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Isolation and Identification of Bacteria from Activated Sludge and Compost for Municipal Solid Waste Treatment System

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Abstract. Municipal solid waste has to be stabilized prior to discharge in order to reduce biological activity and prevent or slow the release of harmful chemicals into the environment and reduce odour production. The consortium Microorganisms (CM) has been proposed as a new technology to assist in the treatment and disposal of sewage sludge, conforming to strict environmental regulations. The aims of this study are the isolation and identification of bacteria from municipal sewage and sludge that were collected from domestic sewage facility. It was culture and isolate under sterile condition in order to examine the microbial in each sample. The identification is based on several testing which is morphology and motility test, physical test and biochemical test. Based from the result obtained from the study of isolation and identification of the bacteria in municipal sewage, *Bacillus* family which is *Bacillus Megatharium* and *Bacillus Licheniformis* was identified except for *Bacillus Aporrhoeus*. Eight strains of bacteria were isolated from municipal sludge under aerobic or anaerobic conditions at 37°C. On the basis of their physiological and biochemical characters, and in comparison with different strains, one strain was identified as belonging to *Bacillus megatherium* and two strains were tentatively identified as *Nitrosomonas sps.* and *Nitrobacter sps*.

Keyword: waste water treatment, municipal waste, consortium microorganisms, sewage waste, sludge degradation

1. Introduction

There a few factors attribute to the municipalities problem such as treatment, disposal and recycling of sewage sludge. Basically the solid waste from a municipality contains the biodegradable organic materials with a significant amount of inorganic matter. For example, sludge exhibits wide variations in the physical, chemical and biological properties (Szymanski & Patterson, 2003). In the view, (Ujang *et al.,* 2002) has reported that untreated and inadequate treatment of wastewater such as municipal sewage has principal contaminations which fall into the categories of Nitrogen, Phosphorus, pathogenic organism, heavy metals and trace organics. The pathogen includes bacteria, viruses, protozoa and helminthes. All of these pollutants have the potential to create public health and environmental problem, particularly in urban areas. Currently, there are a number of methods being used to dispose of sewage sludge from disposal to landfill to land application. Although there are many methods used, there are numerous concern raised regarding the presence of constituents including heavy metals, pathogens and other toxic substances. This requires the selection of the correct disposal method focusing on efficient and environmentally safe disposal. According to the (Gotvajn *et a.l*, 2009) has reported that the biological methods are usually preferred over the physicochemical in removing the majority of pollutants in wastewater treatment. Besides that, (Zhao *et al.,* 2008) has reported that aerobic biological wastewater treatment systems utilize mixed microbial consortia to

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transform organic and inorganic pollutants to innocuocus by products, allowing municipal and industrial wastewater to be released to the environment without detriment. Therefore, biological methods seem to offer effective solution for in order to improve the municipal wastewater treatment. The used of biological method is to introduce contact with bacteria which feed on the organic materials in wastewater treatment. Organic and nitrogen pollution act as nutrient substrate for the purifying biomass in biological method. There are a few goals of this method which are to reduce labour time, enhance BOD/COD removal, degrade wide range of organic waste, increase the system efficiency, reduce sludge build up and reduce hydrogen sulphide cost. The advantage of using biological systems is that, operation takes place at ambient temperature. This consequently can save the energy consumption. The operations are located at outdoor and this implies that the system must be able to operate at seasonally varying temperature

Bacteria in soil occur singly and in aggregates. To isolate and identify the type of bacteria in gram of soil, the soil must be diluted and mixed thoroughly so that the aggregates are broken up such that the suspension of singles cells is achieved. The cell suspension is then serially diluted so that from some dilutions

In nature, microbial population do not segregate themselves by species but exist with a mixture of many other cell types. These populations can be separated into pure culture. These cultures contain only one type of organism and are suitable for the study of their cultural, morphological, and biochemical properties. The techniques commonly used for isolation of discrete colonies initially required that the number of organisms in the inoculums be reduced (James and Natalie, 7th ed., microbiology laboratory manual).

