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# Porphyromonas Levii: A Missing Link In Periodontitis

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**Abstract**: The presence of flora on the tooth surface poses a serious hazard to one's health. It causes dental caries by eroding the enamel and Periodontitis by producing a biofilm. In the oral cavity, more than 2000 species are thought to exist. Because all flora cannot be cultivated via plating or other readily available methods, genetic tools must be used. Biofilm production appears to be an important opportunity for these oral infections, according to current findings. Metagenomic approaches necessitate more careful selection and assessment of sample variety, and obtaining the necessary nucleic acid from the sample is difficult.

**Keywords:** Metagenomics, Periodontal disease, Polymicrobial Biofilm, Dental plaque,Oral microbiome.

## INTRODUCTION

The oral cavity is made up of a complex system of tissues and organs that work together to provide a human with many functions[1,2]. Mucosae and teeth, in particular, constitute two key sites for microbial colonisation. Oral cavity infections have well-known systemic consequences. Oral diseases affect nearly 2 billion people, according to studies. It has also long been known that various bacteria are common in specific medical conditions, offering the door to understanding the link between floral identification, count, or pattern and specific diseases[3,4]. Biofilm production appears to be an important opportunity for these oral infections, according to current findings. As a result, the microbial makeup in various clinical circumstances is unknown. Because most bacteria are uncultivable, traditional microbiological methods are currently ineffective. It is contrasting to the traditional genomic segment in many ways[5] Leucocytes are continually patrolling the periodontium, on the other hand.Infection occurs despite this defence system[6], indicating that leucocyte secretions and cellular recognitions are ineffective[7]. While it may not be able to evaluate all genes and link them to oral flora, one element that could have a role is the HLA antigen. Furthermore, if a correlation exists between HLA and periodontal flora, the clinician can adapt the treatment technique accordingly, leading to a better prognosis[8,9]. Our dental cavities are home to a slew of microorganisms, one of which, Porphyromonas levii, is of particular interest since it can be utilised to treat a variety of diseases. A bunch of Micro-organisms inhabit our oral cavities, out of which, Porphyromonas levii is of interest due to the fact that it can be used as a therapeutic mean for the Oral microbiome[10] The majority of species previously categorised as Bacteroides have been reassigned into new genera. Bacteroides levii[11]. This species shares a high degree of similarity with members of the genus Porphyromonas[12] based on biochemical, chemical, and comparative 16s rRNA sequence analysis. As a result, Bacteroides levii (Holdeman, Cato, and Moore) was reclassified as Porphyromonas levii comb. now under the genus Porphyromonas[13]. Porphyromonas levii is a Gram-negative, anaerobic bacterium from the Porphyromonas genus that was isolated from the rumen of a bovine[14]

## DESCRIPTION OF ORGANISM

## SCIENTIFIC CLASSIFICATION

**DOMAIN**: Bacteria
**PHYLUM**:Bacteroidetes
**CLASS**:Bacteroidia
**ORDER**:Bacteroidales
**FAMILY**:Porphyromonadaceae
**GENUS**:Porphyromonas
**SPECIES:** P. levii
**BINOMIAL NAME**: Porphyromonas levii (Johnson and Holdeman 1983) Shah et al 1995

## TYPE STRAIN

ACM 5042, ATCC 29147, CCUG 21027, CCUG 34320, HAMBI 467, JCM 13866, LEV, Lev 1, NCTC 11028, VPI 10450, VPI 3300

## SYNONYMS

Bacteroide melaninogenicus subsp.levi, Bacteroides levii Gram-negative, obligately anaerobic, nonsporeforming, nonmotile rods or coccobacilli. Cells in broth are 0.5 to 1 by 2 to 5 pm. Cells from solid media are coccobacilli or short rods. Colonies on blood agar plates are smooth, shiny, convex, and 1 to 2 mm in diameter and darken from the edge of the colony toward the

center between 4 and 8 days. Protoheme is the major porphyrin produced, but traces of protoporphyrin also occur. Succinate stimulates growth and can replace the requirement for protohaem. Most other commonly occurring sugars such as arabinose, cellobiose, maltose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sucrose, trehalose, and xylose are not fermented. Growth is markedly affected fected by the presence of protein hydrolysates such as trypticase, proteose peptone, and yeast extract[15]. Some amino acids such asparagine, tryptophan, and phenylalanine and glutamine are utilized[16]

