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ชื่อโครงการ	Isolation and screening cellulolytic bacteria from the fecal pellets of earthworm
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Isolation and screening cellulolytic bacteria from the fecal pellets of earthworm

Miss Saowalak Khema

Project Submitted in Partial Fulfillment of Requirements for the Bachelor Degree in Environmental Science

Department of Environmental Science

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การคัดเลือกและคัดแยกแบคทีเรียที่มีความสามารถในการย่อยสลายเซลลูโลสจากมูลไส้เดือน

นางสาวเสาวลักษณ์ เขมา

โครงงานนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรบัณฑิต

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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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Project title: Isolation and screening cellulolytic bacteriafrom the fecal pellets of earthworm

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Department: Environmental Science

Project advisor: Supawin Watcharamul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of Requirements for the Bachelor Degree

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เสาวลักษณ์ เขมา : การคัดเลือกและคัดแยกแบคทีเรียที่มีความสามารถในการย่อยสลายเซลลูโลสจากมูลไส้เดือน
อาจารย์ที่ปรึกษา: อาจารย์ ดร.ศุภวิน วัชรมูล

การศึกษานี้มีวัตถุประสงค์เพื่อคัดแยกและคัดเลือกแบคทีเรียที่มีความสามารถในการย่อยสลายเซลลูโลสมาจากมูลไส้เดือน African night crawler (*Eudrilus eugeniae*) และ เพื่อศึกษาผลของแบคทีเรียที่มีความสามารถในการย่อยสลายเซลลูโลสต่อการเพิ่มความอุดมสมบูรณ์ของดิน แบคทีเรียที่มีความสามารถในการย่อยสลายเซลลูโลสถูกคัดแยกมาจำนวน 115 สายพันธุ์ แล้วมี 10 สายพันธุ์ที่สามารถย่อยสลายเซลลูโลสได้ดี ได้แก่ CB96, CB58, CB84, CB114, CB10, CB73, CB11, CB22, CB72 และ CB53 ตามลำดับซึ่งสามารถวัดได้โดยแกรมไอโอดีน หลังจากนั้นนำแบคทีเรียที่คัดเลือกมาเพิ่มจำนวนแล้วใส่ลงดินและทำการวัดปริมาณไนโตรเจนและความสามารถในการแลกเปลี่ยนไอออนบวกของดินที่เปลี่ยนแปลงไปซึ่งมีความสัมพันธ์กับความอุดมสมบูรณ์ของดิน พบว่าหลังจากใส่แบคทีเรียที่คัดเลือกลงไป 4 สัปดาห์ ดินมีปริมาณไนโตรเจนและความสามารถในการแลกเปลี่ยนไอออนบวกเพิ่มขึ้นอย่างมีนัยสำคัญ จากการศึกษาพบว่าแบคทีเรียที่คัดแยกจากมูลไส้เดือนนั้นสามารถในไปประยุกต์ต่อการพัฒนาและปรับปรุงดินได้ในอนาคต

คำสำคัญ : แบคทีเรียที่มีความสามารถในการย่อยเซลลูโลส, ความอุดมสมบูรณ์, มูลไส้เดือน

Saowalak Khema : Isolation and screening cellulolytic bacteria from the fecal pellets of earthworm
Project advisor : Supawin Watcharamul, Ph.D.

This study aims to isolate and screen cellulolytic bacteria from the fecal pellets of African night crawler (*Eudrilus eugeniae*) and investigate the effect of cellulose degradation of cellulolytic bacteria on soil fertility. A total of 115 isolates of bacteria were isolated and ten isolated bacteria that have the highest HC capacity from the measurement of Gram Iodine method were selected as follows: CB96, CB58, CB84, CB114, CB10, CB73, CB11, CB22, CB72, and CB53, respective. After that, ten cellulolytic bacteria were enriched and added into the soil and measured nitrogen content and the cation exchange capacity of the soil which was related to soil fertility. It was found that after 4 weeks, the amount of nitrogen and the cation exchange capacity in the soil was increased from before adding the bacteria significantly. From the study, it was found that isolated cellulolytic bacteria from the fecal pellets of earthworm can be applied to the development and improvement of soil quality in the future.

Keywords: cellulolytic bacteria, soil fertility, Pill- earthworm

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Saowalak Khema

CONTENT

CONTENT (CONT.)

LIST OF TABLES

LIST OF FIGURES

LIST OF LIST OF EQUATIONS

CHAPTER 1
INTRODUCTION

1.1 Research rationale

In soil, there is a great variety of microorganism which play an important role in maintaining soil fertility (Hasan, 2000).They have functions in the process of decomposition and nutrient circulation (Vikram *et al*., 2007). One of the important microorganisms is cellulolytic microorganism such as fungi, bacteria and actinomycetes. Especially bacteria that have a higher growth rate and can produce complex enzymes to degrade cellulose. In addition, bacteria inhabit a wide variety of environmental niches (Maki *et al*., 2009). They can produce cellulases which convert cellulose into soluble sugars and glucose. (Bhat and Bhat, 1997). Sugar derived from microbial degradation is used as a carbon source for microorganisms in soil to grow (Gunina and Kuzyakov, 2015). When the amount of carbon in the soil increases, the active microorganisms (Monard *et al*., 2008).

