International Journal of Advanced Information in Engineering Technology (IJAIET)
Vol.5, No.4, April 2018 ISSN: 2454-6933

Isolation, Characterization and Identification of Bacteria from Organic Waste

Anjali.K.Ullas, Anitha .K

Malabar College of Engineering and Technology, Kerala Technological University

anjali.midhumol.777@gmail.com, anithasubashk@gmail.com

***Abstract*— The complex molecules in wastes are decomposed by many microorganisms, which are either soil borne or air borne. These organisms have many applications in the production of organic manure and bio-fertilizers. These isolated microorganisms could be used as industrial microorganisms and for mitigation of pollution. Organic waste samples were collected from agro based industries and from the agriculture fields consisted of both biotic and abiotic components with different physiochemical parameters such as pH, moisture, carbon content, nitrogen cellulose, hemicelluloses, lignin and starch. In present investigation, the study is to isolate most frequently occurring active strains adapted to the organic waste physical-chemical conditions and having good biodegradation potential. These bacterial isolates were identified on the basis of colony morphology, Gram staining, biochemical tests and using selective and differential media. After identification of the bacteria from organic waste, the usefulness of the bacteria in different field was also analyzed in this project.**

Keywords –*agar medium, bacteria, biochemical test, organic waste*

I. INTRODUCTION

The highly toxic organic compounds have been synthesized and released into the environment directly or indirectly over a long period of time by industrial and agricultural activities. Agro wastes include solids, liquids and gases. The production and improper disposal of agro wastes has become a major pollution issue round the globe. Everyday huge quantity of waste is generated in all the developing and developed countries. Biological decomposition of organic waste such as fertilizers, pesticides and agro wastes are the most important and effective way to remove these compounds from the environment. Bacteria, actinomycetes, fungi, algae and protozoa are the major microorganisms found in soil which decompose soil organic materials, of which bacteria are most prominent and most abundant. Microbes use the waste for their own metabolism and finally produce some simple and useful compounds which are important for soil health, plant growth and overall eco-balance. Microorganisms have the ability to interact, both chemically and physically with substances, leading to the structural changes or complete degradation of the target molecules. Therefore, the present study was aimed to focus at the importance of isolation, characterization and identification of bacteria from waste dumping soils.

A. Bacteria

Bacteria constitute a large domain of prokaryotic microorganisms. Typically a few micrometers in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria also live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterized, and only about half of the bacterial phyla have species that can be grown in the laboratory (https://en.wikipedia.org/wiki/Bacteria).

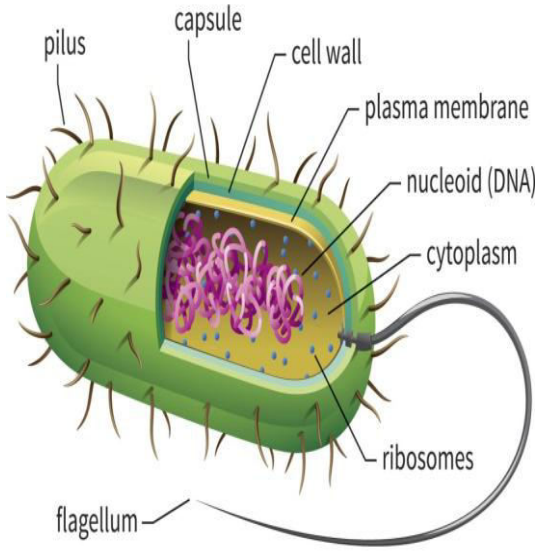

Fig.1. Bacteria

II. METHODOLOGY

The organic waste such as compost soil and food waste soil has been collected from Minaloor, Thrissur, (10.6253° N, 76.2218° E) for the microbiological analysis. The samples are collected from a depth of 4 cm to 8 cm. Samples were collected in the month of January. Soil samples were collected (approx 300g) in clean, dry and sterile polythene bags using sterilized spatula.