## METAGENOMICS: AN OVER VIEW

Despite efforts to uncover the links between bacteria and human health[17,] , little is known about the species and functions of the microbial community linked to oral disorders.[18] Large efforts have been made to characterise the composition of the human microbiome at various body regions in order to increase our understanding of the interactions between bacteria and human hosts.[19,20,21,22,23,24] Gram-negative Periodontal infections such as Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia are frequently isolated from tooth plaques in periodontal patients and were once thought to be distinct periodontal pathogens[25]. Following that, researchers discovered a substantial link between the quantities of various cultivable bacteria (such as Prevotella intermedia, Fusobacterium nucleatum, Selenomonas noxia, Actinobacillus actinomycetemcomitans, and Eubacterium nodatum) and periodontal disease[26,27,28,29,30] There have been no studies that have examined the functional differential between oral microbiomes in healthy people and periodontal disease patients. The genetic content and functional potential of a microbial community can be screened via metagenomic sequencing. Many molecular biological techniques, such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA Fingerprinting (RPAD), Denaturing Gradient Gel Electrophoresis (DGGE), Quantitative Real-time Polymerase Chain Reaction (qPCR), and others, have been used in the last two decades to identify and classify uncultivable oral microbial species[31,32,33]. Recently, next generation sequence technologies (NGS) have enabled the investigation of a large number of microorganisms in various environments without the need for bacterial culture. Using DNA sequencing to investigate various environmental niches, many novel microbe species have been discovered. To examine uncultivated oral microbial populations, two basic DNA sequencing methodologies have been extensively used: 16S rRNA sequence analysis and metagenomics[34,35,36,37,38,39,40,41].

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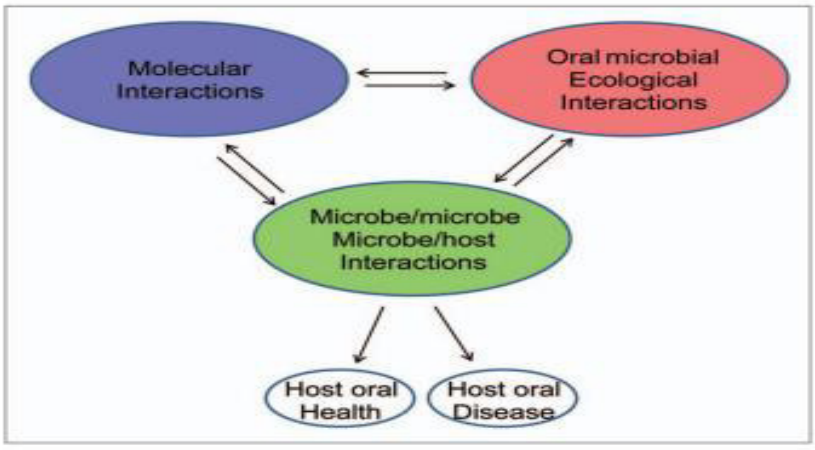



## AIMS AND OBJECTIVES

- To find a missing link of Organism- **Porphyromonas levii in Periodontitis.**
- To elaborate on the incidence and prevalence of cultivable and non-cultivable flora in the sub gingival plaque samples in Indian population
- To associate pathogens with chronic and aggressive periodontitis.

## METHODOLOGY

**Study type: Cross sectional study**

- **Group I:** Aggressive periodontitis
- **Group II**: Chronic periodontitis
- **Group III:** Healthy subjects with absence of periodontitis

• **Sample:** Sub gingival plaque

### ➢ SUBJECT SELECTION

**Inclusion criteria**

- Aggressive periodontitis patient
- Clinical finding with greater than 4mm of pocket depth
- Radiographic findings with arc shaped bone loss in incisors and molar
- Chronic periodontitis patients.

- Clinical findings with greater than 4mm of pocket depth

**Exclusion criteria**

- Antibiotic therapy within the past 3 months
- Pregnant women
- Systemically unhealthy
- Smokers

