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Isolation of solid waste microflora

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ABSTRACT

Udupi district is a town located near Mangalore, Karnataka state. Temperature ranges from 30 to 35 degrees centigrade in daytime and is around 22 - 22°C ± 2°C during night. Humidity is normally high most of the time. The rainy season is from April to September.Solid waste samples weighing approximately 2 to 2.5 Kgs were randomly taken from Karkala and Udupi districts and then transferred into the nylon bags and brought to the laboratory for further evaluation for the incidence of bacteria.The objective of the project includes the collection of the Solid waste sample, isolation of the microflora and then the identification of these micoflora. Different methods were adopted for the isolation of the micro organism. Some of these methods are Blotter method, Pour plate method,and Agar syringe method for bacteria. Identification of bacteria was done based on their colony and biochemical characteristics. Identification of fungi is carried out based on their colony and morphological characteristics, spore formation and biochemical reactions.The bacteria were isolated and identified to the species level. Bergey's Manual of Determinative Bacteriology was used as reference for identification.

KEYWORDS

Solid waste;
Agar syringe;
Bergey's manual;
Pour plate method.

INTRODUCTION

Over the years, there has been a continuous migration of people from rural and semi-urban areas to towns and cities. The uncontrolled growth in urban areas has left many cities deficient in infrastructural services such as water supply, sewerage and municipal solid waste management .It is estimated that about 1, 00,000 MT of Municipal Solid Waste is generated daily in the country. Per capita waste generation in major cities ranges from 0.20 Kg to 0.6 Kg. Generally the collection efficiency ranges between 70 to 90% in major metro cities whereas in several smaller cities the collection efficiency is below 50%. For obtaining a long term economic solution, planning of the system on long-term sustainable basis is very essential .Solid Waste (SW) is unwanted materials disposed off by man, which can neither flow into streams nor escape immediately into the atmosphere. These non-gaseous and non -liquid residues result from various human activities. These cause pollu-

tion in water, soil and air. Waste is an unavoidable consequence of satisfying man's needs for food, water, air, space, shelter, and mobility. In any material process, by product recovery or recycling can substantially alter waste quantity and quality, but all processes eventually produce some waste.

MATERIALS AND METHODS

Collection of domestic organic waste-isolation, identification of microflora

The waste collected in a clean plastic container of 500gm capacity. Container closed with air tight lid and carried to the laboratory. All residential waste shall be collected at least bi-weekly. Many specific kinds of microorganisms can be obtained from organic wastes by the creation of an artificial environment for them in the laboratory which will enhance their growth over competing organisms. Characteristics of the organisms which give them special advantages over others are exploited in the formulation of culture media and the choice of incubation conditions. Ultimately we want to achieve the following:

IDENTIFICATION OF MICROFLORA

Identification of bacteria

The isolated bacterium is then identified according to Bergey's manual specifications by examining its microscopic characters, microscopic morphology, antibiotic susceptibility and biochemical characters. (Bergey's 1981).

RESULTS AND DISCUSSION

The pour plate method, to isolate the microbes in the solid waste sample was carried out and clumped colonies were obtained. The isolated colonies were selected and sub cultured on nutrient agar plates by streak-plate method. The pure culture obtained was used for further analysis. The results of various analyses are shown in the tables given below.

MR-VP test

If the solution turns crimson to ruby pink, it indicates it's a positive result. While no change in color indicates a negative result. Test organism S1, S2, S4 showed negative reaction to Methyl red test, while test organism S3 showed positive reaction to methyl red test. Test organisms S1, S2, S3showed negative test result for Voges-Proskauer Test, while test organism S4 showed positive result.

Indole test

A red color in the alcohol (upper) layer is a positive result. Absence of red coloration indicates negative result. Test organisms S2 and S3 showed positive reaction to indole test. This can be concluded since red color was seen in the upper alcohol layer when kovac's reagent was added. The other organisms S1 and S4 gave negative result on the addition of kovac's reagent.

MR-VP test

If the solution turns crimson to ruby pink, it indicates it's a positive result. While no change in color indicates a negative result. Test organism S1, S2, S4 showed negative reaction to Methyl red test, while test organism S3 showed positive reaction to methyl red test. Test organisms S1, S2, S3 showed negative test result for Voges-Proskauer Test, while test organism S4 showed positive result.

Motility test

If migration away from the line of inoculation is evident then you can conclude that the test organism is motile. Lack of migration away from the line of inoculation indicates lack of motility. The entire test organism showed negative result for this test.

Citrate test

Citrate positive is indicated by a color change of the medium from green to blue. For citrate negative organism these is no change in color of the medium. Test organism S3 and S4 showed positive result for citrate test, while test organism S1 and S2 showed negative result.

Endospore staining

The endospore stain green and the vegetative cells stain red for positive endospore staining. The cells stain red in the case of negative endospore staining.

Test organisms S1, S2, S3 and S4 all give negative result for endospore staining.

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TABLE 1 : Results of biochemical tests

Test organism	Gram staining rod/cocci	IMViC Test				Endospore formation	Motility test
		Indole test	Methyl red test	Voges-proskauer test	Citrate test		
S1	Positive cocci	- (No color change)	-	-	-	-	-
S2	Positive rod	+	-	-	-	-	-
S3	Positive rod	+	+	-	+	-	-
S4	Positive cocci	- (No color change)	-	+	+	-	-

TABLE 2 : Results of biochemical tests

Test organism	Starch hydrolysis	Casein hydrolysis	Manitol test	Oxidase test	Catalase test	Hydrogen sulphide test	Nitrate reduction test
S1	-	-	-	+	+		
S2	-	-	-	-	-		
S3	+	+	-	+	-		
S4	-	-	+	+	+		

Starch hydrolysis

A positive starch hydrolysis reaction is represented by the presence of a clear zone surrounding the microbial colonies. Negative reaction is indicated by the presence of dark blue coloration of the medium.

Test organism S3 gave positive result for starch hydrolysis test while organisms S1, S2 and S4 gave negative result for this test.

Casein hydrolysis

A positive result is represented by the presence of a clear zone surrounding the bacterial growth while the absence of clear area around the growth of an organism shows negative result.

Test organism S3 gave positive result for casein hydrolysis test while organisms S1, S2 and S4 gave negative result for this test.

Mannitol test

The conversion of media color from red to yellow indicates positive hydrolysis of mannitol whereas the presence of red color of the media indicates negative result for mannitol test. Test organism S4 showed positive result for mannitol test while organisms S1, S2 and S4 gave negative result for this test.

Carbohydrate utilization

The change of color from red to yellow indicates the production of acid by the fermentation of sugar (glucose, sucrose and maltose) by the micro organism. The production of gas is shown by the accumulation of gas in Durham's tube. The test organisms S2, S3 and S4 ferment glucose to produce acid but only organism S4 produced gas. Test organism S1 does not ferment glucose. The test organism S3 and S4 ferment sucrose to produce acid but gas was produced by only by the organism S4. Test organisms S1 and S2 do not ferment sucrose. The test organism S4 fermented lactose to produce gas but the other organisms S1, S2 and S3 does not ferment lactose.

TABLE 3

Test organism	Carbohydrate utilization test			Cellulase test
	Glucose	Lactose	Sucrose	
S1	-(No Gas)	-(No Gas)	-(Gas Produced)	+
S2	+(No Gas)	-(No Gas)	-(No Gas)	-
S3	+(No Gas)	-(No Gas)	+(No Gas)	+
S4	+(Gas Produced)	+(Gas Produced)	+(Gas Produced)	+

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