# Isolation and Characterization of Bacteria from Solid Waste

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***Abstract:*** **- Solid Waste can also be defined as waste type that includes predominantly household waste (domestic waste) with sometimes the addition of commercial waste collected by a municipality within a given area. They are in either solid or semisolid form and generally exclude industrial hazardous wastes. Micro organisms that dwell in solid wastes are grouped under Solid Waste Microflora (SWM). The most common organisms that are generally found in solid waste are bacteria and fungi. These micro organisms use the components of the waste as the substrate for their growth. They grow and multiply on these wastes by utilizing the various components that make up the solid waste. Further a wide variety of pathogenic microorganisms have been reported to be present in these organic wastes. Not significant study made in the field of solid waste micro flora in the study area. So, we are interested to investigate the Bacteria present in solid wastes and to find out its applications.**

***Key words*: Bacteria, Solid Waste, pathogenic**

## I. INTRODUCTION

Since, the beginning of human kind has been generating waste in the form of bones and other parts of animals they slaughter for their food or the wood they cut to make their carts, with the progress of civilization, the waste generation become more complex in nature. The end of 19th century the industrial revolution saw the rise in world consumers, industrialization leads to urbanization.

Solid Waste can also be defined as waste type that includes predominantly household waste (domestic waste) with sometimes the addition of commercial waste collected by a municipality within a given area. They are in either solid or semisolid form and generally exclude industrial hazardous wastes. The solid waste disposal industry divides solid waste into four major categories for disposal depending on the state in which they are disposed (Mohapatra, 2006).

The waste generated in urban usually said that municipal solid waste (MSW).28.8% of India population residing in urban area (census 2001).It is predicted 41% reside in cities by 2011. In India waste quantity increase 46 million tons in 2001 to 65 mllion ton in 2011.(Kumar and Gaekwad, 2004**)**. The quantity of MSW is usually expressed as a per capita basis.

Table:1.1 The MSW generated in India.(Teri, 1998).

| Year | Per capita waste generation | Urban municipal waste MT/Yr |
|---|---|---|
| 1971 | 375 gm/day | 14.9 MT/Yr |
| 1981 | 430 gm/day | 25.1 MT/Yr |
| 1991 | 460 gm/day | 43.6 MT/Yr |
| 1997 | 480 gm/day | 48.1 MT/Yr |

A waste is said to be hazardous if it is infectious, meaning containing viable microorganisms or their toxins which are known or suspected to cause disease in animal or human (Yakowitz, 1988). Waste disposal poses threat to both man, animals and the soil. Like chemical hazards, aetiologic agents might be dispersed in the environment through water and wind. Poisonous plants, insects, animals and indigenous pathogens are biologic hazards that might be encountered at the waste site (Khupe,1996).

*Micro organisms in Solid Waste*

Micro organisms that dwell in solid wastes are grouped under Solid Waste Microflora (SWM). The most common organisms that are generally found in solid waste are bacteria and fungi. These micro organisms use the components of the waste as the substrate for their growth. They grow and multiply on these wastes by utilizing the various components that make up the solid waste. Further a wide variety of pathogenic microorganisms have been reported to be present in these organic wastes. (Amalraj, *et al*., 2006).

Not significant study made in the field of solid waste micro flora in the study area. So, we are interested to investigate the microorganisms present in solid wastes and to find out its applications.

## II. MATERIALS AND METHODS

*Identification of Bacteria*

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

*Sample collection and Isolation of Bacteria :*

A total of 21 Solid waste samples were collected in different season from 2006-2009,from different stations of a waste-dump site at Udupi in every season two times samples are collected The samples were examined for temperature, pH and for the frequency of isolation of viable aerobic bacteria. The mean temperature values of the solid waste ranged from 27$^{o}$C to 28$^{o}$C while the mean pH values ranged from pH 5.4 to 7.9. Each Solid waste sample was thoroughly shaken in 10ml of normal saline. An liquot(1.0ml) was transferred into the next test tube and diluted serially in one-tenth stepwise to 10-5 dilution (Paul andClark, 1988). From the dilution of 10-4 of each soil sample, 0.1ml aliquot was transferred aseptically onto freshly prepared Nutrient agar plates and spread with a sterile bent glass rod (Paul and Clark, 1988; Harrigan and McCance, 1990). The dilution of $10^{-4}$ was used in plating for bacteria because the dilution of $10^{-3}$ gave a confluent growth while $10^{-5}$ gave fewer growth. The inoculated plates were inverted and incubated at 37$^{o}$C for 24- 48 hrs after which the plates were examined for growth. The discrete colonies which developed were counted and the average counts for duplicate cultures were recorded as total viable bacteria in the sample.

