Screening and identification of bacteria from various historical and archaeological areas in Vellore and Chennai for pharma benefits

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ABSTRACT

Bacteria are cosmopolitan and grow in any type of environment. They are present everywhere and are usually found on the walls of houses. Among a large pool of bacteria only some are able to survive based on the toxic substances produced by their sporulated colonies. The objective of this project is to screen out and identify the predominant bacterial species present in the samples collected from the walls of old buildings. A significant amount of gram positive bacteria can be seen in the entire wall samples chiseled out from very old buildings and houses. Simple growing of bacterial colonies by pour plate technique and then Grams staining, Catalase, Coagulase and Heamolytic test, IMVIC tests of those colonies confirms the presence of a bacterial species similar to *Streptococcus,* tentative screening, staining and biochemical identification will be discussed.

KEY WORDS: Historical, Bacteria, Archeological Area, Pharma Benefits, Catalase, Coagulase, Grams staining, Heamolytic.

1. INTRODUCTION

Bacteria are the most important part of our life and they are present everywhere. The bacteria can multiply anywhere and their growth depends on various environmental factors. (Welton, 1972), Bacteria may be surviving in the walls of building as vegetative mode or spore status and can be used as indicators to determine the age of building, or construction strategies.

The bacterial colonies can be located in crevices or on walls of buildings along with fungal colonies. The growth of these bacterial colonies depends on various factors such as dampness (Gili Regev-Yochay, 2006), humidity, moisture content, sunlight etc. It is seen that as the buildings become old the walls tend to become damper and hence favour the growth of bacterial colonies on them (Chester and Moskowitz, 1987; Rajkowska, 2014). A lot of bacteria are present in the surrounding but only a few are able to survive for a long duration on the walls of old buildings. So our aim is to screen and identify the prime species present on the walls (Graeme, 2006). These bacterial species cause specific diseases also which can be averted once the causative species are identified for pharmacological benefits (Anderson, 1997). In this potential field research is rudimentary. This is the first attempt in India to study the microbial presence in old monuments, archaeological evidence monuments and temples to determine the age and building materials.

2. MATERIALS AND METHODS

Collection of samples: Vellore Fort, A Temple in Chennai, A housing colony in Chennai.

Protocol:

Collection: The samples were collected in sterilized Eppendorf tubes using fork and sealed aseptically (Yantian Ma, 2015). Four strains of bacterial colonies were screened and two potential strains were tentatively studied by performing staining, morphological and biochemical tests.

Observation of Bacteria: To observe the bacteria by simple pour plate technique, a solid agar media was prepared by taking 150 mL water in a conical flask. A suitable amount of nutrient agar in the flask with agar powder to solidify the contents in the flask. The media and the Petri dishes were sterilized. Two drops of anti- fungal agent was added into conical flask to prevent the growth of fungi. After this the plates were formed by taking 1.5 gm from each sample into the Petri dishes and adding media on top of the sample.

Slant Tube Culture- Preservation of Bacteria: This technique is used to preserve the colonies of the sample for a longer time. The agar media was prepared and poured in the test tubes. The test tubes were slanted at 75^0- 80^0 angles till the solidification of agar media. The sample was inoculated on the slant surface of tubes and they were incubated for 24 hours.

For detection of the type of bacteria present certain biochemical tests indole, citrate, TSI (Total Sugar Iron), methyl red, Vogus proskauer test (IMVIC), Hi media KIT tests- orinithine utilization, urease activity, phenyl alanine activity, nitrate reduction, H_2S production, glucose utilisation, adonitol utilisation, lactose utilization, arabinose utilization, sorbitol utilization and lysine utilisation were performed.

Grams Staining: Gram staining is a technique used to draw a distinction between two large groups of bacteria based on their dissimilar cell wall constituents. In this technique cell walls of Gram-positive bacteria retains crystal violet due to presence of a thick peptidoglycan layer and hence colonies appear violet. The Gram negative bacterium

colonies appear red as it retains the saffranine dye in its peptidoglycan layer as it loses its crystal violet stained outer cell wall in ethanol wash. The grams staining was performed and the results were positive (fig.2.1-2.4).

