



## **Screening and Characterisation of Alkaliphiles from Sewage effluents**

**Ganga. G**

Dept of Biochemistry and Industrial Microbiology

Sree Ayyappa College Eramallikara Chengannur

Email id ;drgangasac@gmail.com

### **ABSTRACT**

*The term alkaliphiles are used for a microorganism that grows optimally or very well at pH values above 9 but cannot grow at the near-neutral pH value of 6.5. Alkaliphiles have made a great impact in industrial applications. Sewages come under alkaline environments. So the study of alkaliphiles has been done by collecting the sewage water sample from Vyttila Kochi. The sample was cultured Isolated and checked for the pH tolerance level varied from 6.5 -11. Twenty-three isolates were able to thrive at a pH level varied from 9-11. Isolated were identified based on morphological and biochemical characteristics. These cultures were further tested for 3 subsequent cultures for their susceptibility to Alkalinity. Then the individual organisms were identified using morphological characters, biochemical tests, and referring to Bergy's manual. Most of the strains obtained were Acinetobacter / Moraxella sp. The organisms identified include Acinetobacter / Moraxella sp, Lactobacillus sp., Enterobacteriaceae sp, Brucella / Bordetella / Bacteroides sp, Staphylococcus, Neisseria / Veillonellasp, Corynebacterium sp. Most of these organism are commonly grown in neutral pH and are turned in to alkyliphiles hence further study is needed to understand its molecular mechanism. Since most of this isolates were opportunistic pathogens the role this alkyliphiles in antibiotic resistance also needs to be carried out.*

**Keywords:** Alkaliphiles, Isolation and Identification, Sewage effluents

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### **INTRODUCTION**

Life in an extreme environment never draws much attention since extreme conditions are always considered to be hostile to support any form of life. Extremophiles are a group of an organism are that well adapted to grow in this habitat due to their Molecular-level adaptation to thrive the extreme temperature, pH, salinity, pressure, etc. Alkaliphile are extremophiles that are very well adapted to thrive in high alkaline conditions. Many Prokaryotic, Eukaryotic organisms as well as some archaeobacteria have come under this category. They often live at high alkaline pH ranging from 9-13. [1-2]. Alkaliphiles are well adapted to thrive in alkaline conditions because of their structural, bioenergetic, metabolic, physiological mechanisms well suited to live in an alkaline habitat. In addition to that extracellular and intracellular enzymatic cellular mechanisms provides them to maintain osmotic balance, as well regulate the growth and reproduction Alkaliphiles have multiple adaptations well suited to maintain their growth in a diverse niche. In an alkaline environment, they play a good role in bio-cycling of carbon, recycling of Nitrogen, and biotransformation of sulfur compounds [3].

Alkaliphiles are always considered to be novel organisms due to their great role in many Industrial Applications. They have wide applications as deodorizers due to their ability to remove foul odors and Hydrogen sulfide gas from the environment. Alkaliphiles are used in. They have a great role in booming and bioleaching applications. These organisms had received great attention in research due to their versatile application in the biotechnology industry. Most of the alkaliphiles reported having biosurfactant activity hence is used in the production of detergents and stain removal application in the laundry. (4-5). Due to its ability to produce antimicrobial, they are extensively exploited in antimicrobial research. (5-9). Alkaliphile have a good adaptation in Sewage and its effluents, thus considered to be a well suited alkaline environment for its growth. [10] In the present study, we have attempted to isolate and characterize alkaliphilic bacteria from a nearby sewage effluent in Ernakulam Dist.

## MATERIAL AND METHODS

### Sample collection:

The sewage water samples for the present study were collected five different sites from vyttilla Kochi. Samples were aseptically collected later pooled together and processed immediately after the collection.

### Isolation of bacteria:

The bacteria were isolated and identified in this study was based on Berge's manual of bacteriology One liter of sewage water sample was collected in a sterile bottle for sample processing. Bacterial samples were isolated from sewage water and were conducted using the quadrant streaking method. A loopful of water sample was streaked in the plates containing Luria Broth (LB) medium with different pH (6.5, 7, 8, 9, 10, 11, and 12) and thereafter incubated for 24 hours. Morphologically different bacteria isolates were sub-cultured into corresponding LB agar having their respective pH. Isolates were further stored at 4°C for future use.

### Identification of bacteria:

#### Morphological identification

Cultural characteristics, Gram staining techniques were the major tests utilized to carry out the morphological identification of bacteria. Gram staining of identification was based on methods (11 -12)

**Spore staining method** :Spore staining was done to determine the spore-forming bacteria. A differential staining technique (Schaeffer-Fulton method) is used to distinguish between the vegetative cells and the endospores (13)

#### Biochemical Characterization of Alkaligenous bacteria

1, Catalase test: The presence of catalase enzyme was observed by using 30% hydrogen peroxide and Peroxidase enzyme production was observed.

