# Detection of *Propionibacterium* in Samples by Metagenomic Analysis that Collected from Patients Suffering Acne Vulgaris in Babylon Province

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## Abstract

By the metagenomic analysis detected the microorganisms in acne disease. The Taxonomic assignment of the sequences determined classified them into diverse microorganisms. In present study the microorganisms were bacteria (95.29%), eukaryote (4.23%), viruses (0.29%), archaea (0.04%), other sequences(0.15%) and unclassified sequences (0.00)these results determined for PA1. similarly, the PA2 was contains bacteria (96.52%), eukaryote (3.15%), viruses (0.18%), archaea (0.05%), other sequences(0.08%) and unclassified sequences (0.00). The identified and taxonomic of the bacteria that apparent high presenting 95.29% and 96.52% for each PA1and PA2 respectively. According this results taxonomic of bacteria to genus present to detected the important genus was infection acne. The PA1 sample was similarity with PA2. The genus bacteria for (PA1 and PA2) were *Corynebacterium* (34.67%, 40.32%), *Propionibacterium* (32.23%, 24.04%), *Staphylococcus* (24.54%, 24.18%), *Malassezia* (1.13%, 0.86%), *Bacillus* (0.92%, 1.99%), *Pseudomonas* (0.54%, 1.17%), *Escherichia* (0.36%, 0.52%), *Acinetobacter* (0.33%, 0.58%), unclassified (0.32%, 0.20%), *Stenotrophomonas* (0.31%, 0.51%), *Meiothermus* (0.30%, 0.36%) and *Enterococcus*(0.17%, 0.45%) whereas the PA2 have 0.18% of *Streptococcus* but the PA1 not appearance *Streptococcu.*

***Keywords:*** *Babylon Province, Propionibacterium acnes, the metagenomic analysis, acne vulgaris.*

## Introduction

*Propionibacterium acnes* is a nonspore- forming, gram- positive, anaerobic, pleomorphic rod whose end products of fermentation include propionic acid(1). *P.acnes*is considered an opportunistic pathogen, causing a range of infections as well as being associated with a number of inflammatory conditions. Itis primarily recognized for its role in acne vulgaris where it is thought to contribute to the inflammatory phase of the condition(2).

*Propionibacterium acnes*, which are a normal inhabitant of the skin, produce fatty acids that inhibit the growth of fungi on the skin.(3). However, when it becomes trapped inside the hair follicle, it may grow and cause inflammation and acne infection(4).

Metagenomic functional analysis revealed that same of chemical pollutant that effected the structure and function of microbial community(5). Metagenomic analysis was applied to illustrate the metabolic potential of microbial consortium for the degradation of polluted soil, and this could provide additional information of function conducted by un-culturing bacteria(6). The field of metagenomic developed as a consequence of the diversity that prokaryotic diversity was much greater than previously realized and that the prokaryotic population.(7)

The classification method of metagenomics sequencing data can be divided into two categories according to different sample data processed method: one is based on the sequence of marker genes such as 16SrRNA (marker gene metagenomic) and other is based on whole-genome sequencing fragment (shotgun-sequences metagenomic)(8).

## Materials and Method

**Samples Collection:** 2 samples (Name these two samples PA1 & PA2) from two patients were subjected for sampling which include both skin sites (comedown) for the sampling was forehead from both sexes and the age of patient was 17 and 18 years. These patients were diagnosed by dermatology physician, according to the signs and symptoms,, in addition to be having risk factors that were determined by the information about patients . In this study, patients with recent usage of local antibiotic treatment and usage cosmetic material were excluded from sampling.

**DNA Extraction for Gram positive Bacteria:** DNA extraction was carried out according to the genomic DNA purification kitsupplemented by manufactured company (Gene aid, UK).

**Whole Genome Sequencing (WGS) and Analysis:** 2 samples of DNA isolated from patients with acne were selected for whole metagenome sequencing based on the next-generation sequencing technique. After extraction of Genomic DNA, the extracted DNA subjected to quantification by Nano Drop instrument to estimate the DNA concentration according to manufacturing's instructions. In addition, the condition of the DNA was assessed by gel electrophoresis method to evaluate the presence or absence of DNA in the sample, where 1µl of DNA loaded to 1% agarose gel and run at 160V for 30min. Following this step, only successful samples were submitted to Macrogen company (Korea) for whole metagenome sequencing (Paired-ends) using the Illumina NovaSeq 6000 platform. The resulted raw reads were processed by further bioinformatics tools.