*Culture media*

Nutrient Agar (NA): (Difco manual,1969)

Formula for 1L

Beef extract: 3 g

Peptone : 5 g

Agar : 15 g

Distilled water : 1000ml

pH: 6.8± 0.2

*Protocol for preparing nutrient agar***:** (Modified from Hatcher, 1965)

Place a large Buchner funnel on top of a 2 liter Erlenmyer flask. Place a disc of filter paper into the Buchner funnel. Lay the filter paper down while pouring a bit of water over the paper to ensure there are no air bubbles under the paper. Weigh out 15 g of Difco bacto agar on the digital scale and dump this on top of the pre-moistened filter paper in the Buchner funnel. Slowly, pour approximately 1 L of ddH2O into the Buchner funnel..When the water has almost entirely drained, fill the funnel top with dd H2O again. Leave this to drain overnight. Check the $p^{H}$ of the medim. Adjust $p^{H}$ with NaOH or acid according to the requirements. Plug with cotton plug and autoclave at 121$^{0}$c and 15psi for 20 minutes. Transfer approximate 15 ml of media in to a sterilized Petriplates.

*Spread plate method (Micklos,1990)*

Method for the isolation of bacteria from the collected sample is Spread plate method. Spread plate is useful for quantifying micro organisms that grow on solid medium.

*Technique for obtaining pure culture of the microbial isolate*

*Streak Plate Method (Hendricks, 2005)*

The streak plate method offers a most practical method of obtaining discrete colonies and pure cultures. This method is done in the following way:Using a sterilized loop, the micro organism is picked from spread plate culture. It was then streaked on solidified labelled nutrient agar plate to make a series of parallel non overlapping streaks onto the solid agar. The plates were incubated at 35ºC for 24 - 48 hours.

*Bacterial Colony Morphology : (Zaved, 2008)*

Procedure: The largest, well-isolated colonies were observed with the naked eye to determine general shape and chromogenesis.The Quebec colony counter was used since it has a magnifying glass, and a light behind the plate stage for better observation.

*Pure culture: (Zaved, 2008)*

One single colony was identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate. The plates were then incubated at 30°C or room temperature. Pure cultures were checked from nutrient agar plates .Gram stain was determined to re-check the identical cell morphology and gram reaction comparing to the original colony. At this point, another nutrient agar plate were re-streaked with the correct colony, and pure cultures were-checked using the above steps. After achieving a pure culture, the same colony was streaked onto a nutrient agar.. These cultures were incubated for 1 day in the refrigerator. The isolates from soil were used for further experiments.

*Schaeffer-Fulton method for staining endospores (Schaeffer,* ***1933.)***

Air dry and heat fix the organism on a glass slide and cover with a square of blotting paper or toweling cut to fit the slide. Saturate the blotting paper with malachite green stain solution and steam for 5 minutes, keeping the paper moist and adding more dye as required. Alternatively, the slide may be steamed over a container of boiling water.Wash the slide in tap water.Counterstain with safranin for 30 seconds. Wash with tap water; blot dry. Examine the slide under the oil immersion lens (1,000X) for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink.

## III. RESULTS AND DISCUSSION

The mean temperature ($^{0}$C) and $P^{H}$ values of soil samples on the dump site are as shown in Table1 and 2 respectively. Generally, the temperature values ranged from 18 $^{0}$C to 37 $^{0}$C. The mean temperature value is 27.5. The pH values ranged from pH 5.4 to 7.4. The mean pH was 6.4. The different value

of bacteria for different solid waste samples in different seasons like Rainy, Winter and Summer are as follows