Fig.2. Compost Soil

Fig.3. Food waste Soil

A. Physicochemical analysis of organic waste

Soil tests measure the microbial composition and activity of different soil horizons. The physicochemical characteristics of soil influence the rate of biomass production and the activity and composition of microorganisms. Seasonal changes in soil moisture, soil temperature, pH organic carbon, nitrogen, lignin, starch, cellulose and hemicelluloses can have a large effect on soil microorganisms, which, in turn, affect the ability of the soil to supply nutrients to plants through the turnover of soil organic matter. Therefore, the collection of representative soil samples is extremely important and sampling should always be performed taking into account the above-mentioned heterogeneity associated with many soil types. Soil pH was measured using pH meter, where as the soil temperature was measured using soil thermometer. The moisture content was analyzed by weighing the soil sample before and after oven drying. Spectrophotometric analysis as done in order to find nitrogen, cellulose and starch content. The standard titration method as well as laboratory analytical method was followed in order find the organic content, lignin and hemicelluloses content.

B. Total count of bacteria

Pour plate method is used for isolation of bacterial colony. Four different test tubes were taken and 9 ml of sterile distilled water was added in three test tubes and 10ml of water was added in one test tube.1g of soil sample was collected from the organic waste soil and was mixed in 10ml of sterile distilled water. 1ml of the suspension from the above solution was taken and added to 9ml of distilled water containing test tube.1ml of solution from 10^{-1} dilution was transferred into flask containing 9ml of distilled water to get dilution of 10^{-2} . Similarly, 1ml of solution was serially transferred from 10^{-2} to 3rd test tube containing 9ml water to get dilution of 10^{-3} spread into three different petriplates. Then Plate count agar medium is poured into petri plates. The Petri plates were then incubated at 37^{o}c for 24hrs.The colonies were taken observed and counted using digital colony counter. The bacteria can thus be isolated and counted by calculating C.F.U. i.e., Colony Forming Unit.

C.F.U. = no of colonies/inoculums size (ml) X dilution factor C.F.U/ml

C. *Gram stain*

The Gram stain is almost always the first step in the preliminary identification of a bacterial organism. A smear was prepared by placing a drop of water on the slide and then transferring microorganism to the drop of water with a sterile cooled loop. It was mixed and spread by means a circular motion of the inoculating loop. Smear was air dried and heat fixed Crystal violet is the primary stain used first and stains all cells purple. Its function is to impart its color to all cells in order to establish a colour contrast. Gram‘s iodine, used as mordent in which this reagent is not only a killing agent, but also serves as a mordant a substance that increases the cells affinity for a stain. It does this by binding to the primary stain, thus forming an insoluble complex. The resultant crystal violet iodine complex serves to intensify the colour of the stain. At this point, all cells will appear purple black. Ethyl alcohol, 95% is used as decolorizing agent in which this reagent serves as a dual function as a protein-dehydrating agent and as a lipid solvent. By using this Gram negative bacteria become colorless. Safranin is used to stain red those cells that have been previously decolourized. Thus safranin is a counter stain since only gram negative cells undergo decolourization, they may now absorb the counterstain. Gram positive cells retain the purple colour of the primary stain and gram negative bacteria appear as pink color.

D. *Culturing of certain species of bacteria and various biochemical tests*

For E-coli, Enterobacter aerogenes, Proteus mirabilis, Serratia species, Staphylococcus, Bacillus species the standardization of medium was prepared in similar manner. First of all the 50 gm soil was mixed up with 450 ml of Butterfield’s Buffered phosphate water and being serially diluted. For finding out E-coli the culture media used is Violate Red Bile Agar (VRBA) on which the diluted sample is poured. After incubation at 35 ^{0}C for 24 hours purple color colony are formed in which it is transferred to Brilliant Green Bile Broth (BGLB). After incubation at 35 ^{0} C gas formation was noted after 24 to 48 hours in order to confirm the E-coli species. Enterobacter aerogenes shows Greenish-metallic sheen in EMB Proteus mirabilis show negative result in EMB and Purple colonies in Carman agar. Serratia species show Creamy-yellow colonies in Carmon agar. Baird-Parker agar is used to Staphylococcus speciesand provided an incubation period of 45 to 48 hours at 35-37°C. Colonies of Staphylococcus aureus are circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an

outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. Coagulase test was conducted in order to identify Staphylococcus aureus and Staphylococcus epidermidis. In case Bacillus species Mannitol Yolk Polymyxin agar (MYP) is used by providing incubation for 18-24 hours at 30°C. Bacillus cereus colonies are usually a pink on MYP with precipitate zone.

