Polyethylene degradation by bacteria extracted from Galleria mellonella's gut

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ABSTRACT

From the problems of plastic waste in Thailand which occurs about 3-4 million tons per year and inefficiently waste management cause the continuous problems for humans and animals. Incomplete decomposition of plastic into small pieces produces "microplastics" which can be contaminated in the body by the food chain system. One of the methods of microplastic degradation is using bacteria. Moreover, waxworms and mealworms can eat plastics. Therefore, this research aims to identify bacteria extracted from the gut of waxworm and determine the efficiency of polyethylene degradation by extracted bacteria. The waxworms were fed with polyethylene to stimulate the formation of bacteria in their guts. The bacteria were aseptically extracted from 9 gut samples and cultivated. After that, biochemical test and biodegradation test of bacteria from each gut sample were carried out. The polyethylene was added to bacterial culture and measured their mass before and after cultivation for 7 days. The results showed inconclusive bacterial species, but they are possibly gram positive and non-mobile bacteria. Moreover, the weights of polyethylene could be obtained with technical errors.

INTRODUCTION

According to the problem of plastic trashes,

About 3-4 tons per year

About 1.03 million tons are in the ocean

Mostly found plastic bags, straw and water bottle cap.

Ocean Conservancy, 2018

Microplastic is the piece of plastic with 1-5 nm size. It causes one of the problems of the plastic that contaminated in the ocean by UNEP.

Nowadays, microplastics start to cause concern for the environment impact. These microplastics are in toothpaste, shaving cream and others. In addition, they are found in fibers of fishing equipment and products from synthetic fibers. When they are washed, more than 100 fibers are found in 1 liter of water. Or even contaminated plastic waste in the environment both in the ocean and the ground will become microplastics as well(Chanchai Kahapana,2018).

Some aquatic animals have contaminated microplastics.

The disposal of microplastics is therefore an important issue to save the environment. One of the methods to decrease the number of microplastics is using bacteria to degrade plastics. It is known that waxworm can eat and degrade plastic. This degradation is caused by the enzymes of bacteria that are in the intestine of the worms. (Jun Yang, 2014) Thus, the aims of this project are classifying the species of bacteria extracted from the intestine of waxworm (Galleria mellonella) and determine the efficiency of polyethylene degradation by bacteria extracted from gut of Galleria mellonella to be used for further study of the disposal of microplastic.

HYPOTHESIS

- In *Galleria mellonella*'s gut has more than 1 species of bacteria.
- If *Galleria mellonella* was fed with plastic, *Galleria mellonella*'s gut will generate bacteria that can degrade the plastic.
- Bacteria that has been extracted can degrade polyethylene as the same as In the *Galleria mellonella*'s gut.

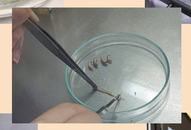
OBJECTIVE

- To classify the species of bacteria fond in gut of waxworm
- To study of the process of extracting bacteria from gut of waxworm
- To determine the efficiency of polyethylene degradation by bacteria extracted from gut of waxworm

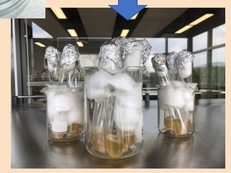
Methodology

1. Extracting bacteria from waxworm or *Galleria mellonella*

- Feed the waxworms with PE for 10 days. Select 10 (1.5 *cm* long)
- Make the waxworms faint in freezer that about -55°C.
- Dissect the shank part of waxworms and use forceps to clamp the gut out and put it in 10 *mm* of Nutrient Broth in sterile condition for 24 hours.
- Use Inoculating loop to spread out, Spread plate and Streak plate technique, the Nutrient broth with the waxworm's gut on Nutrient agar and then put them in Incubator at 35 °c for 24 hours.



waxworm's gut

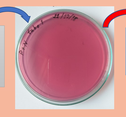


2. Classifying species of bacteria by using Agar and Biochemical Test

By using Agar

- MacConkey Agar

an indicator, a selective and differential culture medium for bacteria designed to selective isolate Gram-negative and enteric bacilli on lactose fermentation



Observe the color changed after 24 hours

- Motility Test

Motile bacteria can move by using structure called flagella. Prepare a semisolid agar medium in a test tube. Inoculate with a straight wire, making a single stab down the center of the tube to about half the depth of the medium. Incubate at 35°c for 24 hours. Observe the growth of bacteria in the tube

By using Biochemical Test

- Urease Test

Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. Use Inoculating loop to spread the bacteria on slant urea agar by using streak plate technique and keep them in Incubator at 35°c for 48 hours. After that, use dropper to drop 2 drops of Phenol red. Observe the color changed.