An earthworm is an animal in the phylum Annelida that have an important role in soil ecosystems. The main function of the earthworm is to decompose dead plants to release of nutrients from them (Darwin, 1892). Lignocellulose decomposition from earthworms are caused by microorganisms in the gut of earthworms, which are capable of releasing the enzymes to decompose (Fujii *et al*., 2013). Cellulolytic microorganisms from the gut of earthworms such as bacteria and fungi which can degrade cellulose into sugars using endogenous cellulases, an enzyme that is wide variety in invertebrates (Watanabe and Tokuda, 2001; Tanimura *et al*., 2013). When earthworms excrete into the soil, the microbes enter the soil and affect cellulose degradation and soil fertility. Moreover, the fecal pellets of earthworm were also used to make fertilizer to improve soil quality. Soil fertility can be measured from parameters as follow, soil pH, organic matter, cation exchange capacity, total nitrogen, total phosphorus, available phosphorus, and available potassium (Darilek *et al*., 2009).

Based on recent research, cellulolytic bacterial augmentation has been found to increase soil fertility. Isolated cellulolytic bacteria were collected from different chickpea (Cicer arietinum L.) agricultural fields of Patiala District, Punjab, India (Singh and Prakash, 2010). They can significantly increase the amount of phosphorus and organic matter. In 2015, Bhowmick and Sengupta studied isolated cellulolytic bacteria which were collected from soil in Nadia district of West Bengal. They can solubilize phosphate, potassium and fix nitrogen

In this research, the researchers focuses on isolate and screen effective cellulolytic bacteria from the fecal pellets of earthworm and the effects of cellulolytic bacteria on cellulose degradation in soil from the fecal pellets of earthworm when it is excreted into the soil to improve soil quality. To be used to improve soil fertility in the future.

1.2 Objectives

1) To isolate and screen cellulolytic bacteria from the fecal pellets of earthworm

2) To investigate the effect of cellulose degradation of cellulolytic bacteria on soil fertility

1.3 Scope of study

Source of cellulolytic microorganisms was collected from the fecal pellet of African night crawler (*Eudrilus eugeniae*) from Wanrung farm (33/790 Moo 8, Phimonrat, Bang Bua Thong, Nonthaburi). Isolated and screened strains were augmented into soil from vegetable plot behind Chamchuri 10 building. Soil fertility was measured from soil cation exchange capacity, total nitrogen for 28 days.

1.4 Expected outcome

1) Obtain effective cellulolytic bacteria from the fecal pellets of earthworm

2) Isolated and selected cellulolytic bacteria from the fecal pellets of earthworm are effective in cellulose degradation in soil

CHAPTER 2
LITERATURE REVIEW

2.1 Soil microorganisms

Microbes are small organisms that are too visible to the naked eye. They are found in many habitats such as water, soil, air and food (Löhnis and Fred, 1923). In soil, soil microorganisms are important for biogeochemical cycles. For example, bacteria in nitrogen cycle. They use N compounds as an electron receptor to increase energy for body tissues and proteins production. Some microorganisms can convert nitrate to ammonium or gaseous products. This process can make nitrogen stability in the soil (Rütting *et al*., 2011). Nitrogen fixation by microorganisms occurs through nitrogenase as intermediates which contain 2 proteins (dinitrogenase and nitrogenase reductase).

2.2 Cellulose

Cellulose is an organic compound that has a formula $(C_6H_{10}O_5)_n$, which is a polysaccharide, a polymer of β-1,4-glucan chain with β-1,4-linkage bonds as shown in Figure2.1. Cellulose can be synthesized by plants, algae, some bacteria, and some animals (Richmond, 2000). Mostly, cellulose is derived from plants, accounting for 40% of the carbon-based part of the plant, which is a component of plant cell walls. Cellulose may be found in the pure form in the plant, but it is most often mixed with hemicelluloses, lignins, and other (Hon, 1996)

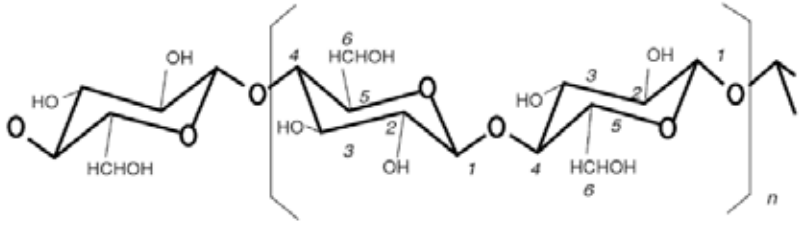


Figure2.1 Structure of cellulose

2.3 Cellulose biodegradation of cellulolytic microorganism

Because the structure of the cellulose is insoluble and difficult to decompose. Cellulose degradation requires specific enzymes that come from cellulolytic microorganisms that produce enzymes that are combined between Endo-β- (1,4) -glucanases (EC 3.2.1.4), Exo-β- (1,4) -d-glucanases (EC 3.2.1.74 and EC 3.2.1.91), and β-d-glucosidases and cellobiases (EC 3.2.1.21) (Hobdey *et al*., 2015).

2.4 DNS method

3,5-dinitrosalicylic acid (DNS) method is the measure of reducing sugars concentration by Sumner, which has been further developed by Miller in 1959. This method is a color measurement technique caused by redox reactions between 3,5-dinitrosalicyclic acid and the reducing sugars as shown in Figure2.2. The reaction of carbonyl group on reducing sugar to oxidize into the carboxyl group by 3,5-dinitrosalicylic acid, which has yellow color that is reduced to 3-amino-5-nitrosalicylic acid which has redbrown color and can measure the absorbance by spectrophotometer at a wavelength of 540 nm.