For the standardized medium of Klebsiella and Salmonella species, 50 gm of sample taken and mixed up with 450 ml of buffered peptone water. n case of Klebsiella species ,the soil sample was diluted by serial dilution method, followed by spread plate method using blood agar medium. It is also pour plated in Mac conkey agar .Incubated at 37 ^{0} C for 24 hours. Positive result is observed by forming non hemolytic mucoid colonies on blood agar and lactose positive mucoid colonies in Mac conkey agar. For Salmonella species, Xylose Lysine Deoxycholate (XLD) agar is used and provided incubation at 37 ^{0} C for 24 hours. Agar containing Cetrimide has been used successfully to isolate and also to detect the presence of low numbers of Pseudomonas aeruginosa by providing incubation at 35-37°C for 16–48 hours. Colonies are flat and spreading with serrated edges and a metallic sheen. Colonies are surrounded by blue-green pigment.

In order to identify Shigella species, standardized medium is prepared by mixing 25 g sample into 225 ml Shigella broth to which novobiocin (0.5 µg/ml) has been added. Adjust pH, if necessary, to 7.0 ± 0.2 with sterile 1 N NaOH or 1 N HCl. Place flask in anaerobic jar. Incubate jars at 44 deg. C in a forced air incubator for 20 hours. Agitate enrichment culture suspension and streak on a_MacConkey agar plate and incubate for 20 hr at 35°C .Shigella colonies are slightly pink and translucent in nature. After culturing each type of bacteria species, various biochemical tests such as coagulae, catalase, oxidase, citrate tests etc and motility test were carried out.

III. RESULTS AND DISCUSSIONS

A. *Physicochemical analysis of organic waste*

Based on the physicochemical analysis of the five different organic waste samples the result is tabulated in the table 1.The moisture content is maximum for food waste and minimum for compost soil. The pH varies from 4.3 to 8.6.The temperature is much higher for compost soil. The carbon content and nitrogen content is much higher for food waste soil .Cellulose and hemi cellulose content is maximum for compost soil. The maximum lignin content and Starch content is is found in food waste soil. Moisture content, pH, Temperature, play a major role in the increase of the microbiological activity of the compost soil and food waste soil. So before identifying the bacteria, the physical and chemical properties of the soil sample must be analyzed properly.

TABLE I
PHYSICOCHEMICAL ANALYSIS OF ORGANIC WASTE

Sl no.	Physicochemical properties	Compost soil	Food waste soil
1	Moisture (%)	64.08	83.54
2	pH	8.6	4.3
3	Temperature(^{0}C)	34	24.6
4	Carbon (%)	62.5	69.3
5	Nitrogen (%)	0.65	0.76
6	Cellulose (%)	29.4	26.5
7	Hemi cellulose (%)	23.1	19.6
8	Lignin (%)	7.6	28.9
9	Starch (%)	23.9	49.1

B. *Total count of bacteria*

By plate count techniques the colony forming unit per gram of soil sample was found out. The food waste contains maximum amount of bacteria when compared to compost soil. It is due to the presence of optimum physical and chemical parameters. The bacterial count is being tabulated in table II.

TABLE II
TOTAL COUNT OF BACTERIA

Sl no.	Organic waste	Bacterial count (cfu/g)
1	Compost soil	2,10,000
2	Food waste soil	2,13,000

Fig.4. Bacterial isolation in compost soil

Fig.5. Bacterial isolation in food waste soil

C. *Identification of bacteria from organic waste*

The common bacteria, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Shigella sp., Proteus mirabilis, Pseudomonas aeruginosa, Bacillus anthracis, Bacillus subtilis, and S. epidermidis are dominating species in the soil samples collected from compost soil and food wastesoil and it is shown in table III and table IV. The physiochemical properties of soil play an important role in the growth of microorganism. These isolated compost soil bacteria are also used in the seasonal and off season crop production with different environment condition. These microorganisms are to supply nutrients to crop, to encourage plant growth; for example, through the production of plant hormones and to control or inhibit the activity of plant pathogen. The presence of Enterobacter species, Escherichia coli and Klebsiella indicated the possibility of fecal contamination. This could have been enhanced by unhygienic practices and well as poor sanitary conditions .The presence of Staphylococcus aureus must have also been due to poor sanitary condition of the site. The common type of bacteria isolate occurring in soil and various biochemical test to be done is obtained by referring the journal reference no. 4.