Catalase Test: The enzyme catalase is produced to neutralise the bactericidal effects of toxic oxygen metabolites like H_2O_2 by microorganisms living in aerobic environments to protect them. The Catalase enzyme plays a major role in mediating the splitting of H_2O_2 into oxygen and water. Hence to ascertain the bacterial cell isolated from sample as aerobic or anaerobic (capable to produce catalase enzyme), a small inoculum of sample is mixed with hydrogen peroxide solution (3%). This causes rapid splitting of hydrogen peroxide into water and oxygen which is evident from evolution of oxygen bubbles if the bacterium is aerobic. The anaerobic bacterium lacks this enzyme and thus lack of bubbles is an evidence of sample containing the anaerobic bacteria.

Catalase-positive bacteria can be strict aerobe or a facultative anaerobe. They all utilize oxygen as a terminal electron acceptor in respiration. The bacteria showing negative test may be anaerobe or a facultative anaerobe that only ferment and do not utilize oxygen as a terminal electron acceptor in respiration (i.e. *streptococci*). The Catalase test was done to assure the presence of facultative anaerobes in the samples (fig.5.1).

Coagulase Test: Coagulase test is used to recognize different *Staphylococcus* sp. based on the production of Coagulase enzyme by them. Coagulase is an enzyme produced by Coagulase-positive bacterium (*S. aureus*) that cross links α and β chain of fibrinogen present in plasma and converts it into fibrin clot. As a result of this conversion clumping takes place as individual cocci sticks together. The clumping is evident from the milky (increase in turbidity) appearance in sample if it is positive (Kloos and Schleifer, 1975). A sample of Coagulase- negative *Staphylococcus* does not give any milky appearance on inoculation of sample. The Coagulase test was performed to detect the presence of *Staphylococcus* sp. in the samples (fig.5.2).

Haemolytic Test: The haemolytic reaction is useful in differentiation of the *Streptococci*. An agar media containing 5%v/v of animal blood is prepared by mixing blood agar powder commonly containing trypticase soy agar (TSA) as base medium with blood. The purpose of using trypticase soy agar is that it favours the growth of all bacteria. Other base media may be substituted if control strains of specific genera are to be studied. The haemolysis is of three types- alpha, beta and gamma. A complete clearance around the colony is the evidence of β- haemolysis (Layer, 2006). A partial clearing of green colour is interpreted as α- haemolysis. No change in media suggests γ- haemolysis.

Blood agar solution was prepared by adding suitable amount of blood agar powder in distilled water. This solution was then sterilized and 5%v/v blood was put into it allowing the media to solidify. Afterwards the plates were streaked with samples and incubated for 24 hours at 35^0C (fig.3.1-3.5).

3. RESULTS & DISCUSSION

Several tests were performed and following results were obtained:-Staining, haemolytic test on blood agar plate, catalase and coagulase test show that the microorganism is Gram positive, catalase, coagulase negative but starin 4 is positive and Heamolytic test Alpha haemolysis by strain 1 and beta lysis by all strains.

Table.1. Identification Tests

Name of the test	Strain I	Strain II	Strain III	Strain IV
Grams' staining	Gram positive	Positive	Positive	Positive
Catalase test	Negative	Negative	Negative	Positive
Coagulase test	Negative	Negative	Negative	Positive
Heamolytic test	Alpha	Beta	Beta	Beta

Table.2. Gram negative kit test (biochemical tests for identification)