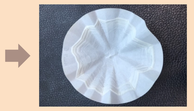
- Methyl Red and Voges-Proskauer Test

MR-VP tests were used to distinguish between members of the family Enterobacteriaceae and used to characterize other groups of bacteria including Actionobacteria. Culture bacteria in MR-VP broth for 48 hors in 35°c. After that, observe the color change for Voges-Proskauer test. Then, use dropper to drop 2 drops of MethyL red and observe the color changed for Methyl red test.

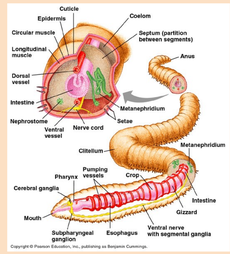
- Catalase Test

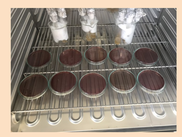
Catalase is an enzyme.
A drop of 3% H_2O_2on the slide with bacteria.
Observe a lack and weak bubble production.

3. Testing degradation ability of bacteria



- Weight 0.3 g of Polyethylene in centrifuge tube and pour ethanol, flood cover the polyethylene, into every tube.
- Incubate the bacteria that already examined and characterized in Nutrient broth at 35°c for 24 hours.
- Prepare Mineral Salt Medium (MSM) 11 Erlenmeyer Flasks 50 ml per each.
- Use micropipette to collect 100 microliters of bacteria in Nutrient broth and put it in MSM.
- Put polyethylene in to every flask.
- Weight polyethylene after the experiment..
- Put PE into every flask





RESULTS

Classify species of bacteria by using Agar and Biochemical Test

Tube	1	2	3	4	5	6	7	8	9	+ control	- control
MacConkey	-	-	-	-	-	-	-	-	-	+	-
Motility test	-	-	-	-	-	-	-	-	-	+	-
Urease test	+	-	-	-	-	-	-	+	+	+	-
Methyl red test	+	+	+	+	+	+	+	+	+	+	-
Catalase test	+	-	±	-	-	-	-	+	+	+	-

MacConkey Agar

Positive	Negative
• Noncoliform	• Gram-positive

Motility test

Positive	Negative
• Motile bacteria	• Non-motile bacteria

Urease test

Positive	Negative
• Proteus spp • Cryptococcus spp • Corynebacterium spp • Helicobacter pylori • Brucella spp	• *Escherichia* • *Shigella* • *Salmonella*

Methyl red test

Positive	Negative
• *E. coli* • *Yersinia* sps	• *Enterobacter aerogenes* • *Klebsiella pneumoniae*

Catalase test

Positive	Negative
• *Staphylococcus* • Enterobacteriaceae	• *Streptococcus* • *Enterococcus*

Testing ability of degradation of bacteria

เขTube	-control	2	3	5	6
Mass of Polyethylene before testing with bacteria(g)	0.300	0.300	0.300	0.300	0.300
Mass of Polyethylene after testing with bacteria(g)	0.2847	0.3564	0.3869	0.3598	0.3533

DISCUSSION & CONCLUSION

Extraction of bacteria from waxworm or *Galleria mellonella* has done from 9 waxworms. Then, Bacterial culture on the Nutrient Agar by using streak plate technique. The colonies are very similar. So, there was one type of bacteria that has been found in each plate.

Classification species of bacteria by using Agar and Biochemical Test has done from bacteria in Nutrient Broth and Nutrient Agar. From MacConkey Agar test, all plates are negative. Therefore, it is gram-positive bacteria. From Motility test, all plates are negative. It can be concluded that it is Non-motile bacteria. From other Biochemical test, in each test, it could be concluded in many different genus and species of bacteria, according to the table.

Testing ability of degradation of bacteria, in this process the changed mass of polyethylene was considered. The mass of polyethylene in tube 2, 3, 5 and 6 are increased because the errors from Nutrients broth in powder form that did not separate from Polyethylene because the size filter paper that cannot separate them.

Future Plans

- Observe the surface of polyethylene by using Scanning electron microscope before and after.
- Make the control to find the precise number of Polyethylene mass in Testing ability of degradation of bacteria process.

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