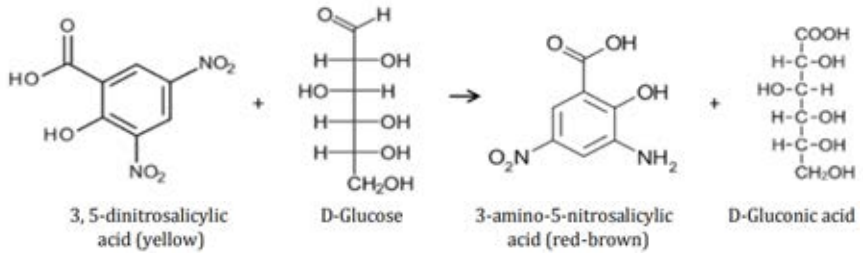


Figure2.2 Conversion of reducing sugars by DNS (Garriga *et al*., 2017).

2.5 Earthworms

The earthworm is an invertebrate animal in the phylum Annelida. They live in the soil in the soil that are related to soil structures and nutrient cycling in the soil. Earthworms decompose organic matter such as plant litter and mammalian dung (Lee, 1985). They are able to directly decompose organic matter which is not related to microorganisms and indirectly related to intestinal microorganisms (Swift *et al*. 1979). Although earthworms are in poor soil conditions, they can develop the digestive system together with microorganisms to be able to digest nutrients into energy (Edwards, 2004).

2.6 Related research

According to a study by Singh and Prakash in 2010, bacteria have contributed to improving soil quality. This study focused on available phosphorus in the soil, found that cellulolytic bacteria that are isolated from the soil significantly increase the amount of phosphorus in the soil.

CHAPTER 3
MATERIALS AND METHODS

3.1 Source of the microorganisms

Cellulolytic bacteria were isolated from the fecal pellets of African night crawler (*Eudrilus eugeniae*) from fertilizer which produced by Wanrung Farm.

3.2 Soil sample

Soil samples for cellulose studies were collected from the vegetable plot area behind Bhaloem Rajakumari 60 (Chamchuri 10) building because it is an area that needs soil improvement to be suitable for cultivation (13°44'34.2"N 100°31'43.9"E).

3.3 Culture media[1]

1. Carboxy Methyl Cellulose(CMC) agar plates
2. Luria-Bertani agar medium (LB)
3. Bushnell Haas medium (BHM)

3.4 Chemicals and Equipment

1. Chemicals

The chemicals and reagents used in the experiment are described in the appendix.

2. Equipment

The devices used in this research are as follows:

1. Incubator
2. Drying Oven
3. Centrifuge
4. Analytical balance
5. Autoclave
6. Spectrophotometer

[1]Formulas of culture media are shown in **APPENDIX A**.

3.5 Experiment

Isolation and screening cellulolytic bacteria

Cellulolytic bacteria were isolated from the fecal pellets of African night crawler (*Eudrilus eugeniae*) from Wanrung farm. They were measured parameters include temperature, water content, and pH (Guber *et al*., 2015).

One gram sample was incubated in culture media with 100 ml Bushnell Haas medium (BHM) and carboxymethyl cellulose (CMC) 72 h at 37°C 120 rpm. One milliliter of inoculated media was transferred to fresh medium and incubated repeatedly (Lo *et al.*, 2009). After 3 times, the 3 rd inoculated media was serial dilution. One milliliter of inoculated media was diluted in 9 ml of saline solution (0.85% NaCl (w/v)) and spread on CMC agar media plates as shown in Figure 3.1. They were incubated 24 h at 37°C. The number of bacteria that can be observed was measured by the colony-forming unit (CFU) as Equation 1. At least 100 strains of growing bacteria are isolated for re-streak on CMC agar for purification of bacterial strain. The morphological appearances were observed by microscope. Followed by store them on CMC agar at 4 °C.

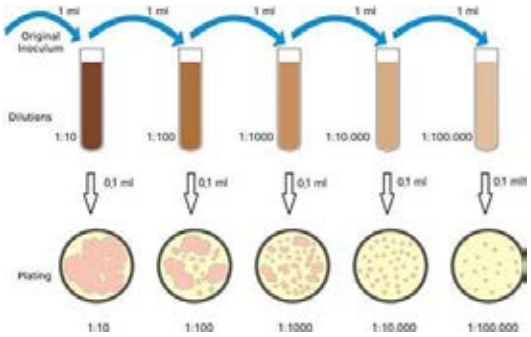


Figure 3.1 Serial dilution (Alves and Cruvinel, 2016)

Cellulolytic bacterial strains were screened using Gram's iodine (Kasana *et al*., 2008). Isolated bacteria were inoculated on CMC plates 24 h at 37 °C and then flooded with Gram's iodine for 3–5 min. The clear zone was observed and determined the hydrolysis capacity to compare the hydrolysis capacity calculated from Equation 2 for select strains to test cellulase activity.

$$\text{CFU/ml} = \frac{\text{No. of colonies x dilution factor}}{\text{Volume of culture plate}} \quad \text{(Equation 1)}$$

$$\text{Hydrolysis capacity (HC value)} = \frac{\text{Diameter of clearzone}}{\text{Diameter of colony}} \quad \text{(Equation 2)}$$

Cellulase activity assay

Isolated colonies were transferred to 50 ml of LB Broth medium and incubated 24 h at 37°C. Cultures were centrifuged 10,000 rpm at 4°C to obtain supernatant as a source of crude enzyme. Reduced sugar was detected with DNS method (Miller, 1959). Half of milliliter of supernatant was mixed with 1ml of 1% CMC solubilized in acetate buffer (pH 4.8) and incubated 60 min at 50°C. Reactions were stopped by adding dinitrosalicylic (DNS) acid reagent 3 ml and boiled 5 min then they kept to cool and measured the absorbance with a wavelength of 540 nm. 1 unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol of reducing sugars per minute by using Glucose as a Standard.