*Pseudomanas spp* 2.38x 106 CFU, during Rainy 3.38x 106 CFU Winter and 1.38x 104 CFU in Summer. *Bacillus pasteurii* 1.5x 105 CFU, Rainy 1.38x 103 CFU Winter and 2.48x 104 CFU in Summer. *Staphylococcus aureus* 0.98x 106 during, CFU Rainy 0.99x 103 Winter and CFU 0.78x 105 CFU in Summer.*Corynebacterium kutscheri* 0.01x 104 CFU, during Rainy 0.02x 106 CFU Winter and 0.04x 106 CFU in Summer. *Aeromonas s*pecies 0.38x 105 CFU during, Rainy 0.56x 106 CFU Winter and 0.46x 106 CFU in Summer. *Micrococcus roseus* 0.32x 106 CFU during Rainy 0.31x 105 CFU Winter and 0.32 x 105 CFU in Summer. *Corynebacterium xerosis* 0.02x 106 CFU during, Rainy 0.02x 103 CFU Winter and 0.05x 106 CFU in Summer. *Aeromonas veronii* 0.05x 103 CFU, Rainy 0.02x 103 CFU Winter and 0.05x 106 CFU in Summer. *Aeromonas hydrophila* 0.07x 106 CFU, Rainy 0.07x 104 CFU Winter and 0.03x 106 CFU in Summer *Pseudomanas spp* recorded the highest total number of 2.50 x 105 CFU while *Corynebacterium xerosis, Aeromonas veronii* and *Aeromonas hydrophila* recorded the lowest total number during the sampling period. The types of bacteria and their frequency of isolation (indicated as percentages of total bacterial count) in the dumpyard of the waste are as shown in Table 3.The frequency of isolation of the bacterial isolates ranged from *Pseudomanas spp*-27.50%,*Bacillus pasteurii* 22.25% ,*Staphylococcus aureus*19.5%, *Corynebacterium kutscheri*1%*Aeromonas* species 17% *Micrococcus roseus*4%, *Corynebacterium xerosis* 0.5%*Aeromonas veronii* 2.0%

This is not surprising since the degree of acidity (pH) reported for the soil (pH 5.4 to 7.9) would favour the proliferation of bacteria than that of fungi.The present study more or less similar to Obire,2002 he states that**,** A total of 48 Solid waste samples were collected fortnightly in the months of June, July and August 1995,from four different stations of a waste-dump site. The samples were examined for temperature, pH and for the frequency of isolation of viable aerobic heterotrophic bacteria and fungi. The mean temperature values of the soils arranged from 27$^{o}$C to 28$^{o}$C while the mean pH values ranged from pH 5.4 to 7.9. The mean total viable aerobic heterotrophic bacteria population ranged from 0.38 x $10^{6}$ CFU/g soil to 2.00x$10^{6}$ CFU/g soil. The bacteria with their frequency of isolation from the waste-dump soils were: *Arthrobacter spp (4.7%), Bacillus spp (15.2%), Escherichia coli*(12.1%), *Klebsiella spp* (9.6%), *Micrococcus spp* (2.5%), *Proteus spp* (10.2%), *Pseudomonas spp* (5.4%), *Serratia spp* (2.5%),*Staphylococcus spp* (21%) and *Streptococcus spp* (16.8%). Only *Bacillus spp, E. coli, Staphylococcus spp* and *Streptococcus spp* were isolated from all the stations our investigation has also shown that seasonal influence can affect microbial proliferation. The second sampling in the month of June (which had a mean monthly rainfall value of 147.0mm) which is the beginning of the heavy rains, recorded the lowest number of total viable aerobic heterophic bacteria (2.67 x $10^{6}$), while the fourth and sixth samplings carried out in the months of July and August (which had mean monthly rainfall value of 318-517mm) considered as peak of the rainy season, recorded the highest number of total viable aerobic heterotrophic bacteria (5.07 x $10^{6}$)and the lowest number of total viable fungi (9.4 x $10^{4}$) for all the stations, respectively. This showed that, bacteria and fungi of the waste dump site respond differently to seasonal influence.