2.Oxidase test: The presence of cytochrome, oxidase enzyme was observed by observing the ability of the test organism to oxidase the substrate tetramethyl para-phenylenediamine dihydrochloride forming a colored end product, i.e indophenol.

3. Glucose fermentation test: The ability of an organism to ferment carbohydrate and acid or acid with gas are production was observed. All the isolate from effluent water was tested by this method and was observed for the fermented end-products. Prepare broth media LB Media(Himedia -India). with phenol red and 1%, carbohydrate was taken Equal amount of test organism were aseptically inoculated in to the broth, Alternatively, inoculate each test tube with 1-2 drops of an 18-24 h brain-heart infusion broth culture of the desired organism. Incubate tubes at 35-37°C for 18-24 hours. Longer incubation periods may be required to confirm a negative result.

## RESULTS

### Isolation of bacteria

All the test was carried out and results were observed based Berge's manual of bacteriology.

#### Screening of alkaliphiles

After overnight incubation at 37°C, the colonies were observed and counted. The total number of colonies obtained from Luria Broth is 100 CFU/gm. The organism was allowed to grow at various pH 6.5 -pH12 and the results are shown in figure 1- 4

About 23 different colonies thrived in pH range varies from 9-11 were selected. Twenty three selected organisms were tested for its susceptibility to high alkaline pH by growing in three consecutive subcultures. For these plates were selected and sub cultured into their respective alkaline medium and were kept in the incubator for overnight incubation at 37°C. Colony characteristics and colony morphology, Gram staining, and Biochemical characters were observed from each growth. The result showed that growth pattern morphology and biochemical characters were the same in all the tests. The sub cultured organisms were then identified by biochemical tests. (Figure6 ).

#### Phenotypic test:

On Gram staining, gram-positive and gram-negative bacteria were identified from the sub-cultured bacteria. Gram-negative bacteria were more than gram-positive ones. About 18 gram-negative and 5 gram-positive (Figure 6) bacteria were identified.

#### Spore forming test;

The majority of organisms were obtained in the study were non-spore formers except the members of Firmicutes

#### Biochemical test:

From the isolated organisms 16 were oxidase-positive and 7 were oxidase negative, 16 strains were positive to catalase test, and glucose fermentation test was done in which 22 were glucose-fermenting. The results of alkaliphiles.

Total twenty three alkilophiles were screened and identified based on Morphological staining and biochemical characterisation three major phyla of bacteria were identified and their percentage of distribution is given in figure 7.