Soil quality analysis

The soil from the vegetable plot area behind Bhaloem Rajakumari 60 building is collected about 3 kg by using a shovel to dig the soil into a wedge shape of about 0-15 cm deep. After that, collect the soil beside the hole as shown in Figure 3.2. The physical properties of the soil are measured including temperature, pH and water content (Guber *et al*., 2015). After that, the soil is collected. The collected soil was put in a plastic bag.

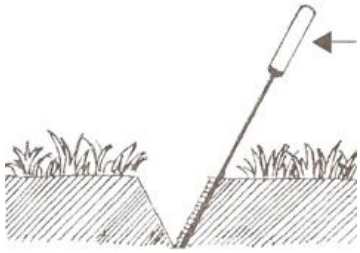


Figure 3.2 Soil sample collection (Rice Department of Thailand, 2019)

The soil that was collected was air dried and sifted the soil through a sieve of 2 and 0.5 mm. Then weighed 60 grams of soil and put it into a plastic jar as shown in Figure 3.3. After that, add 25 ml of distilled water and 1 ml of cultures in LB culture medium and incubate at 37 ° C for 4 weeks. The quality of the soil that changes each week is measured as follows: Nitrogen content, cation exchange capacity (CEC)

a. b.

Figure 3.3 Plastic jar

Nitrogen content is measured by the Kjeldahl method and the cation exchange capacity is measured by the NH_4OAc method. Compare the difference of nitrogen content and cation exchange capacity values between before and after isolated cellulolytic bacteria were augmented into the soil by using Paired t-Test and then conclude the results of using cellulolytic bacteria to increase soil fertility.

CHAPTER 4

RESULTS

4.1 Isolation and screening cellulolytic bacteria

Cellulolytic bacteria were obtained from the fecal pellets of African night crawler (*Eudrilus eugeniae*) from Wanrung farm as shown in Figure 4.1. Their temperature was 28 °C. The acidity was 7 and the moisture content was 2%RH.

a. b.

Figure 4.1 the fecal pellets of African night crawler (*Eudrilus eugeniae*)

Cellulolytic microorganisms in the fecal pellet of earthworm were enriched in culture medium consisting of Bushnell Haas medium (BHM) and carboxymethyl cellulose (CMC), which are carbon sources. After 3 times, the 3rd inoculated media was serial dilution and incubated on CMC agar media plates 24 h at 37°C as shown in Figure 4.2. A total of 115 isolates of bacteria were isolated and re-streaked on CMC agar for purification of bacterial strain.

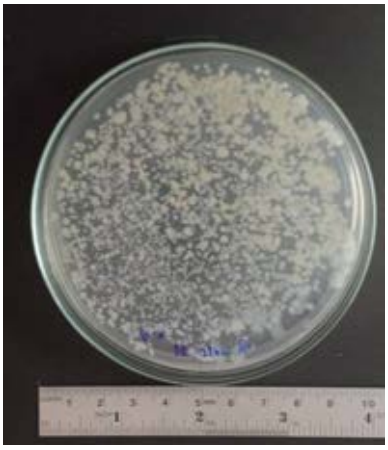


Figure 4.2 Cellulolytic bacteria that grow on CMC agar at concentration 10^{-3}

After that, cellulolytic bacterial strains were screened using Gram's iodine to determine the hydrolysis capacity. Ten isolated bacteria that have the highest HC capacity were selected as follows: CB96, CB58, CB84, CB114, CB10, CB73, CB11, CB22, CB72,and CB53 which have the HC capacity as follows 5.24, 4.34, 3.88, 3.72, 3.57, 3.34, 3.11, 2.86, 2.91, and 2.87, respective as shown in Figure 4.3.

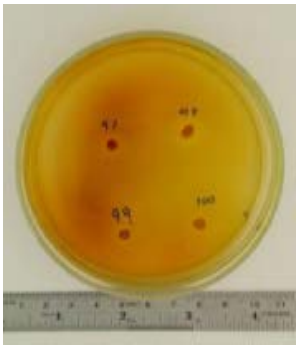


Figure 4.3 Clear zone of cellulolytic bacteria caused by Gram's iodine

4.2 Cellulase activity assay

Ten selected bacteria were enriched in LB broth medium and centrifuged to obtain supernatant as a source of crude enzyme. Cellulase activity were measured with DNS method by reduced sugar measurement. Crude enzyme was incubated with 1% CMC solubilized in acetate buffer (pH 4.8) and mixed with dinitrosalicylic (DNS) acid as shown

in Figure 4.4. After that, all samples were measured the absorbance with a wavelength of 540 nm.

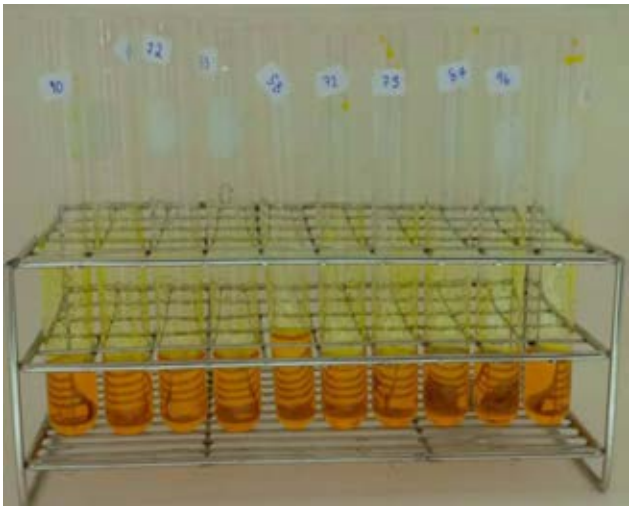


Figure 4.4 Color from the reaction between reducing sugar and dinitrosalicylic (DNS) acid

From the experiment, the absorbance at 540 nm of the reaction of all selected was 0 compared to the standard glucose as shown in Figure 4.5 the reduced sugar value of all samples is about 0 mg / ml.