The raw data were analyzed by several bioinformatics tools. All bioinformatic approaches which used to analyze the study sequences were depend on either using command-line tools and bioinformatics softwires on open-source operating system, Linux (Version: Ubuntu 18.04.3 LTS, Canonical Ltd., UK), Windows-based program (CLC Genomics Workbench version 20.0.3) or using web-based servers such as the Galaxy platform (https://galaxyproject.org)(9). Before WGS analysis, the raw data undergo quality control by FastQC (Version, 0.11.5) (10) to evaluate quality of reads and calculating the basic statistics (such as total number of bases, reads and GC content). After quality control, raw reads were subjected to preprocessing steps to reduce biases in analysis by trimming out bases of low quality, adapter sequences, the Poly-G tail and human DNA. Trimmomatic (version 0.36) tools (11) and CLC Mapper (CLC Genomics Workbench version 20.0.3). The filtered raw data have undergone for further processing steps summarized with its tools and references in Table (1).

**Table (1): The Bioinformatics tools or programs which used for analyze the filtered raw data**

| Processing type | Tool/Programs | Ref. |
|---|---|---|
| Assembly | CLC *de novo* assembly | (12) |
| Visualizing the *de novo* assembly | Bandage (v0.8.1) | (13) |
| Ordering contigs | Mauve (v2.4.0) | (14) |
| Taxonomy classification | MG-RAST version 4.0.3 | (15) |
| Visualizing annotated Taxonomy | Krona | (16) |
| Manipulation of the SAM/BAM files | SAMTools | (17) |

## Results and Discussion

By the metagenomic analysis detected the microorganisms in acne disease. The Taxonomic assignment of the sequences determined classified them into diverse microorganisms. In present study the microorganisms were bacteria (95.29%), eukaryote (4.23%), viruses (0.29%), archaea (0.04%), other sequences(0.15%) and unclassified sequences (0.00) these results determined for PA1. similarly, the PA2 was contains bacteria (96.52%), eukaryote (3.15%), viruses (0.18%), archaea (0.05%), other sequences(0.08%) and unclassified sequences (0.00) depended on the Pie chart in Figure (1) and (2).