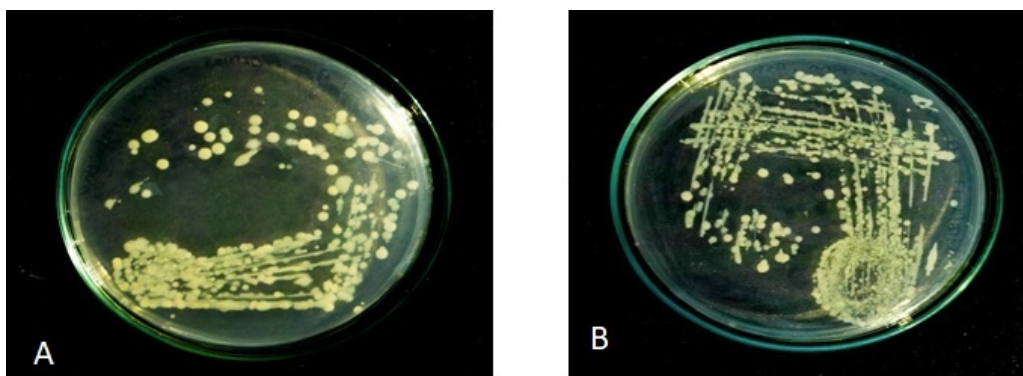


Figure 1 Organism grown at normal ( pH 7) plate A and Plate B

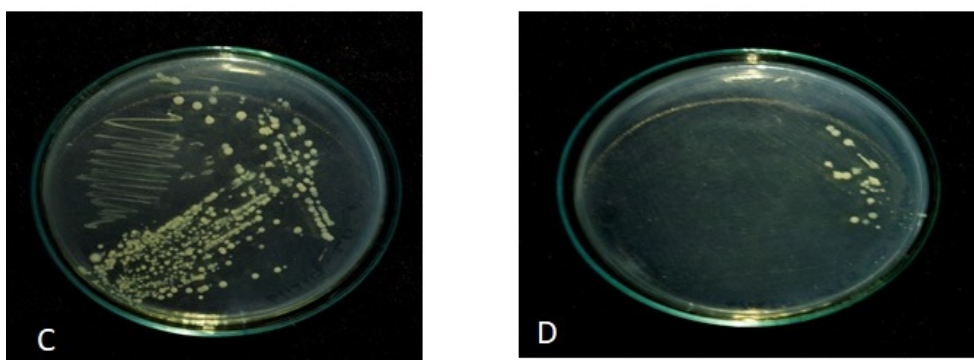


Figure 2 Organism grown at alkaline ( pH 9) plate C and Plate D

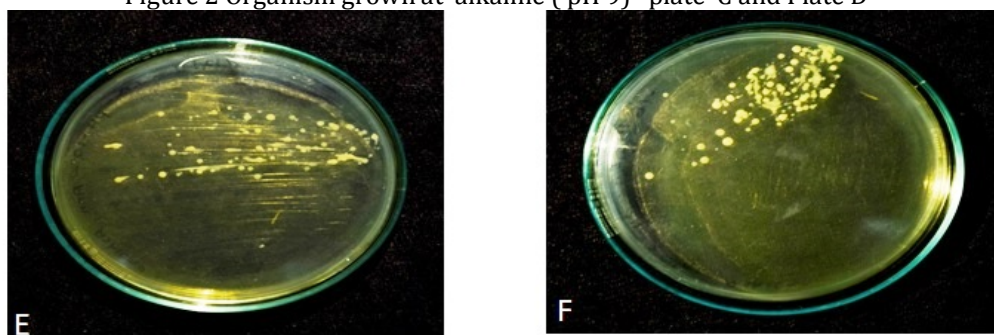


Figure 3 Organism grown alkaline ( pH 10) plate E and Plate F

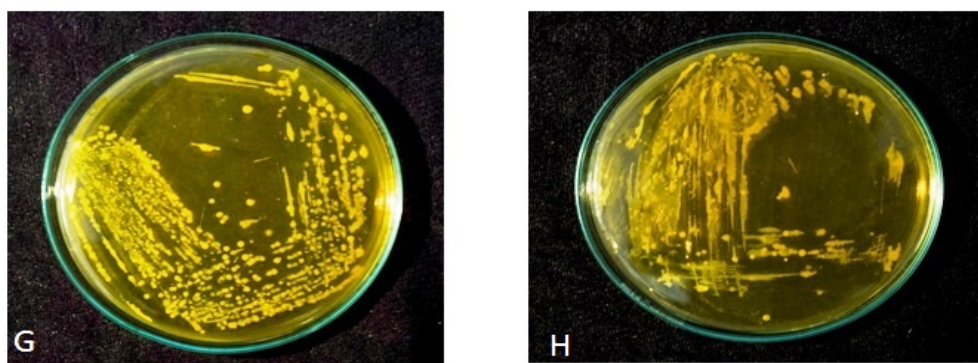