The bacterial isolates were presumptively identified by means of morphological examination and some biochemical characterization. E.coli, Salmonella Enterica, Serratia marcescens, B. Cereus, Pseudomonas aeruginosa, B. subtilis, B.megaterium, Staphylococcus aureus are the isolated species from food waste soil. The parameters investigated include colony characteristics, shape, spore, motility, Gram's reaction, catalase production, urease production, Voges- Proskauer (V-P) reaction, Indole production, Nitrate reduction, citrate utilization, carbohydrate metabolism (acid-gas production), starch hydrolysis, hydrolysis, Casein hydrolysis. Isolates from genus Bacillus were differentiated from other isolates on the basis of spore staining, gram staining, catalase, starch hydrolysis and MR test. As all the Bacillus sp. contain thick peptidoglycan, Catalase and amylase enzyme. Some of strains of bacilli were found to be variable for MR tests.
9 strains of bacteria are able to find out from compost soil and 8 strains of species were obtained from food waste soil.

TABLE III
BACTERIA IDENTIFED FROM COMPOST SOIL

Sl no.	Bacteria isolate	Gram stain	Catalase	Oxidase	NR	VP	MR	Indole	Citrate	Urease	Motility
1	**E.coli**	-ve bacilli	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	Motile
2	**Klebsiella pneumoniae**	-ve bacilli	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	Non motile
3	**Enterobacter aerogenes**	-ve, bacilli	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	Motile
4	**Shigella sp.**	-ve rods	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	Non motile
5	**Proteus mirabilis**	-ve, bacilli	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	Motile
6	**Pseudomonas aeruginosa**	-ve, bacilli	+ve	+ ve	+ve	-ve	-ve	– ve	+ve	-ve	Motile
7	**B. subtilis**	+ve, bacilli	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	Motile
8	**Staphylococcus aureus**	+ve , cocci in clusters	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	Non motile
9	**Staphylococcus. epidermidis**	+ve , cocci	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	Non motile

TABLE IV
BACTERIA IDENTIFED FROM FOOD WASTE SOIL

Sl no.	Bacteria isolate	Gram stain	Catalayse	Oxidase	NR	VP	MR	Indole	Citrate	Urease	Motility
1	**E.coli**	-ve bacilli	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	Motile
2	**Salmonella Enterica**	-ve, rod	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	Motile
3	**Serratia marcescens**	-ve rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	Non motile
4	**Pseudomonas aeruginosa**	-ve, bacilli	+ve	+ ve	+ve	-ve	-ve	– ve	+ve	-ve	Motile
5	**B. Cereus**	+ve , bacilli	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	Non motile
6	**B. subtilis**	+ve , bacilli	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	Motile
7	**B.megaterium**	+ve , bacilli	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	Non motile
8	**Staphylococcus aureus**	+ve , cocci in clusters	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	Non motile


Fig.6. E.coli

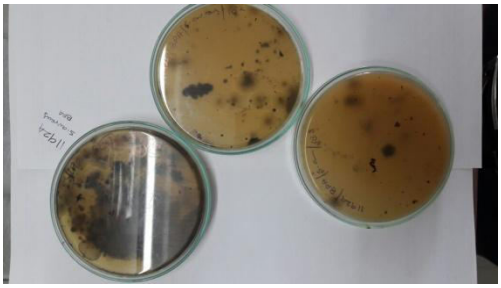
Fig.9. S.aureus

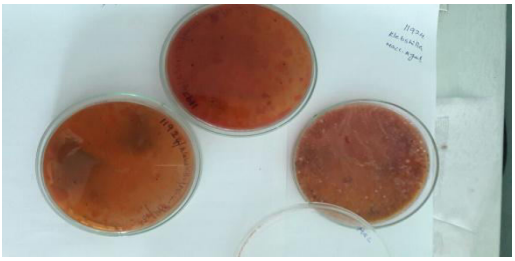
Fig.7.Klebsiella pneumonia

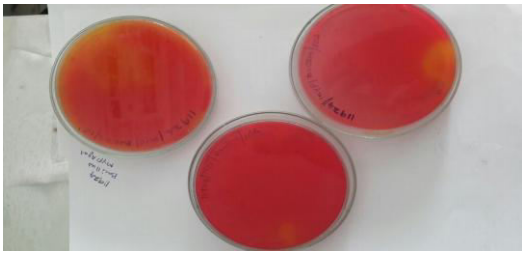
Fig.10. Bacillus cereus

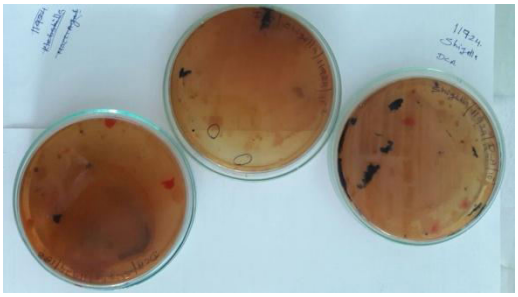
Fig.8. Shigella spp.

