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Identification of Predominant Bacteria present in Skin that causes Malodour in Garments when combined with Sweat

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Abstract: ***Sweat or perspiration is characterized as the moisture exuded from sweat glands through the pores of the human skin. It varies from person to person based on a number of factors that includes age, sex, food habits, temperature, occlusion and humidity [1]. About 25% of the water is given off by the body under normal sedentary conditions[2]. Textiles used as clothing are constantly in contact with not only the microbes present in the environment but also the microorganisms present on the skin surface. When these organisms come in contact with perspiration, it is broken down into acids. Sweat by itself does not contain any odour, but when broken down into acids and due to the rapid multiplication of bacteria in sweat, it produces an obnoxious odour. When collected, sweat is said to be an colourless liquid, salty in taste, and having a rancid smell [3]. Odours produced from the human body is unhealthy and unhygienic, and can be viewed as an embarrassing problem by the society. Previous studies have demonstrated that the ecology of microbes present in human skin is highly complex, and it varies between individuals [4]. In order to address the problem of malodour caused due to sweat, it is imperative to identify the microorganisms present in the sweat. This investigation aims at identification of bacterial flora in axillary sweat using the 16S rRNA sequencing protocol by collecting sweat samples from healthy individuals.***
Keywords: Perspiration, microorganisms, predominant bacteria, malodour, quantitative and qualitative analysis.

I. INTRODUCTION

Textiles used in clothing such as apparel, inner clothing, socks and stockings that are in close contact with skin provide an ideal environment for the attachment of bacteria present on human skin. It is transferred either by direct contact or through sweat. There are 3 million to 4 million sweat glands present in the human body [1]. Sweat by itself does not contain any bacteria. Bacteria thrives in warm and moist conditions provided by the skin. The unpleasant smell is due to the rapid multiplication of bacteria breaking down sweat into acids and they feed on it. Thioalcohols are formed when sweat is broken down by acids which has a smell equal to onion, sulphur or meat [5]. Body odours are an easily understandable marker of hygiene. In all forms of human malodour formation, bacteria plays a central role. Malodours thus are a comprehensible sign of microbial action, and the strong focus of modern society on hygiene makes malodours ever more objectionable [7]. Additionally, microbes can lead to multiple problems including textile discoloration, stains and fiber damage, unpleasant odour, and a slick and slimy feel on the skin [6].

II. METHOD AND MATERIALS

A. Quantitative Analysis

Sweat samples were collected from human subjects belonging to the City of Bangalore, Karnataka, which enjoys a tropical climate. The mean age of the subjects was between 20-40 years; who were all in good health, and who did not consume any medication or antibiotics for at least 1 month immediately preceding the study. They were told not to use deodorants, talcum or moisturizing lotions for at least three days before the sweat sampling. They were instructed to conducted their normal routine duties for the day. Subaxillary sweat was collected from 50 subjects (25 women and 25 men) after sewing a square linen swatch measuring 5 x 5 cm (scoured and sterilized) on the armscye of their blouses and shirts. The sweat soaked linen swatches were collected in sterile 10 mL saline test tubes until it was transported to the laboratory for bacterial identification and quantitative analysis. The sweat swatches were pooled together for bacterial enumeration. About 5 replicates were performed. Dilutions of 10^6 were carried out by suspending the samples x1 in Phosphate buffered saline (PBS). Nutrient Agar plates were incubated for 24 hours at 37^0C. The volume plate was 0.1 ml. Colony forming units are single bacteria or clumps of bacteria which are able to form colonies on the medium used. The CFUs were determined using the following formula.

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$$\text{CFU} = \frac{\text{No. of .colonies x Dilution factor}}{\text{Volume plated (ml)}}$$

Colony forming units (CFU) were calculated by counting the number of viable clonogenic cell expressed as CFU/mL. They depict the number of cells that are viable to proliferate and can form colonies. The number of colonies were comparatively less in females samples compared to male samples. From the Plate 1 and Table 1, it can be seen that the CFUs differ in males and females significantly.