The isolates were grouped accorded to their colonial morphology and cell characteristics. The colony were counted and re-isolated in pure culture using the medium on which they had grown as described by (Njoku *et al.* 1990). Isolates were thereafter subjected to biochemical test as described by (Collins and Lyne 1984) and (Ogbulie *et al.* 1994). The probable identifies of the isolates were determined as recommended by

2. Experimental

2.1. Material

Soil samples were collected from Indah Water Konsortium Sdn Bhd. The Gram's iodine stain, Gram's crystal violet stain, Gram's safranin solution, Nigrosin stain solution, nutrient broth, ethanol, Barrit's reagent A, Barrit's reagent B and methyl red - Voges Proskauer (MRVP) broth purchased from Permula Chemical Sdn Bhd (Malaysia). Malachite green solution and Nitrate reduction test purchased from Sigma Aldrich (M) Sdn Bhd. Anaerogen, and anaerobic indicator purchased from Thermo Scientific Microbiology Sdn Bhd (Malaysia).

2.2. Dilution of Sample

1 g of soil sample was weighed and added into 99 ml of distilled water. Then, 1 ml from the sample was taking out and added into 9ml of distilled water. This step was continuously repeated until fifth dilution. 0.1 ml from each dilution bottle was streak on the agar plate. For each plate, hockey stick was used to spread the dilution on the medium. The plates was sealed with parafilm, labelled and kept in the 30°C kept in incubator for 24 hour.

2.3. Isolation of microorganism

The soil samples had been taken from municipal sewage and municipal sludge. Isolation of these microorganisms until get single colony had been done by using serial dilution method and streaking method. Twenty plates of agar plate were prepared aseptically. After agar plate was ready, the fast growing of bacteria plate is taken. The different shape of bacteria on the plate is chosen. By using aseptic technique, the selective bacteria on the agar plate are streaked. The plate is divided into 4 parts. The streak agar plate is sealed and kept in 30°C incubator for growing. After 24 hours, purification of bacteria was done doing by another spread plate technique and incubation. Besides that, from the previous plate, gram staining is done. Samples was observed under microscope and characterized based on (Lozano *et al*., 2009).

2.4. Identification of microorganism

Biochemical test were performed by standard procedure based on Bergey's manual. For the identification of these microorganisms from municipal sludge, several biochemical identification methods such as Gram stain, spore forming, strict anaerobes, starch hydrolysis, Voges-Proskauer and swollen cell test had been used. There are several biochemical identification methods done of municipal sludge such as Gram stain, starch hydrolysis, citrate test, and Voges-Proskauer.

2.5. Maintenance of microorganisms

All the microorganism cultures were maintained at 4 ^{o}C in nutrient agar. All the cultures were sub-cultured at 15 days interval.

3. Result and Discussion

The isolation process is a procedure of isolation the mixture of colonies to a single colony. This process was done by using streaking method to obtain pure cultures. The sample of soil was added with 1 liter pure water to obtain solution sample before transferred into nutrient agar plate. It is important that the numbers of colonies developing on the plates are not being too large. On crowded plates some cells may not form colonies, and some colonies may fuse, leading to erroneous measurements. Thus, to obtain the appropriate colony number, the samples need to be diluted. This solution samples were diluted up to 10^{-5}. By using spread plate method, the diluted samples were transferred into nutrient agar plate and the bacteria were grown on it. From the observation, these samples take about three until four days to growth on the plate. Figure 4.1 shows the growth of the bacteria on plate after 5 days.

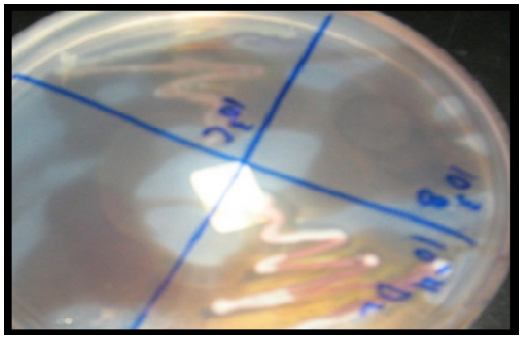

Figure 4.1: Image of sample 10^{3}C and $10^{-4}D_2$

Based on Bergey's manual, microorganism that should be obtained from municipal sewage are *Bacillus licheniformis, Bacillus megatherium and Bacillus aporrhoeus*. After performing the starch hydrolysis test, clear zone was detected as a positive result in the present of Bacillus bacteria. There were sample S8, S3 and S4.