Figure 4 Organism grown at alkaline ( pH 11) plate G and Plate H



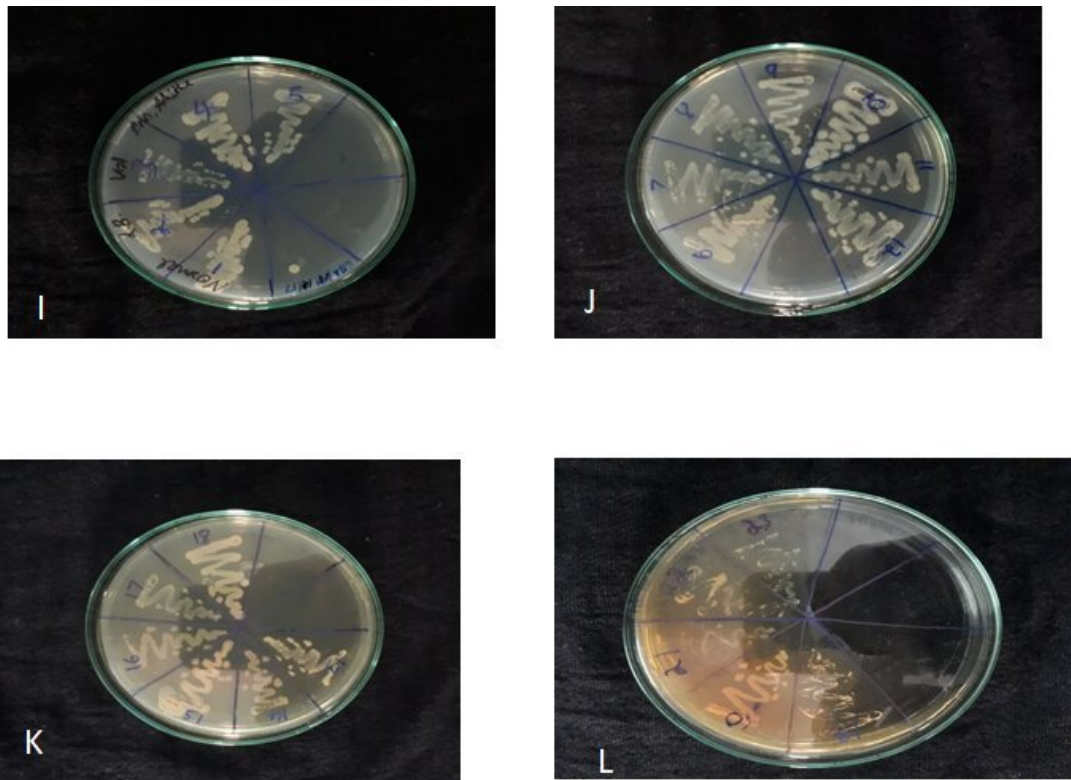


Figure 5 Selection of Alkaliphilic organism based on various pH conditions  
Plate I (pH7), plate J (pH9) plate K (pH10) plate L(pH11)

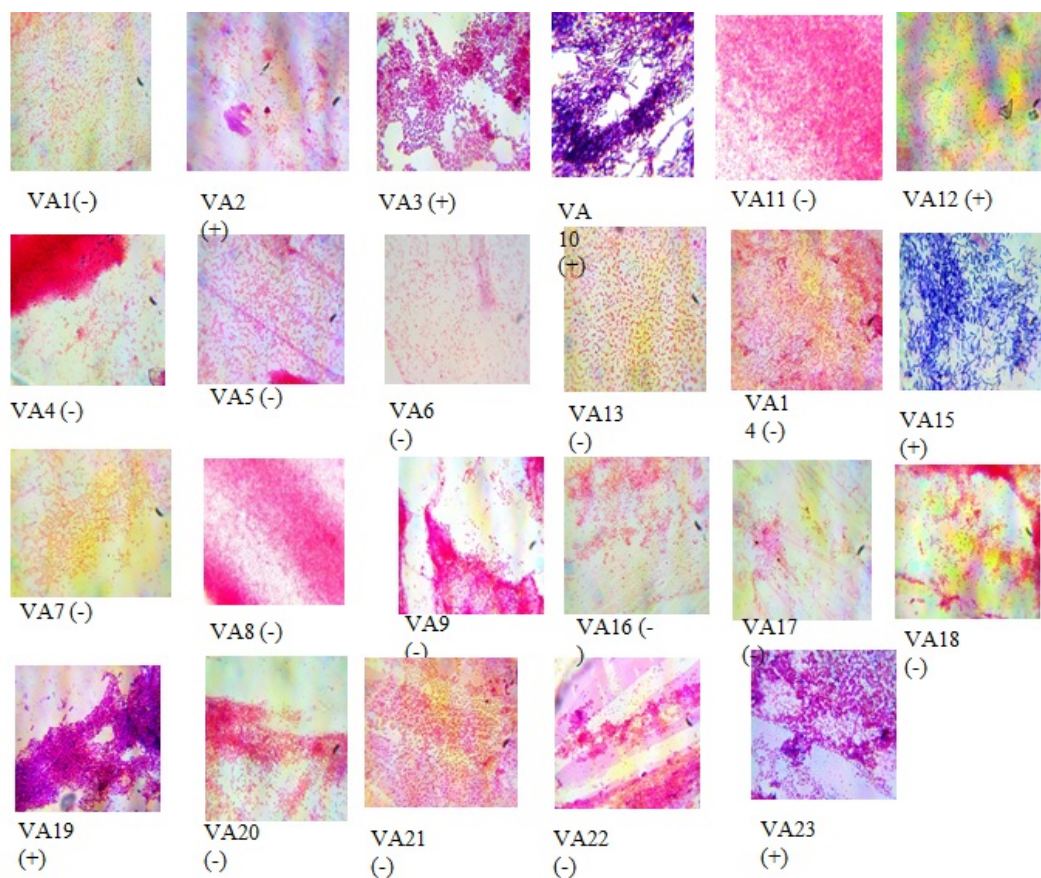


Figure 6 : Phenotyping of 23 Alkaliphilic isolates (VA1-VA23) Gram positive (+) Gram Negative (-)