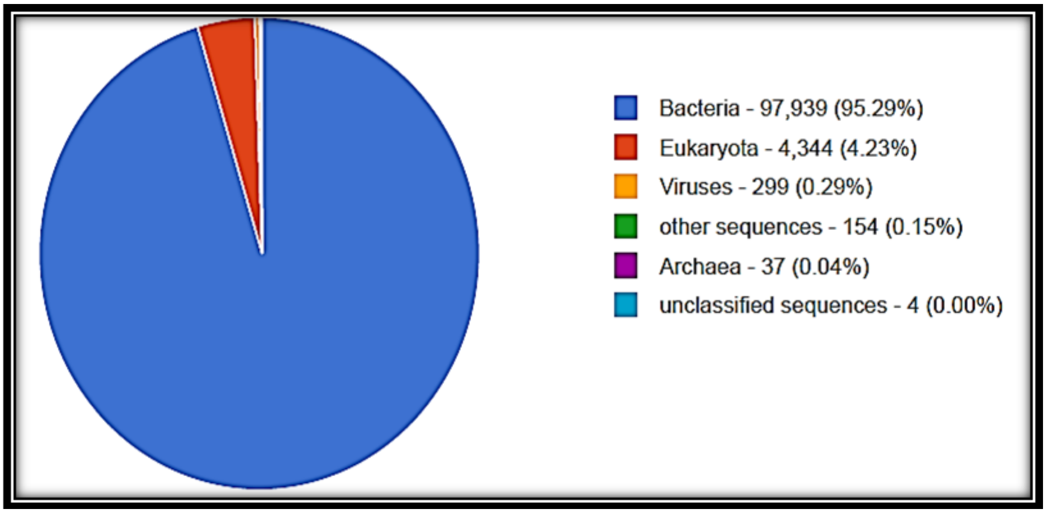


**Figure (1): Pie chart represents the distribution of domain taxa of PA1.**

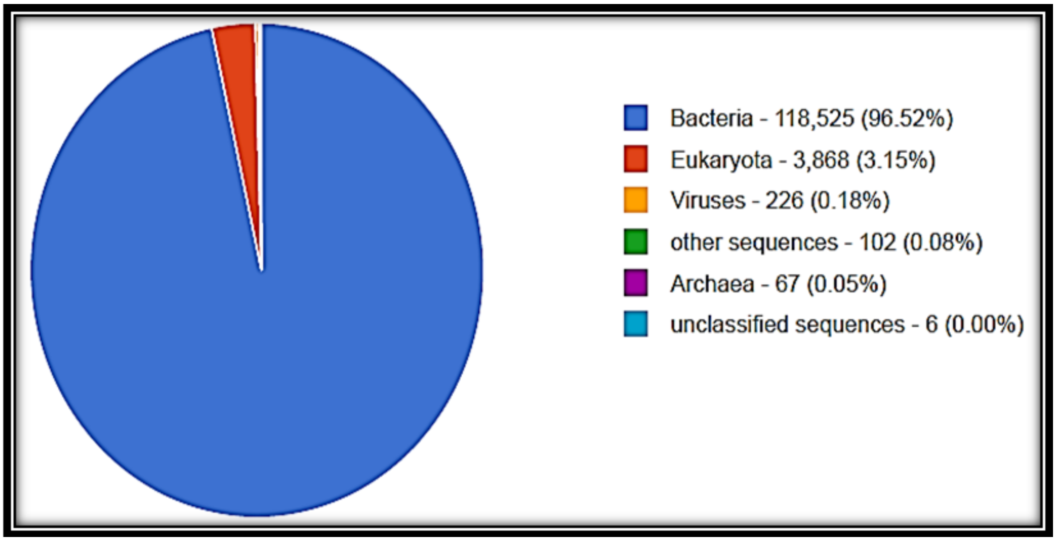


**Figure (2): Pie chart represents the distribution of domain taxa of PA2.**

The bacteria in the above results was higher than other microorganisms in two samples. It was 95.29% and 96.52% for each PA1and PA2 respectively. These results detected the bacteria is a major source in acne diseases compared with other microorganisms additionally several studiesidentified bacteria in acne vulgarism. So many factor increasing the bacteria in acne lesions may be the bacteria have multi –virulence factors, generation time, resistant to inhibiters growth that secreted by skin and other factors.

Correlated the results with (18) The surface of the skin is cooler than the core body temperature and is slightly acidic, and squares are continuously shed from the skin surface as a result of terminal differentiation.

The results agreements with (19) they documented The microbiome includes bacteria, fungi, viruses, parasites, and micro-eukaryotes which play significant role in dermatological disorders.

The metagenomic analysis as important part of this study was to identified and taxonomic of the bacteria that apparent high presenting 95.29% and 96.52% for each PA1and PA2 respectively. According this results taxonomic of bacteria to genus present to detected the important genus was infection acne. The PA1sample was similarity with PA2. The genus bacteria for (PA1 andPA2) were *Corynebacterium* (34.67%, 40.32%), *Propionibacterium* (32.23%, 24.04%), *Staphylococcus* (24.54%, 24.18%), *Malassezia* (1.13%, 0.86%), *Bacillus*

(0.92%, 1.99%), *Pseudomonas* (0.54%, 1.17%), *Escherichia* (0.36%, 0.52%), *Acinetobacter* (0.33%, 0.58%), unclassified (0.32%, 0.20%), *Stenotrophomonas* (0.31%, 0.51%), *Meiothermus* (0.30%, 0.36%) and *Enterococcus*(0.17%, 0.45%) whereas the PA2 have 0.18% of *Streptococcus* but the PA1 not appearance *Streptococcus* such as according to the pie charts in below in the Figures (3) and (4).