Table 1 - Colony forming units in replicates of 5

S.No.	Male sweat samples	Female sweat samples
1.	$7.1\pm 0.01 \times 10^6$	$4.1 \pm 0.01 \times 10^6$
2.	$5.2 \pm 0.11 \times 10^6$	$3.5 \pm 0.11 \times 10^6$
3.	$4.5 \pm 0.03 \times 10^6$	$4.3 \pm 0.04 \times 10^6$
4.	$6.4 \pm 0.04 \times 10^6$	$6.3 \pm 0.04 \times 10^6$
5.	$10 \pm 0.02 \times 10^6$	$3.6 \pm 0.03 \times 10^6$

Plate 1 - Colony forming untis (Males)

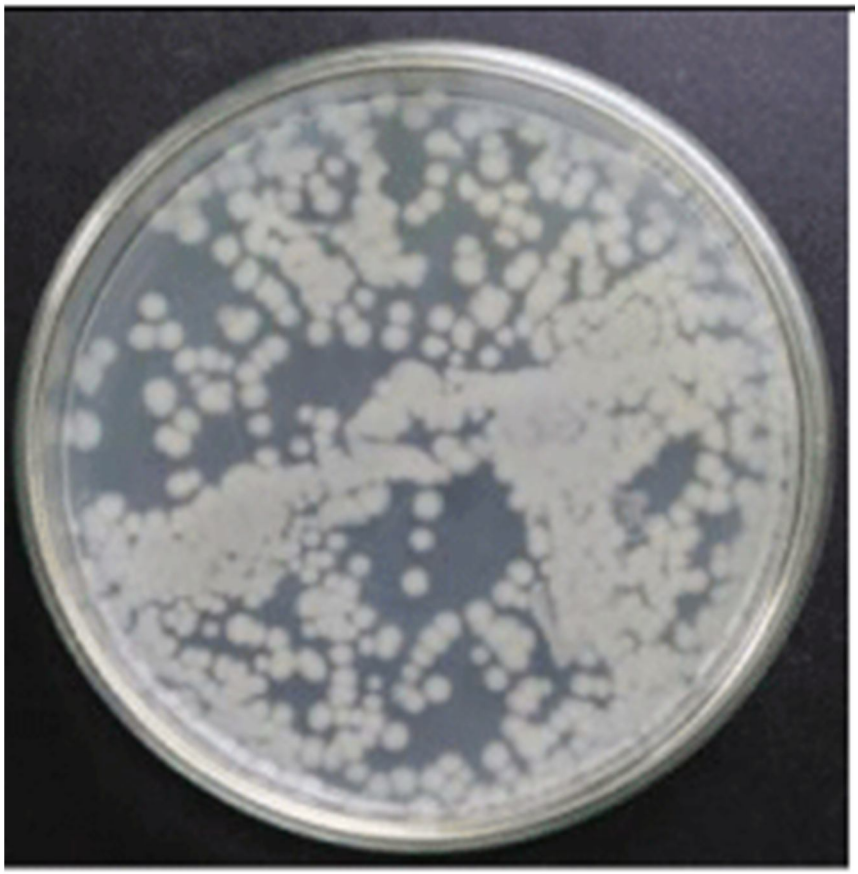
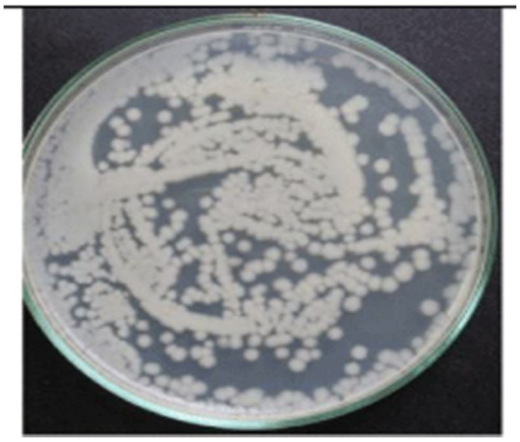


Plate 2 - Colony forming units (Females)



B. Qualitative Analysis

Bacterial Identification based on 16S rRNA Gene Sequencing:

After quantitative analysis of the sweat, about 10 colonies were inoculated into 10 different 5 ml LB broth tubes and incubated at 37^0C overnight.