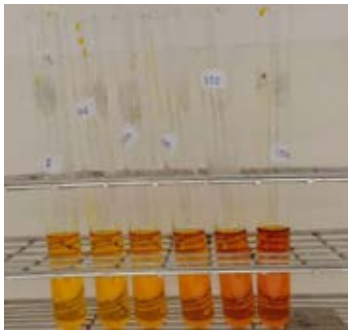


Figure 4.5 Color of standard glucose in each concentration

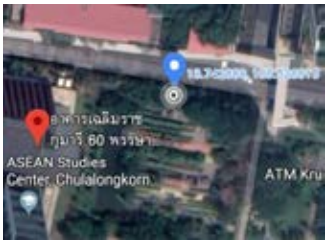
4.3 Soil quality analysis

Soil properties

The soil from the vegetable plot area behind Bhaloem Rajakumari 60 building (13°44'34.2"N 100°31'43.9"E) were collected on 11 March 2019 at 5.30 PM as shown in Fiugure4.6 and measured soil properties as shown in Table 4.1.

a.

b.



c.

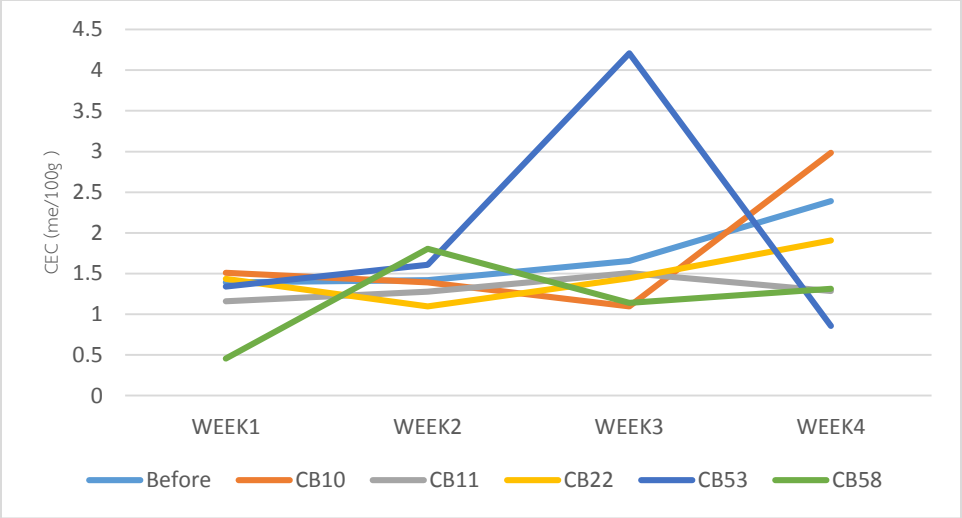
Fiugure4.6 soil sampling locations

Table 4.1 Soil properties

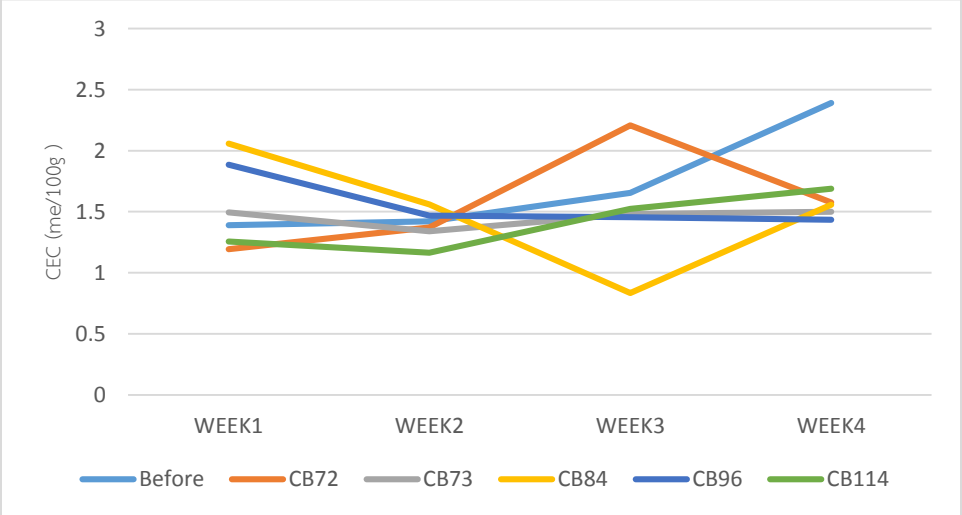
Parameter	Measured value
Temperature (°C)	30
pH	5.4
Moisture content (%RH)	8
Total kjeldahl nitrogen (%)	0.029254
Total phosphorus (mg/g_{soil})	0.6266
Organic matter (%)	2.51
Cation Exchange Capacity (me/100g)	1.113305

Cation Exchange Capacity

Cation exchange capacity (CEC) is measured by the NH_4OAc method. The measurement results are as shown in Figure4.7



a.



b.

Figure4.7 CEC comparison chart for each species of cellulolytic bacteria

Based on statistical analysis by using Paired t-Test, it was found that after adding isolated cellulytic bacteria into the soil, the cation exchange capacity in the soil was

increased from before adding the bacteria significantly from Table6.6 showed that p-value < 0.05 in **APPENDIXB**. The cation exchange capacity that has changed from the addition of individual cellulytic bacteria is similar due to p-value> 0.05, except CB11 that is different from CB72 and CB84. CB72 that is different from CB84 significantly (p-value <0.05).