Test	Colour Change	Strain 1	Strain 4
Citrate Utilization	Green to Blue	+ve	+ve
Lysine Utilization	Olive green to light purple/dark purple	-ve	-ve
Ornithine Utilization	Olive green to light purple/dark purple	+ve	+ve
Urease Activity	Orangish yellow to pink	+ve	+ve
Phenylalanine Activity	Colourless to green	+ve	-ve
Nitrate Reduction	Colourless to pinkish red	-ve	-ve
H_2S Production	Orangish yellow to black	+ve	+ve
Glucose Utilization	Pinkish red/red to yellow	-ve	+ve
Adonitol Utilization	Pinkish red/red to yellow	-ve	-ve
Lactose Utilization	Pinkish red/red to yellow	-ve	-ve
Arabinose Utilization	Pinkish red/red to yellow	-ve	+ve
Sorbitol Utilization	Pinkish red/red to yellow	-ve	-ve

Table.3. IMVIC tests

Test	Colour Change	Strain 1	Strain 2	Strain 3	Strain 4
Citrate	Green to blue	+ve	+ve	+ve	+ve
Methyl Red	Yellow to red	-ve	-ve	-ve	-ve
TSI(Total Sugar Iron)	Pink to Red	+ve	+ve	+ve	+ve
Voges Proskauer	Yellow to pink	+ve	+ve	+ve	+ve
Indole	Formation of a ring at the top	+ve	+ve	+ve	+ve

The Grams' staining test confirms that bacteria present are gram positive predominantly (fig.2.1-2.4, table.1).

Most of the samples show negative Catalase test and Coagulase test which is done exclusively for anaerobic bacteria and supports presence of Streptococcus (fig.5.1 & 5.2, table.1).

The samples showing positive support the presence of species similar to Streptococcus.

The haemolytic test which is done exclusively for streptococcus species shows β-haemolysis supporting presence of it (fig.3.1-3.5, table 1).

Further the positive kit test for strain -1 and strain- 4 (fig.4, table.2)

Figure.1.1. Vellore fort sample   **Figure.1.2. Chennai railwaystation**   **Figure.1.3. Vellore fort**
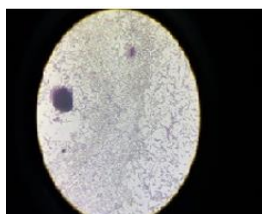

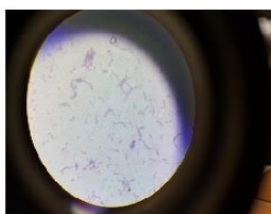**Figure.1.6 Autoclaving**   **Figure.1.5. Autoclaving of sample**   **Figure.1.4.   Preparing agar media**
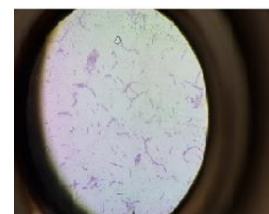
Collection of samples and sterilization

Sample 2.1   **Sample 2.2**   **Sample 2.3**   **Sample 2.4**

Grams' staining

Figure.3.1. Control   **Figure.3.2. Beta haemolysis**   **Figure.3.3. Beta haemolysis**
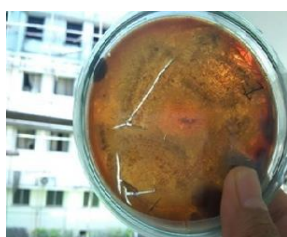

Figure.3.4. Alpha Haemolysis   **Figure.3.5. Test plates for haemolytic test for Streptococcus species**
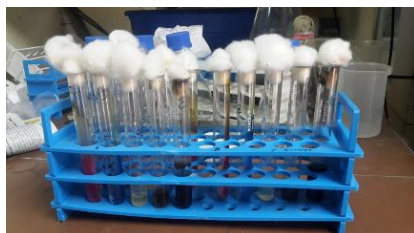
Figure.4. Biochemical kit tests

Figure.5. IMVIC tests

Figure.5.1. Catalase test

Figure.5.2. Coagulase tests

4. CONCLUSION

The results obtained shows the presence of species similar to *Streptococcus* in structure. The other test results confirm their similarity. Earlier research has reported the presence of *lactobacillus, staphylococcus* and *clostridium* which also supports our results.

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