↓

About 1.5 mL of each culture was used for genomic culture.

↓

The culture was kept overnight, and about 1.5 mL was pelleted at 6000 rpm for 5 minutes.

↓

Supernatant was discarded and pellet was suspended.

↓

To which 500µl of Bacterial cell lysis buffer (Guanidium isothiocyanate, SDS, Tris-EDTA), was added and mixed by inverting the vial for 5 minutes. The cells which did not get lysed were further treated with additional detergent and incubated for 10 minutes with gentle mixing till the suspension looked almost transparent.

↓

Equal volume of 100% isopropanol was layered on top of this solution. The two layers were mixed gently till white strands of DNA were seen and until the solution became homogenous.

↓

The strands of DNA were spooled with the help of a pipette tip and transferred into a fresh vial.

↓

About 300 µl of 70% ethanol was added to the spooled DNA.

↓

The spooled DNA was spun to precipitate DNA at 10,000 rpm for 10 minutes.

↓

The supernatant was discarded. The pellet was air dried (without allowing it to dry completely).

↓

About 30 µl of 1X TE (Tris EDTA buffer) was added and the pellet was suspended (incubated for 5 min at 55–60^0C to increase the solubility of genomic DNA).

↓

About 5 µl of the freshly extracted DNA along with 3 µl of gel loading dye was loaded onto the 1% agarose gel and subjected to electrophoresis.

↓

Agarose Gel Electrophoresis was conducted according to protocol. The following bacteria was isolated and identified from human sweat collectively from males and females.

III. RESULTS AND DISCUSSION

Conducted 16S rRNA sequencing to identify the bacterial organisms. About 10 colonies having characteristically distinct morphological features were selected from the plates. The bacteria were differentiated based on their shape, cell wall, and genetic makeup. The shapes ranged from rod-shaped, spiral and spheres. The bacteria identified from the sweat assay are given in the following Table 2. The identified bacteria belonged to the bacillus, staphylococcus, Pseudomonas, Acinetobacter and Exiguobacterium species. There were both Gram-positive and Gram-negative stains. They are numbered from sample 1 to sample 10.

Table 2 - Predominant Bacteria identified from Male and Female Sweat

S.No	Sample	Bacteria Identified	Gram stain
1.	Bacteria 1	Bacillus tequilensis	Gram-positive
2.	Bacteria 2	Enterobacter kobei	Gram-negative
3.	Bacteria 3	Pseudomonas stutzeri	Gram-negative
4.	Bacteria 4	Bacillus cereus 1	Gram-positive
5.	Bacteria 5	Staphylococcus hominis 1	Gram-positive
6.	Bacteria 6	Staphylococcus hominis 2	Gram-positive
7.	Bacteria 7	Bacillus cereus 2	Gram-positive
8.	Bacteria 8	Acinetobacter calcoaceticus	Gram-negative
9.	Bacteria 9	Bacillus safensis	Gram-positive
10.	Bacteria 10	Exiguobacterium profundum	Gram-positive

IV. SUMMARY AND CONCLUSION

From the study conducted it can be seen that the flora present in males and females were markedly different, higher in males than in the female sweat samples. This only proves that that men and women sweat at different rates given the same climatic conditions and average physical work [8]. Several studies have evidenced that men and women sweat at different rates due to their reproductive hormones testosterone in males and estrogen in females [9]. Studies done by Harker et al by spectra data set demonstrates physiological variability in number of metabolites and concentrations present in males and females sweat samples [10].