Total kjeldahl nitrogen

Total kjeldahl nitrogen is measured by Kjeldahl method. The measurement results are as shown in Figure4.8

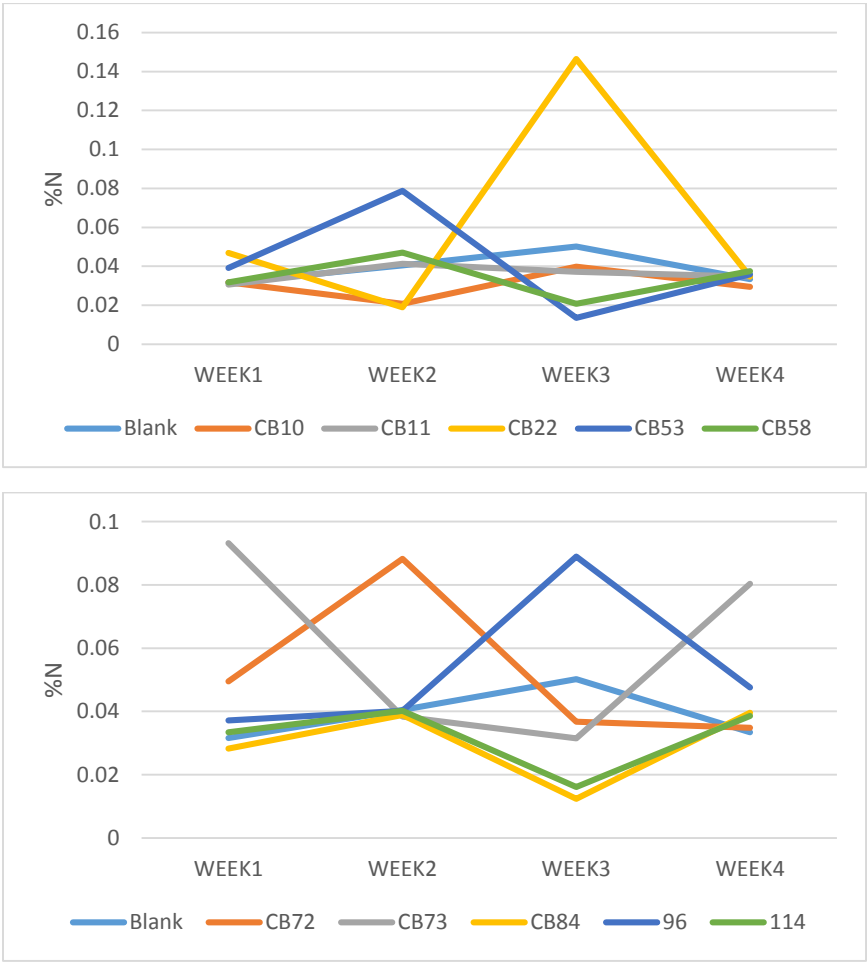


Figure4.8 Total kjeldahl nitrogen comparison chart for each species of cellulolytic bacteria

Based on statistical analysis by using Paired t-Test, it was found that after adding isolated cellulytic bacteria into the soil, the amount of nitrogen in the soil was increased from before adding the bacteria significantly from Table6.4 showed that p-value < 0.05 in **APPENDIX B** The amount of nitrogen that has changed from the addition of individual cellulytic bacteria is similar due to p-value> 0.05, except CB10 that is different from CB53 and CB58. CB22 that is different from CB84, CB96 and CB114 and CB84 that is different from CB114 significantly (p-value <0.05).

CHAPTER 5
DISCUSSION & CONCLUTION

5.1 Isolation and screening cellulolytic bacteria

From observing, the isolation of cellulolytic bacteria from the fecal pellets of earthworm from the Wanrung farm, it was found that the temperature of the fecal pellets of earthworm would vary with the air temperature. The temperature measured before being used is 28 ℃ in the range of 25–35 ℃ (Bui, 2014) which is also suitable for the biological preservation of bacteria. The pH that is measured is 7, which is in the range of cellulose to decompose quickly (Lynd *et al*., 2002). From the measured moisture content, it was found that this moisture content was low, but the cellulolytic bacteria could still grow in this conditions, which is a condition that is not suitable for fungal growth (Borowik and Wyszkowska, 2016). Cellulolytic bacteria can be isolated from the fecal pellets of African night crawler *(Eudrilus eugeniae)* from Wanrung farm 115 isolates. The highest HC value is CB56 equal to 5.24, but all isolated cellulolytic bacteria, as described in Chapter 4, are not able to measure cellulase activity with the DNS method. This may be due to many reasons, such as the crude enzyme may be destroyed before testing the reducing sugar by DNS method from temperature, pH, or other (Pardo and Forchiassin, 1999). Therefore, no enzymes in the CMC degradation, which is cellulose. Therefore, this test could not see the color difference caused by the reaction between, 5-dinitrosalicyclic acid and the reducing sugars.

5.2 Soil quality analysis

According to the study of soil properties from the Bharatem Rajakumari 60 building, the pH value is still in the acid range. The amount of nitrogen content in the soil is less than 0.05%, which is very low, and the amount of phosphorus in the soil is more than 45 mg / kg, which is very high. The amount of organic matter in the soil is between 1.0–1.5%, which is at a moderate level. The CEC value is less than 5 cmol / kg which is very low according to Land Development Department criteria. From this study found that the cellulolytic bacteria can significantly increase nitrogen and CEC values. According to study

by Pramanik *et al* in 2017, the cellulolytic bacteria were found to play an important role in increasing the NO_3-N content in soil. And from Valarini *et al* studies in 2002, the cellulolytic bacteria were associated with CEC. Most of the experiments have better nitrogen content and CEC values. Some of the results may be error because the soil in the experiment is not yet homogeneous.