## SAMPLE COLLECTION

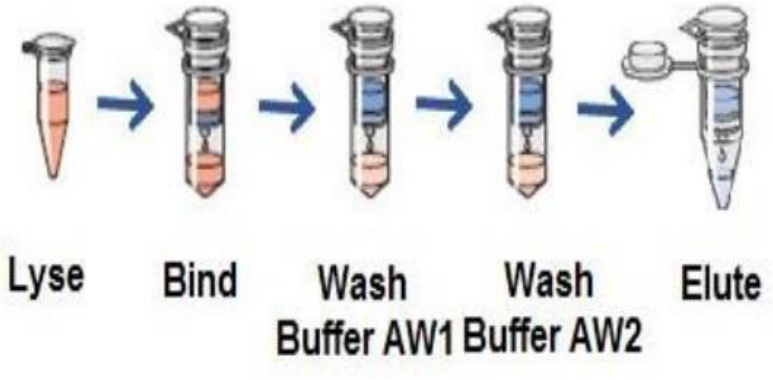
Patients who reported to the OP of Periodontics department were taken for the study. After careful subtraction of supragingival plaque with sterile cotton roll, subgingival plaque will be composed from deep sites in each quadrant using a sterile gracey curette. The subgingival plaque will be assembled in a 9 sterile eppendorf tube covering 1ml of phosphate buffered saline.

## DNA EXTRACTION

The DNA was removed from subgingival plaque samples using DNeasy Blood and Tissue Kit. The removed DNA was enumerated using Qubit 4 Fluorometer.

## PROCEDURE

The collected samples in the Eppendorf tubes were mixed well by inversion and gentle shaking for a few seconds, after which the samples were incubated at $50^0$ C in a water bath for a minimum 15 -20 minutes. After incubation the centrifugation of the cells at 5000 x g (7500 rpm) was done for 10 min and the supernatant was rejected. The pellet was suspended into 180µl buffer ATL and added 20µl proteinase K and mixed thoroughly by vortexing.




**DNA Quantification**

The extracted DNA was quantified using Qubit 4 Fluorometer

**16S rRNA PCR**

A PCR targeting 16S rRNA gene was performed for the three different saliva samples with 25µL reaction volume consisting of broad-range pan 16S rRNA primers.

**16S rRNA PCR amplicon Purification**

All the three saliva samples 16S rRNA PCR amplicons were purified using FavorPrep PCR Purification Mini Kit (Favorgen, Taiwan) and after the purification, amplicons was quantified using Qubit 4 Fluorometer for Nanopore library preparation.

**16S rRNA sequencing using Oxford Nanopore Technologies**

Sequencing was done by third-generation sequencing technology, Oxford Nanopore Technologies (ONT).

**16S rRNAamplicon library preparation and sequencing**

Briefly, 1 µg of 16S rRNA PCR product was used for the end repair process with NEBNext Ultra II End-repair/dA-tailing (New England Biolabs, USA).

**Preprocessing of16S rRNA Metagenome Sequencing Data**

The Fast5 output sequences from the MinION sequencer were basecalled and Demultiplexed using Albacore Softwarev2.0.1 and basecalled Fast5 sequences were converted to Fasta files using Poretools Software v0.5.1.

**Taxonomy Assignment by MG-RAST analysis**

The 16S rRNA processed reads were finally analysed by using MG-RAST server - a metagenomics analysing server

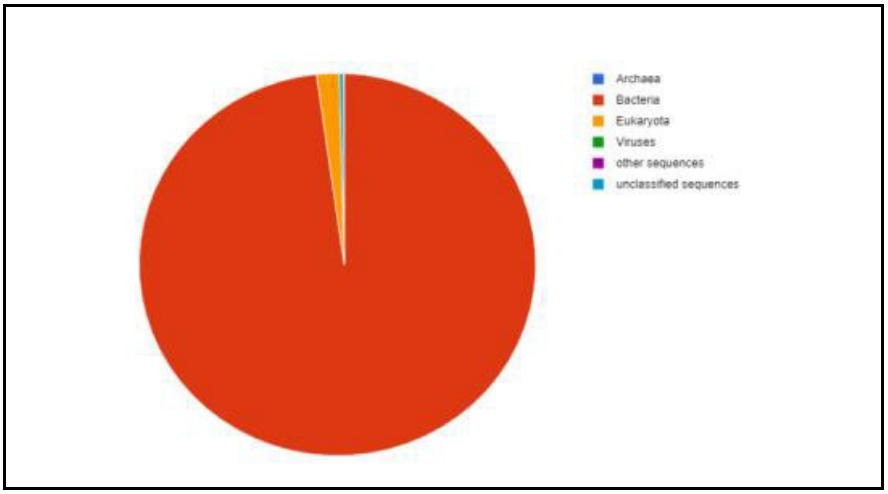
**HLA typing**

HLA typing of 10 samples was done using whole blood by complete locus sequencing by NGS.