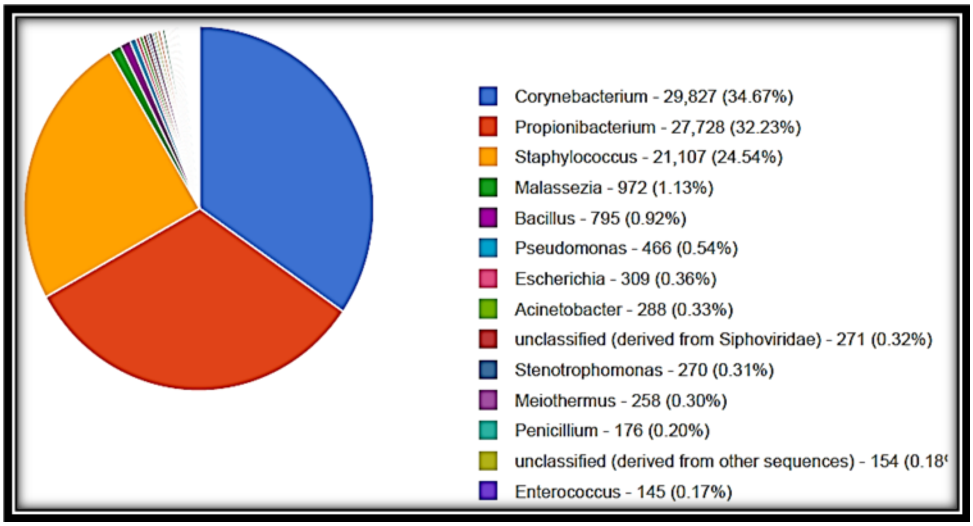
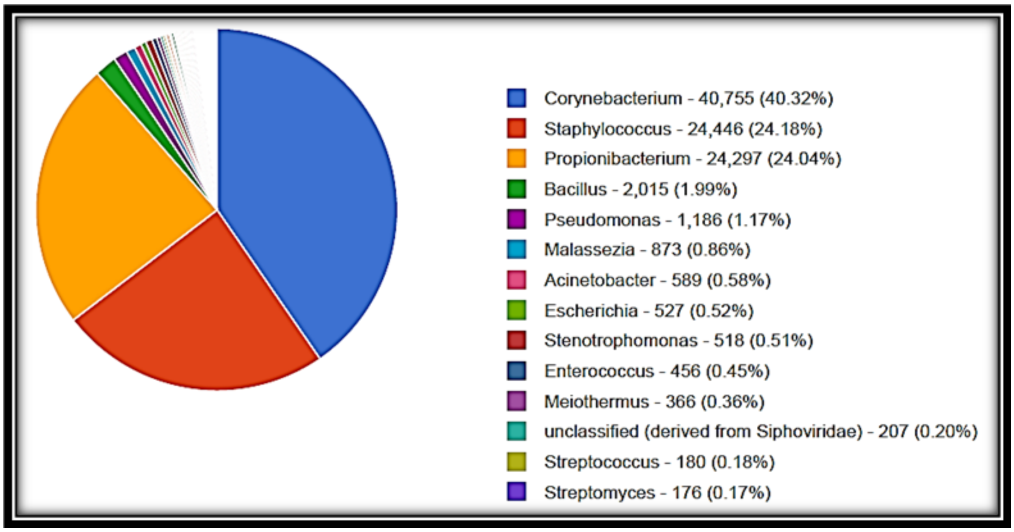


**Figure (3) Pie chart represents the distribution of genus taxa of PA1.**



**Figure(4) Pie chart represents the distribution of genus taxa of PA2.**

The results showed that PA1 and PA2 were similarity in three major bacteria included *Corynebacterium*, *Propionibacterium*, *Staphylococcus* whereas the two samples were significant difference of percentages for the other bacteria. It mainly consists of sebaceous areas, moist areas, dry areas, and sites containing varied densities of hair follicles, skin folds, and skin thicknesses. Sebaceous glands being relatively anoxic support the growth of facultative anaerobes such as acne causing *Propionibacteriumacnes*, which contain lipase-encoding genes that degrade skin lipids of sebum as revealed by full genome sequencing (20).

The primary microbe associated with development of acne is Propionibacterium acnes, also a prominent member of the commensal skin microbiota. Topical and systemic antibacterial drugs have long been used to treat acne, with the efficacy commonly attributed to decreased P. acnes colonization and/or activity(21).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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