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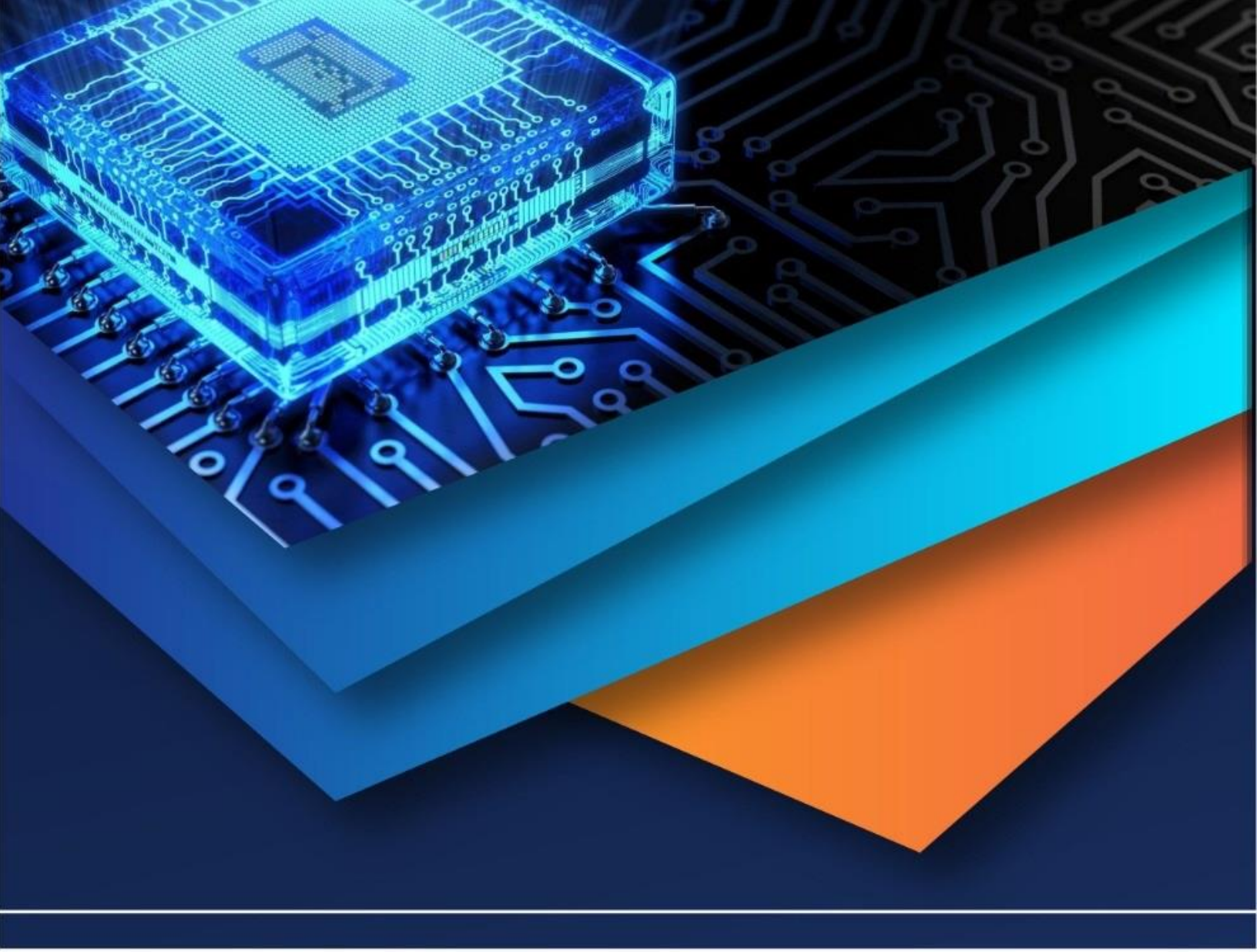

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