5.3 Conclusions

From the study, it was found that cellulolytic bacteria were isolated from the fecal pellets of African night crawler (*Eudrilus eugeniae*) 115 isolates and ten isolated bacteria that have the highest HC capacity from the measurement of Gram Iodine method were selected as follows: CB96, CB58, CB84, CB114, CB10, CB73, CB11, CB22, CB72, and CB53, respective. In the experiment, it was found that are not able to measure cellulase activity with the DNS method. After all isolated cellulolytic bacteria were added into the soil for 4 weeks, it was found that the amount of nitrogen and the cation exchange capacity in the soil was increased from before adding the bacteria significantly**.**

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APPENDIX A

Formulas of culture media

1. Carboxy Methyl Cellulose(CMC) agar plates (Lo *et al.*, 2009)

Chemicals	(g /l)
LB	25
agar	15
carboxymethyl cellulose (CMC)	1%

2. Luria-Bertani agar medium (LB)

Chemicals	(g/L)
Trytone	10
Yeast extract	5
NaCl	10

3. Bushnell Haas medium (BHM)

Chemicals	(g /1000 ml)
CMC	10
$MgSO_4 \cdot 7H_2O$	0.2
K_2HPO_4	1
KH_2PO_4	1
NH_4NO_3	1
$FeCl_3 \cdot 6H_2O$	0.05
$CaCl_2$	0.02

APPENDIX B

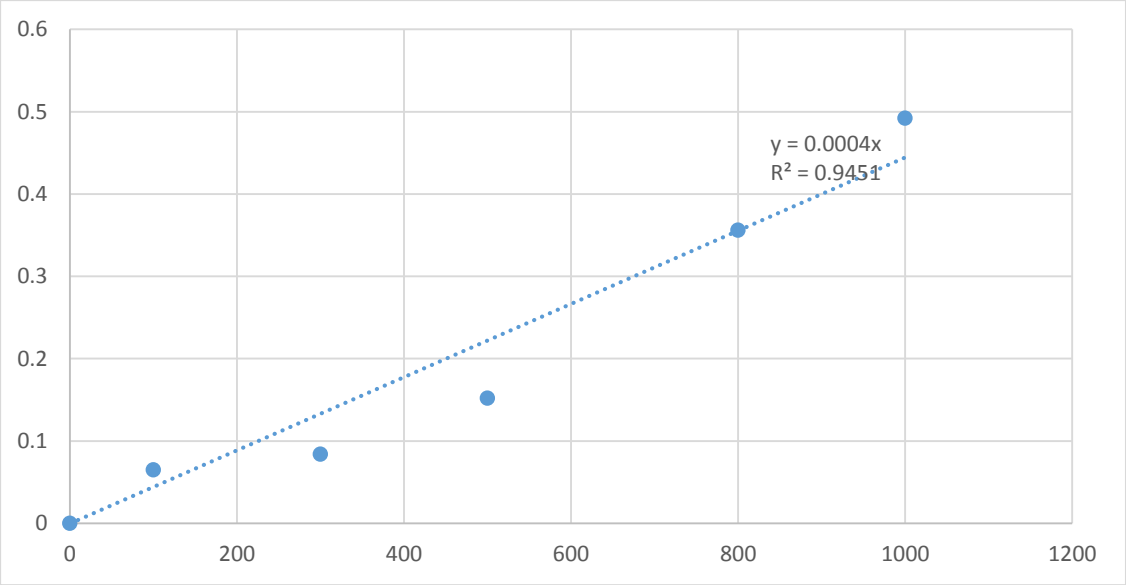

Figure6.1 standard glucose in each concentration

Table6.1 Correlations between cation exchange capacity of each strain of cellulolytic bacteria

Correlations

		blank	CB10	CB11	CB22	CB53	CB58	CB72	CB73	CB84	CB96	CB114
blank	Pearson Correlation	1	.896	.153	.899	-.275	.184	.237	.458	-.169	-.516	.900
	Sig. (2-tailed)		.104	.847	.101	.725	.816	.763	.542	.831	.484	.100
	N	4	4	4	4	4	4	4	4	4	4	4
CB10	Pearson Correlation	.896	1	-.297	.831	-.671	.099	-.219	.354	.274	-.240	.648
	Sig. (2-tailed)	.104		.703	.169	.329	.901	.781	.646	.726	.760	.352
	N	4	4	4	4	4	4	4	4	4	4	4
CB11	Pearson Correlation	.153	-.297	1	.019	.879	.315	.983*	.067	-.996**	-.670	.451
	Sig. (2-tailed)	.847	.703		.981	.121	.685	.017	.933	.004	.330	.549
	N	4	4	4	4	4	4	4	4	4	4	4
CB22	Pearson Correlation	.899	.831	.019	1	-.260	-.266	.166	.787	.002	-.133	.897
	Sig. (2-tailed)	.101	.169	.981		.740	.734	.834	.213	.998	.867	.103
	N	4	4	4	4	4	4	4	4	4	4	4
CB53	Pearson Correlation	-.275	-.671	.879	-.260	1	-.001	.866	.081	-.849	-.271	.119
	Sig. (2-tailed)	.725	.329	.121	.740		.999	.134	.919	.151	.729	.881
	N	4	4	4	4	4	4	4	4	4	4	4
CB58	Pearson Correlation	.184	.099	.315	-.266	-.001	1	.169	-.751	-.396	-.844	-.023
	Sig. (2-tailed)	.816	.901	.685	.734	.999		.831	.249	.604	.156	.977
	N	4	4	4	4	4	4	4	4	4	4	4
CB72	Pearson Correlation	.237	-.219	.983*	.166	.866	.169	1	.250	-.967*	-.596	.565
	Sig. (2-tailed)	.763	.781	.017	.834	.134	.831		.750	.033	.404	.435
	N	4	4	4	4	4	4	4	4	4	4	4
CB73	Pearson Correlation	.458	.354	.067	.787	.081	-.751	.250	1	.001	.310	.677
	Sig. (2-tailed)	.542	.646	.933	.213	.919	.249	.750		.999	.690	.323
	N	4	4	4	4	4	4	4	4	4	4	4
CB84	Pearson Correlation	-.169	.274	-.996**	.002	-.849	-.396	-.967*	.001	1	.727	-.438
	Sig. (2-tailed)	.831	.726	.004	.998	.151	.604	.033	.999		.273	.562
	N	4	4	4	4	4	4	4	4	4	4	4
CB96	Pearson Correlation	-.516	-.240	-.670	-.133	-.271	-.844	-.596	.310	.727	1	-.471
	Sig. (2-tailed)	.484	.760	.330	.867	.729	.156	.404	.690	.273		.529
	N	4	4	4	4	4	4	4	4	4	4	4
CB114	Pearson Correlation	.900	.648	.451	.897	.119	-.023	.565	.677	-.438	-.471	1
	Sig. (2-tailed)	.100	.352	.549	.103	.881	.977	.435	.323	.562	.529	
	N	4	4	4	4	4	4	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table6.2 Correlations between nitrogen content of each strain of cellulolytic bacteria