Table 1: Biochemical testing of microorganism from municipal sewage

Test	S1	S2	S3	S4	S5	S6	S7	S8
Gram Staining	-	-	+	+	+	-	-	+
Spore Staining	-	-	-	-	-	-	-	-
Strict Anaerobes	-	-	-	-	-	-	-	-
Starch Hydrolysis	-	-	+	+	-	-	-	+
Voges-Proskauer	+	+	+	-	+	-	-	-
Cell Diameter	+	+	-	+	-	+	-	+
Citrate	-	-	+	-	+	-	-	-
Swollen Cell	-	-	-	-	-	-	-	-

In Citrate Reduction Test, researchers were able to identify *Bacillus Licheniformis* due to its characteristics of citrate utilization. From the experiment, only sample S5 and S3 shown positive result when the slant changed colour to bluish-green after incubated for 24 hours.

After the first streak, microorganism that was able to observe are *Bacillus* family due to its dominant than others. Figure 4.3 below shown one example of *Bacillus* from first streaking of the sample. According to the following viewed the rod shape of bacteria showed that it is *Bacillus Megatharium* bacteria.

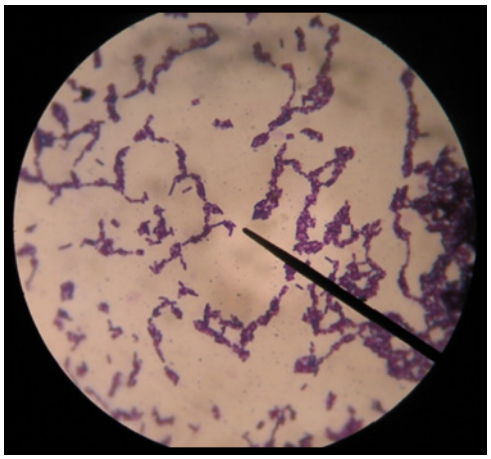

Figure 4.3: Image of Bacillus sp.

Eight strains of bacteria were isolated from municipal sludge under aerobic or anaerobic conditions at 37°C. On the basis of their physiological and biochemical characters, and in comparison with different strains, one strain was identified as belonging to *Bacillus megatherium*. This isolate were able to grow in the temperature range from 37 °C with their optimum growth temperature range being from 60–65°C. The isolated bacilli grew reasonably on sewage sludge in a mixed culture.

Table 2: Biochemical testing of microorganism from municipal sludge

Test	S 1	S 2	S 3	S 4	S 5	S 6	S7	S 8
Gram Staining	-	-	+	-	-	+	+	+
Spore Staining	-	-	-	-	-	-	-	-
Strict Anaerobes	-	-	-	-	-	-	-	-
Starch Hydrolysis	+	+	-	+	+	-	+	+
Voges-Proskauer	+	+	-	+	-	-	-	-
Swollen Cell	+	+	-	+	-	-	-	-

Only S3, S6, S7 and S8 from municipal sludge that were Gram positive, strict anaerobes negative, starch hydrolysis positive, Voges-Proskauer negative and swollen cell negative catalase preliminarily identified as *bacillus megatherium* (Table 1) according to the biochemical and physiological tests described in materials and methods. Figure 4.4 below shown negative starch hydrolysis for S7 of municipal sludge.

4. Conclusion

Based from the result obtained from the study of isolation and identification of the bacteria, Basillus family which is Bacillus Megatharium and Bacillus *Licheniformis* was identified except for Basiclus Aporrhoeus. Further isolation will enhance the chance to get wide variety of bacteria species. For the municipal sludge, only *bacillus megatherium* was identified based on biochemical testing.

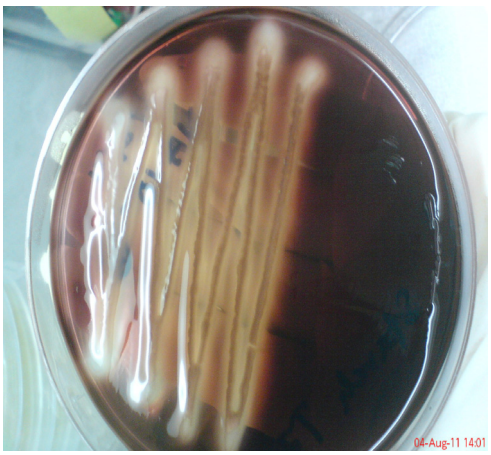

Figure 4.4: Negative starch hydrolysis of municipal sludge

5. Acknowledgements

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