(Marshall and Devinny,1988).Statistical analysis, using analysis of variance. for the data obtained in the present investigation showed that, there was no significant difference in the number of bacteria and fungi at 5% level among the four stations of the waste dumpsite. There were however significant differences in the number of fungi among the different sampling periods (season) at 5% level.The present study shows the types of bacteria and fungi and their frequency of isolation from the waste dump site in Eagle Island. The bacteria isolated from the dump site include, *Arthrobacter* spp., *Bacillus* spp., *Escherichia coli, Klebsiella* spp., *Micrococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Staphylococcus aureus, Staphylococcus* spp., and *Streptococcus* spp. Only *Bacillus, Escherichia coli, Staphylococcus,* and *Streptococcus* were isolated from the stations while *Micrococcus* spp. and *Serratia* sp. were isolated only in station B. The other bacterial isolates occurred in two or three stations. Of the different genera of bacteia isolated from the waste dump site, *Proteus, Staphylococcus* and *Streptococcus* had the highest frequency of isolation in station A, *Staphylococcus* and *Streptococcus* in station B, *Bacillus* in station C, and *Staphylococcus* in station D. The order of decreasing frequency of isolation is *Staphylococcus > Streptococcus > Bacillus > Micrococcus* and *Serratia.*

Generally, the temperature values ranged from 27$^{o}$C to 28$^{o}$C in all the stations. The mean temperature values for station A was 27.5 while mean values for stations B, C and D was 27.6.The pH values ranged from pH 6.5 to 7.9 in Station A; pH 5.9 in Station B; pH 5.4 to 7.9 in Station C and pH 6.3to 7.9 in Station D. The mean pH for stations A,B,C and D. during the sampling period was 7.2, 6.8, 6.8, and 7.1respectively.The temperature of the soil samples from Eagle Island waste dump site ranged from between 27$^{o}$C and 28$^{o}$C. The falls within the mesophilic range of temperatures which is between 20$^{o}$C and 45$^{o}$C. Most microbial species are mesophilic. Hagerty *et al.,* (1973) reported that, during initial composting development, the mesophilic flora predominate and are responsible for most of the metabolic activities that occurs. This increased microbial activityelevates the temperature of the compost, with the subsequent replacement of mesophilic population by thermophilic flora such as *Bacillus, Aspergillus*, and *Mucor* reported in the study.

Among the bacteria isolated, only *Bacillus* spp., *Escherichia coli, Staphylococcus* spp. and *Streptococcus* spp. were isolated from all the stations. Preliminary data collected by our laboratory suggests microorganisms initially present inwastes dominate the composting process. Furthermore,this

data also indicates that under certain conditions, thermo-tolerance spreads rapidly to organisms not believed to be heat resistant. The dissemination of thermal tolerance may take place through similar, complicated mechanisms as are responsible for the increase among bacteria of antibiotic resistance (Davies, 1994; Begley, 1994)

We have isolated an *E. coli* mutant capable of growing at 65$^{o}$C .Having the capacity to degrade cellobiose only at 48$^{0}$C (Droffner,et.al. 1992a). The temperature even in our study area lies between 19-37$^{0}$ C.

**Table:1. Types of Bacteria with frequencies of isolation in the three seasons on the waste dump site**

| Organisum | Frequencies |
|---|---|
| *Pseudomanas spp* MTCC1O206 | 27.50% |
| *Bacillus pasteurii* | 22.25% |
| *Staphylococcus aureus* | 19.5% |
| *Corynebacterium kutscheri* | 1% |
| *Aeromonas* species | 17% |
| *Micrococcus roseus* | 10% |
| *Corynebacterium xerosis* | 0.5% |
| ***Eschresia coli*** | 3.50% |
| ***Corynebacterium spp*** | 0.21 |
| ***Alcaligenes facilis*** | 0.22 |