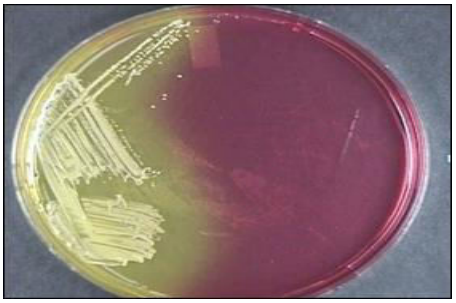
Fig.11. Serratia spp.

Fig.12. Salmonella Enterica

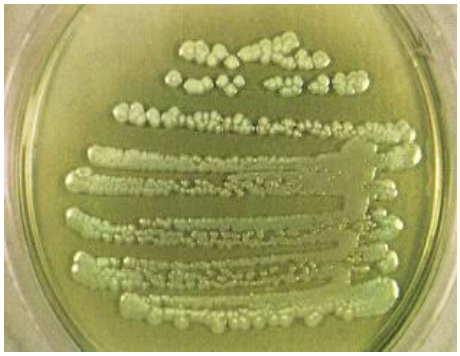

Fig.13. Pseudonomas aeruginosa

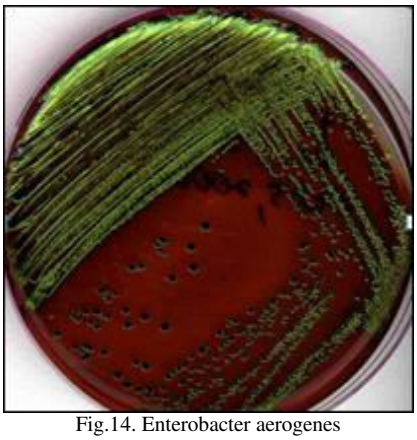

Fig.14. Enterobacter aerogenes

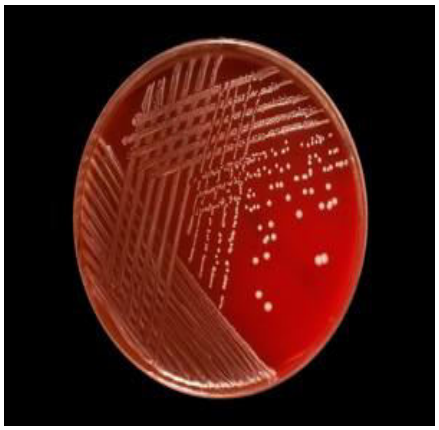

Fig.15. Staphylococcus epidermidis

IV.CONCLUSIONS

The environment where we live is the habitat for various microorganisms; mostly bacteria which are used for various industrial applications like enzymes production, fabric manufacturing, bioremediation, pharmaceutical production, etc. Microorganisms play an important role in composting of organic waste and can be an important contributor to optimal agricultural waste. This study revealed the isolation and identification of diversity of microorganisms which are present in compost and food waste soil habitat. The following are the bacterial isolates from the organic wastes such as E.coli, Klebsilla, Pseudomonas, Bacillus, Micrococcus, Serratia, Enterobactor, Shigella, Proteus spp., Staphylococcus spp., and Salmonella spp. The isolated pathogenic bacteria Klebsilla, can fix N_2 for chick pea plants with soil in different proportion used as inoculants for growth of plants. E. coli has been widely used for recombinant protein production. It introduces a novel expression method in E. coli, the Single Protein Production (SPP) system, in which E. coli is converted into a bioreactor producing only the target protein. Escherichia coli are regarded as a primary choice for the production of biofuels. Here, the microbial productions of liquid biofuels are seemed as the potential to be used either alone or in combination with the present-day fuels. It is identified that successful use of microorganisms for catalysis of biomass into biofuels depends on the organism's ability to produce biofuels in industrial scale at a faster rate and low cost.

