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Culture, Isolation and Identification of Bacteria From Municipal Area of Soil

Sadiqul Ahmed | Part time Lecturer, Department of Botany, Dhing College, Dhing: Nagaon: Assam

ABSTRACT

Diversity of bacteria was studied in Municipal area of soil of Nagaon district, Assam. Nine bacterial species belonging to eight genera have been isolated by growing bacteria in different culture media and following pure culture methods of spread plate and streak plate methods. Isolated bacteria were identified by colony morphology and various biochemical tests, including gram staining, litmus milk reaction, H2S production, NO3 reduction, indole production, MR reaction, VP reaction, citrate use, urease activity, catalase activity, gelatin liquefraction, starch hydrolysis, lipid hydrolysis and fermentation.

KEYWORDS | Diversity, Municipal soil, Bacterial genera, Biochemical test

Summary:
During experimental period soil samples from municipal area were collected and analyzed for the presence of bacteria. For this purpose different culture media and methods of pure culture has been followed. The identification of bacterial genus has been done by different biochemical tests. Finally nine bacterial species belonging to eight genera have been isolated and identified.

Introduction:
The urban area faces the problem of municipal solid waste generated in huge amount. It includes residential, commercial and institutional waste. A large part of such waste contains organic or biodegradable waste. The organic wastes of such site are chiefly decomposed by most of the bacteria. Thus they play an important role in bio-geochemical cycling of nutrients as well as establishing a clean environment. In this study different parameters are used to isolate and identify bacterial genera present in municipal are of soil.

Materials and Method:
Study Area:
Nagaon is a centrally located district of Assam geographically falling at the centre of North-East, India. It is one of the largest district of Assam and bounded by Sonitpur district and Brahmaputra River in the North, Karbi Anglong and North Cachar Hills in the South, East Karbi Anglong and Golaghat district in the East. The total geographical area of the district is 3993 Sq. Km. It extends between 26.2997° N and 92.6984° E. In this study the Dhing area of Nagaon district was selected which is located at 26.47°N and 92.47°E.

Sample Collection:
Soil samples were collected from municipal waste dumping sites of Dhing town, Nagaon District, Assam, India. These sites were covered with different materials i.e., potato peels, sugarcane waste, tree bark, news paper, saw dust, fruit peels, grass, leaves, guar, used tea, wood chips, fruit, different types of plastics etc.

Microbial Isolation and Analysis:
Serial Dilution Agar Plating Method
The serial dilution-agar plating method or viable plate count method is one of the commonly used procedures for the isolation and enumeration of Bacteria, Fungi, and Actinomycetes which are the most prevalent micobes in soil. This method is based upon the principle that when material containing microorganisms is cultured each viable microorganism will develop into a colony, hence the number of the colonies appearing on the plates represents the number of living organisms present in the sample. Here the numbers of colonies appearing on dilution plates are counted, averaged and multiplied by the dilution factor to find the number of cells/spores per grams of the soil sample (Aneja K.R., 2011)

No. of cells/gm = No (average of 3 replicates) of colonies x Dilution factor/Dry wt. of soil

Dilution factor = Reciprocal of the dilution (e.g. $10^{-5} = 10^5$)

Culture Media
The culture media consists of ingredients or chemicals which supports the growth of microorganisms. There are different types of bacterial culture media used in this study are-

Nutrient broth and agar (pH 7.0)
Peptone 5.0 g
Beef extract 3.0 g
NaCl 5.0 g
Distilled water 1000.0 ml

Lactose broth (pH 6.9)
Lactose 5.0 g,
Peptone 5.0 g,
Beef extract 3.0 g.

Starch Agar Media (pH 7.0)
Starch (soluble) 20.0 g,
Peptone 5.0 g,
Beef extract 3.0 g.

Fermentation Media
Peptone 10 g
Carbohydrate source 5 g
Sodium chloride 15 g
Phenol red 0.018 g

Pure culture methods
Spread-plate method:
The spread plate technique is used for separation of a dilute, mixed population of microorganisms so that individual colonies can be isolated. In this technique microorganisms are

spread over the solidified agar medium with a sterile L-shaped glass rod while the petri disc is spun on a turntable. The theory behind this technique is that as the petri dish rotates, at some stage, single cells will be separated from each other by a distance sufficient to allow the colonies that develop to be free from each other.

Streak Plate method:
It is a rapid qualitative isolation method of obtaining discrete colonies and pure culture. It was originally developed by two bacteriologists, Loeffler and Gaffkey in the laboratory of Robert Koch. In this method, a sterilized loop or transfer needle is dipped into a suitable diluted suspension of organisms which is then streaked on the surface of an already solidified agar plate to make a series of parallel, non – overlapping streak. The aim of this exercise is to obtain colonies of microbes that are pure, i.e., growth derived from a single cell/spore.

Biochemical tests
To identify the bacterial genus tests for gram stain, litmus milk reaction, H2S production, NO3 reduction, indole production, MR (Methyl Red) reaction, VP (Voges-Proskauer) reaction, citrate use, urease activity, catalase activity, gelatin liquefraction, starch hydrolysis, lipid hydrolysis and fermentation have been followed.

Results:
Table 1: Identification of bacterial isolates by colony morphology and biochemical tests

Isolate	Gram Stain	Litmus Milk Reaction	H_2S Production	NO_3 Reduction	Indole Production	MR Reaction	VP Reaction	Citrate Use	Urease Activity	Catalase Activity	Gelatin Liquefraction	Starch Hydrolysis	Lipid Hydrolysis	Fermentation			Colony Morphology	Identification
														Lactose	Dextrose	Sucrose		
1	Rod +	Pep	–	+	–	–	±	–	–	+	+	+	±	–	A	A	White, small, opaque, serrate	*Bacillus* sp.
2	Rod –	AGC	–	+	+	+	–	–	–	+	–	–	–	AG	AG	A ±	White, small, opaque, flat	*Escherichia* sp.
3	Rod –	AGC	–	+	–	±	±	+	+	+	–	–	–	AG	AG	AG	Yellow, small, translucent, raised	*Klebsiella* sp.
4	Cocci +	AC	–	–	–	+	–	–	–	–	–	–	–	A	A	A	Orange, small, opaque, raised	*Lactococcus* sp.
5	Rod –	Pep	–	+	–	–	–	+	–	+	+	–	+	–	–	–	Yellow, small, opaque, raised	*Pseudomonas* sp.
6	Rod –	Pep	–	+	–	–	–	+	–	+	+	–	+	–	–	–	Yellowish green, pinpoint, opaque, convex	*Pseudomonas* sp.
7	Rod –	Alk	+	+	–	+	–	+	–	+	–	–	–	–	AG ±	A ±	Gray, translucent, small, even	*Salmonella* sp.
8	Rod –	Alk	–	+	±	+	–	–	–	+	–	–	–	–	A	A ±	White, translucent, pinpoint, convex	*Shigella* sp.
9	Cocci +	A±	–	+	–	+	±	–	–	+	+	–	+	A	A	A	White, opaque, irregular, flat	*Staphylococcus* sp.

Note: A= Acid; G= Gas; C= Curd; Pep= Peptonization; Alk= Alkaline; ± = Variable reaction

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