## Alkaliphiles Isolates

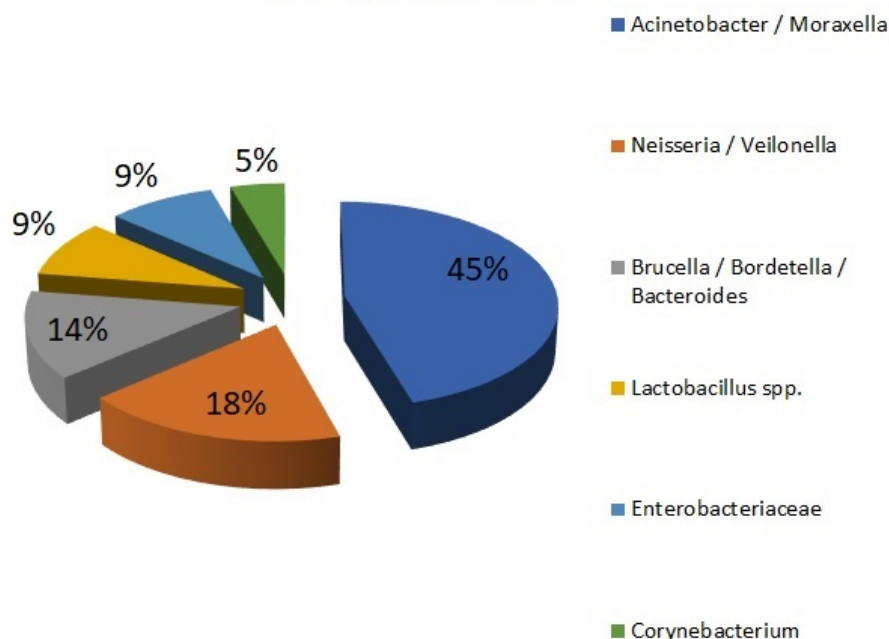


Figure 7: Percentage of distribution of Alkaliphiles isolated from sewage effluents

### DISCUSSION

The sewage water samples were collected from *vyttla Kochi*. Morphologically different colonies of twenty three alkaliphiles were isolated and characterized. All twenty three isolates were grown in alkaline pH up to range of pH11. Bacterial isolates from sewage water samples represent three different phyla such as *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. Of these *Proteobacteria* was the most dominant with 82%. The genus reported were *Acinetobacter*, *Corynebacterium*, *Brucella*, *Lactobacilli*, *Enterobacteriaceae*, *Neisseria*, and *Staphylococcus*. Among which *Acinetobacter* was found to be the most dominant one showing about 45%. The majority of the bacteria are gram-negative and there identified as *Acinetobacter* and *Neisseria*. Alkalitolerant microbes have optimal growth at neutral pH but can grow at pH 10-pH 11. Twenty-three isolates obtained in this study are alkaline tolerant, though they don't belong to the typical alkaliphilic classification the organisms could have started to adopt the alkaline environment. Horikoshi, [4] explained that one of the key features in the adaptation of microorganisms in an alkaline environment is associated with the cell surface, which discriminates and maintains the intracellular neutral environment separate from the extracellular alkaline environment. Irwin [3] reported various prospects of alkaliphiles for the isolation of industrially important for food and industry. The isolate obtained in this study are can be are beneficial in food and industrial application. *Lactobacillus* species are used as starter cultures in industries for controlled fermentation in the production of yogurt, beer, cheese, etc.

Some of the organisms obtained in the study were either pathogens or opportunistic pathogens. Among these, *Acinetobacter* causes diseases, ranging from pneumonia to serious blood infections. *Staphylococcus* can cause food poisoning, cellulitis, impetigo, and toxic shock syndrome. *Brucella* / *Bordetella* cause diseases like brucellosis. *Enterobacteriaceae* is a pathogenic organism that causes various infections including bacteremia, respiratory tract infection, skin, and soft tissue infection, etc. *Neisseria* cause diseases like meningitis and other forms of meningococemia. *Corynebacterium* causes diseases like diphtheria. From this study of alkaliphiles from sewage water, it has been clear that sewages in our localities are turning into a bank of pathogens which can lead to serious health problems for mankind. Interestingly in this study, the occurrence of organism capable to grown at neutral pH had changed in to alkaliphiles. Adaptation mechanism that occurred from neutral to alkaline pH and enzyme involved in the reaction needs to be studied. Since most of the organism observed in this study are opportunistic pathogens and sewages are always prone to be mixed with antibiotics from different sources, further study is needed to understand the role of alkaliphiles in antibiotic resistance and Multiple drug resistance (MDR).

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