The characteristics of Enterobacter aerogenes make it suitable for use in various applications, including biodegradation, bioremediation, water and waste water treatment, biofuel production, biocatalysis and enzyme production. Bacillus species continue to be dominant bacterial workhorses in microbial fermentations. Bacillus subtilis (natto) is the key microbial participant in the ongoing production of the soya-based traditional natto fermentation, and some Bacillus species are on the Food and Drug Administration's GRAS (generally regarded as safe) list. Bacillus strains have also been developed and engineered as industrial producers of nucleotides, the vitamin riboflavin, the flavor agent ribose, and the supplement poly-gamma-glutamic acid.. . Prodigiosin is a red pigment produced as a secondary metabolite by Serratia. The isolation and application of prodigiosin extracted from Serratia marcescens in the colorizations of translucent candles revealed that the pigment prodigiosin can be considered as a possible alternate source of colorant in various industries.

The isolated micro-organisms proteus mirabills could precipitate calcium carbonate. These microorganisms were applied to design self-healing concretes. Broken concrete was treated by a medium culture (MC) containing microorganisms.

REFERENCES

[1] WMF Wan Ishak, S. Jamek, NA Jalanni, NF Mohd Jamaludin "Isolation and Identification of Bacteria from Activated Sludge and Compost for Municipal Solid Waste Treatment System", 2011 International Journal on Biology, Environment and Chemistry IPCBEE vol.24 (2011) © (2011)IACSIT Press, Singapore.

[2] Eze, V.C., Uzoaru, N. and Agwung-Fobellah, D. "Isolation and characterization of microorganisms involved in degradation of sawdust waste in rivers state, Nigeria", Asian Journal of Science and Technology, Vol. 1, Issue 4, pp.044-048, May, 2011.

[3] J. Anitha and Indira A. Jayraaj "Isolation and identification of bacteria from biomedical waste (bmw)", International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 4, Suppl 5, 2012..

[4] Nakuleshwar Dut Jasuja1, Richa Saxena, Subhash Chandra and Suresh C. Joshi " Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan", African journal of microbiology vol.7 (41) pp.4886-4891, 11 October, 2013.

[5] Suganya.S, Judia Harriet Sumathy.V "Isolation and identification of bacteria from covered and uncovered mobile phones" International Journal Of Environmental Sciences Volume 3, No 1, 2012.

[6] Amrita Saha and Subhas Chandra Santra "Isolation and Characterization of Bacteria Isolated from Municipal Solid Waste for Production of Industrial Enzymes and Waste Degradation" Journal of Microbiology & Experimentation 2014, 1(1): 00003.

[7] H. K. Zaved et al"Isolation and characterization of effective bacteria for solid waste degradation for organic manure" Journal of KMITL Sci. Tech. J. Vol. 8 No. 2 Jul. - Dec. 2008.

[8] M. Ram Krishna Rao, Selva Kumar Sivagnanam and Fahmida Banu Syed "Isolation, characterization and identification of predominant microorganisms from agro-waste" Journal of micorbiology Der Pharmacia Lettre, 2016, 8 (5):79-86.

[9]S.Garcha,N.Verma,S.K.Brar"Isolation,characterizationand identification of microorganisms from unorganized dairy sector wastewater and sludge samples and evaluation of their biodegradability" Journal of Water Resources and Industry 16 (2016) 19–28.

[10] Uttiya Deya, Soumendranath Chatterjeeb, Naba Kumar Mondala, "Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation". Journal of Biotechnology Reports 10 (2016) 1–7.

[11] https://en.wikipedia.org/wiki/Bacteria.

[12] https://en.wikipedia.org/wiki/Biochemical_test.

[13] https://en.wikipedia.org/wiki/Culture_media.

[14] https://en.wikipedia.org/wiki/Bacteria_isolation.

Anjali. K. Ullas received B.Tech degree in Civil Engineering from College of Engineering, Trikaripur, Cheemeni in 2015 and is currently pursuing M.Tech in Environmental Engineering from Malabar College of Engineering and Technology.

Anitha.K Assistant Professor in Malabar College of Engineering and Technology, Desamangalam, Thrissur. She took M.Tech from Government Engineering College Thrissur.