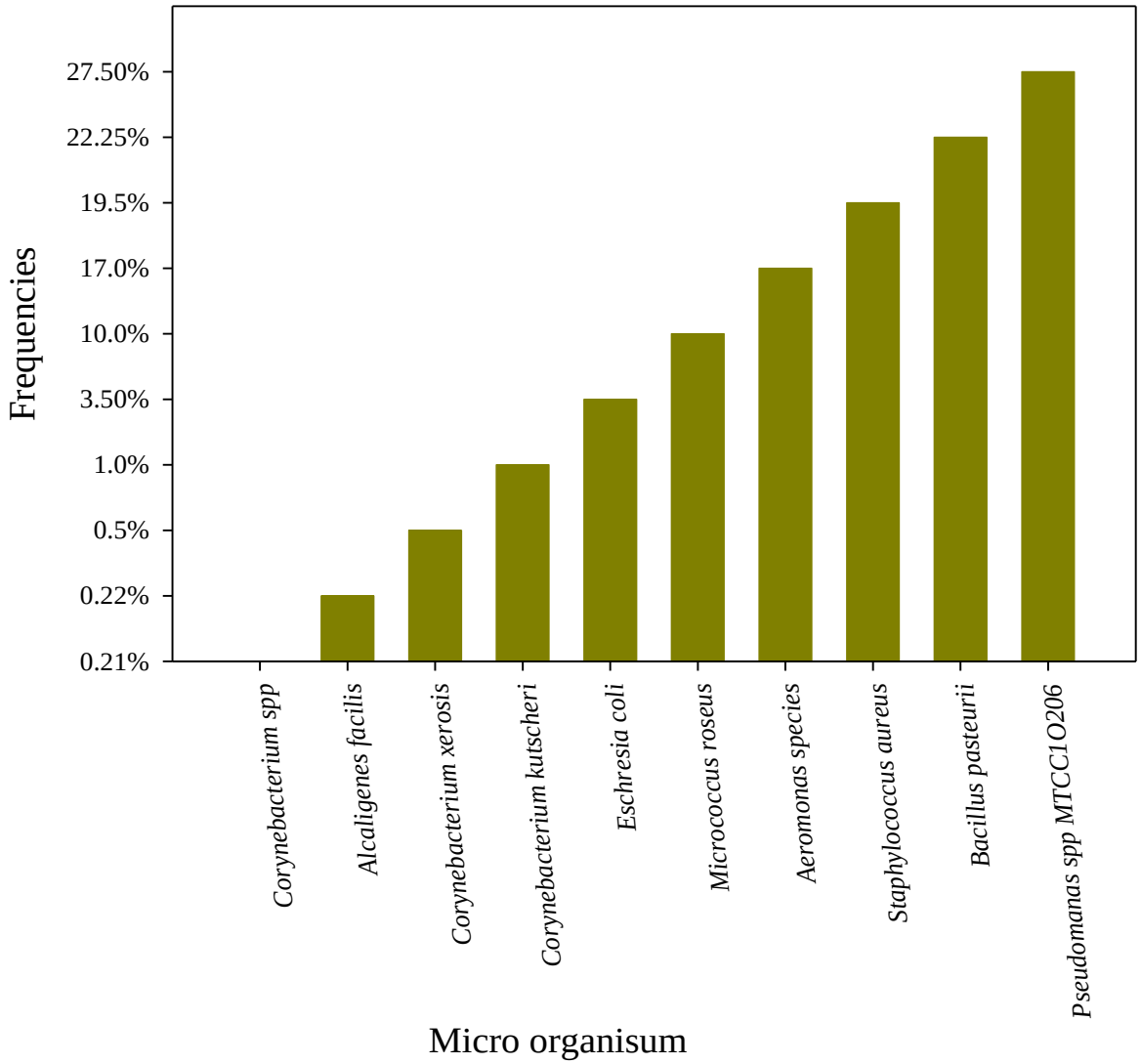


Fig. Types of Bacteria with frequencies of isolation

Table:2 Morphological Characteristics of identified organisms

| | Cell Shape | Colony Form | Colony Elevation | Colony Margin | Gram staining | Endospore formation |
|---|---|---|---|---|---|---|
| *Pseudomanas spp* MTCC1O206 | Diplococci | Filamentous | Raised | Filiform | Negative | Negative |
| *Staphylococcus aureus* | cocci | Irregular | Flat | Lobate | Positive | Negative |
| *Bacillus pasteurii* | Bacilli | circular | Convex | Filiform | Positive | Positive |
| *Aeromonas* species | Bacilli | Irregular | Flat | Undulate | Negative | Negative |
| *Corynebacterium kutscheri* | Bacilli | Rhizoid. | Flat | Undulate | Positive | Negative |

Table:3. Fermentation results of identified organisms

| | Lactose | Sucrose | Glucose | Fructose | Maltose | Mannitol |
|---|---|---|---|---|---|---|
| *Pseudomanas spp* MTCC1O206 | - | + | + | + | + | + |
| *Staphylococcus aureus* | + | + | + | + | + | + |
| *Bacillus pasteurii* | - | + | + | - | - | - |
| *Aeromonas* species | + | + | + | + | + | + |
| *Corynebacterium kutscheri* | - | + | + | + | + | - |



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