Correlations

		Blank	CB10	CB11	CB22	CB53	CB58	CB72	CB73	CB84	CB96	CB114
Blank	Pearson Correlation	1	.433	.607	.787	-.309	-.476	-.007	-.919	-.701	.874	-.765
	Sig. (2-tailed)		.567	.393	.213	.691	.524	.993	.081	.299	.126	.235
	N	4	4	4	4	4	4	4	4	4	4	4
CB10	Pearson Correlation	.433	1	-.433	.895	-.974*	-.994**	-.824	-.043	-.878	.785	-.896
	Sig. (2-tailed)	.567		.567	.105	.026	.006	.176	.957	.122	.215	.104
	N	4	4	4	4	4	4	4	4	4	4	4
CB11	Pearson Correlation	.607	-.433	1	-.006	.498	.405	.625	-.866	.125	.216	.046
	Sig. (2-tailed)	.393	.567		.994	.502	.595	.375	.134	.875	.784	.954
	N	4	4	4	4	4	4	4	4	4	4	4
CB22	Pearson Correlation	.787	.895	-.006	1	-.806	-.917	-.545	-.481	-.959*	.956*	-.995**
	Sig. (2-tailed)	.213	.105	.994		.194	.083	.455	.519	.041	.044	.005
	N	4	4	4	4	4	4	4	4	4	4	4
CB53	Pearson Correlation	-.309	-.974*	.498	-.806	1	.942	.929	-.080	.749	-.724	.791
	Sig. (2-tailed)	.691	.026	.502	.194		.058	.071	.920	.251	.276	.209
	N	4	4	4	4	4	4	4	4	4	4	4
CB58	Pearson Correlation	-.476	-.994**	.405	-.917	.942	1	.757	.091	.923	-.791	.926
	Sig. (2-tailed)	.524	.006	.595	.083	.058		.243	.909	.077	.209	.074
	N	4	4	4	4	4	4	4	4	4	4	4
CB72	Pearson Correlation	-.007	-.824	.625	-.545	.929	.757	1	-.345	.453	-.491	.514
	Sig. (2-tailed)	.993	.176	.375	.455	.071	.243		.655	.547	.509	.486
	N	4	4	4	4	4	4	4	4	4	4	4
CB73	Pearson Correlation	-.919	-.043	-.866	-.481	-.080	.091	-.345	1	.388	-.629	.454
	Sig. (2-tailed)	.081	.957	.134	.519	.920	.909	.655		.612	.371	.546
	N	4	4	4	4	4	4	4	4	4	4	4
CB84	Pearson Correlation	-.701	-.878	.125	-.959*	.749	.923	.453	.388	1	-.842	.983*
	Sig. (2-tailed)	.299	.122	.875	.041	.251	.077	.547	.612		.158	.017
	N	4	4	4	4	4	4	4	4	4	4	4
CB96	Pearson Correlation	.874	.785	.216	.956*	-.724	-.791	-.491	-.629	-.842	1	-.924
	Sig. (2-tailed)	.126	.215	.784	.044	.276	.209	.509	.371	.158		.076
	N	4	4	4	4	4	4	4	4	4	4	4
CB114	Pearson Correlation	-.765	-.896	.046	-.995**	.791	.926	.514	.454	.983*	-.924	1
	Sig. (2-tailed)	.235	.104	.954	.005	.209	.074	.486	.546	.017	.076	
	N	4	4	4	4	4	4	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table6.3 Comparison of nitrogen content between before and after isolated cellulolytic bacteria were augmented into the soil

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Before	.0293	11	.00000	.00000
	After	.0406	11	.01394	.00420

Table6.4 Paired Samples Test of nitrogen content between before and after isolated cellulolytic bacteria were augmented into the soil

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Before - After	-.01134	.01394	.00420	-.02071	-.00197	-2.698	10	.022

Table6.5 Comparison of cation exchange capacity between before and after isolated cellulolytic bacteria were augmented into the soil

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Before	1.1133	11	.00000	.00000
	After	1.6815	11	.57884	.17453

Table6.6 Paired Samples Test of cation exchange capacity between before and after isolated cellulolytic bacteria were augmented into the soil

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Before - After	-.56817	.57884	.17453	-.95704	-.17930	-3.255	10	.009

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