecol Eng 2016; 94: 238-253.

[8] Chan MT, Selyam A, Wong JWC. Reducing nitrogen loss and salinity during 'struvite' food waste composting by zeolite amendment. Bioresource Technol. 2016; 200: 838-844.

[9] Nigussie A, Kuyper TW, Bruun S, de Neergaard A. Vermicomposting as a technology for reducing nitrogen losses and greenhouse gas emissions from small-scale composting. J Clean Prod 2016; 139: 429-439.

[10] Isik K, Gencbay T, Özdemir- Kocak F, Cil E. Molecular identification of different actinomycetes isolated from East Black Sea region plateau soil by 16S rDNA gene sequencing. Afr J Microbİol Res 2014; 8: 878-887.

[11] Ozdemir-Kocak F, Isik K. Molecular identification of Nocardia Diversity in soil by multilocus sequence analysis. BioDiCon 2015; 8(2): 122-133.

[12] Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett AppL Microbiol 1989; 8: 151-156.

[13] Lane DJ. 16S/23S rRNA sequencing. In E. Stackebrandt, M. Goodfellow, J. Wieley (eds) Nucleic acid techniques in bacterial systematics, New York pp. 1991; 115-175.

[14] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013; 30: 2725–2729.

[15] Jukes TH, Cantor CR. Evolution of protein molecules. Academic Press, New York. 1969.

[16] Saitou N, Nei M. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987; 4: 406-425.

[17] ASTM. Standard test methods for moisture, ash, and organic matter of peat and other organic soils. American Society for Testing and Materials. West Conshohocken, PA. 2000.

[18] Barker AV, Volk RJ. Determination of Ammonium, Amide, Amino, and Nitrate Nitrogen in Plant Extracts by a Modified Kjeldahl Method. Anal Chem 1964; 36(2): 439-441.

[19] Artz RRE, Chapman SJ, Robertson AH, Potts JM, Laggoun-Defarge F. FTIR spectroscopy can predict organic matter quality in regenerating cutover peatlands. Soil Biol Biochem 2008; 40(2): 515-527.

[20] Merlin N, Lima VA, Santos-Tonial LM. Instrumental and Experimental Conditions for the Application of Fourier Transform Infrared Analysis on Soil and Humic Acid Samples, Combined with Chemometrics Tools and Scanning Electron Microscopy. J Brazil Chem Soc 2015; 26(9): 1920-1927.

[21] Martinez-Hidalgo P, Galindo-Villardon P, Trujillo ME, Igual JM, Martinez-Molina E. Micromonospora from nitrogen fixing nodules of alfalfa (Medicago sativa L.). A new promising Plant Probiotic Bacteria. Sci Rep-UK 2014; 4: 1-10.

[22] Lin YB, Fan CM, Guo YQ, Di XH, Dong DH, Zhang X, Wei GH. Micromonospora nickelidurans sp. nov., isolated from soil from a nickel-mining site. Int J Syst Evol Micr 2015; 65: 4615-4620.

[23] Zhao Y, Lu Q, Wei Y, Cui H, Zhang X, Wang X, Shan S, We, Z. Effect of actinobacteria agent inoculation methods on cellulose degradation during composting based on redundancy analysis. Bioresource Technol 2016; 219;196-203.

[24] Varma S, Nashine S, Sastri CV, Kalamdhad AS. Influence of carbide sludge on microbial diversity and degradation of lignocellulose during in-vessel composting of agricultural waste. Ecol Eng 2017; 101:155-161.