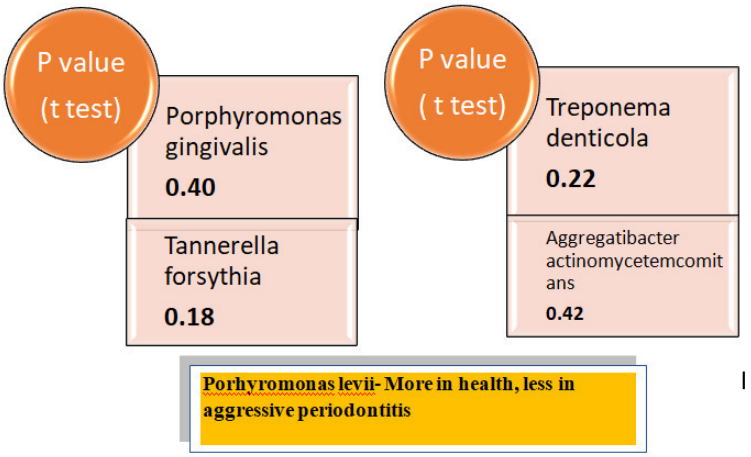
**DNA extraction**

The DNA was extracted from 10 whole blood samples by using QIAamp® Blood Mini Kit . The quality and quantity of the extracted DNA was analysed using NanoDrop.

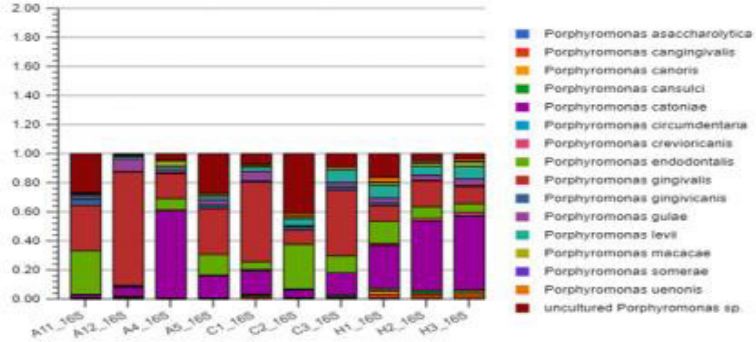
**RESULTS**




The use of metagenomics to the oral microbiome is still in its early stages. However, metagenomics can examine and compare both bacterial and genomic profiles to investigate relationships between microbial diversity, genetic variations, and oral diseases. The oral microbiome appears to be extremely complicated, according to current evidence. The impacts of the microbiome on the host have an impact on oral health and illness. To understand the complex interactions between the microbiome and the host, a way for a better understanding of oral disease. A variety of organisms were detectedand the bacteria in the red complex did not differ significantly between patients with chronic and severe periodontitis..




Novel organism- Porphyromonas levii was found This shows us that, it can be used as probiotics which modify the course of the disease and shift the balance to the favorable side



## DISCUSSION

Metagenomics studies have been conducted in a variety of countries and ethnic groups, and a number of publications have been published as a result. This research is one of the first in the field of Indian population. The Indian populace is projected to enter a new era of periodontal microbiology as a result of this. Aa was discovered in over 50% of the sites in a research by daSilva-Boghossian et al., (2011), but the red complex was found in only around 35% of the sites in Aggressive and Chronic Periodontitis individuals, respectively. Based on literature relating to cultivation and cultivation independent methods of bacterial identification in the oral cavity, Parahitiyava et al., (2010) investigated the oral bacterial flora. They claim that approaches based on culture underestimate the floral population and do not provide adequate information on aetiology and prognosis. In the identification of novel bacterial species, culture-independent approaches were more sensitive. The microbial aetiology and host response in aggressive periodontitis were reviewed by Nibali (2015). "Proven risk variables are only recognised in a limited percentage of AgP cases," they say. They believe that genetically driven dysbiotic alterations in the subgingival microbiota may indicate a susceptibility to fast periodontal tissue loss. In a "comparative genomic analysis" of the known red complex species**,** Endo et al., (2014) discovered novel interactions. They propose various interactions between the species in question, as well as a wide range of specific virulence factors. As a result, they recommend that a new mechanism of floral symbiosis in periodontitis be discovered. Both competitive and cooperative interactions are present in these processes. Uncultured bacteria have been linked to periodontal disease since 1980, according to Socransky et al. They have outlined some of the challenges that microbiologists encounter while studying periodontal pathogens, including difficulty with culturing, the complexity of the microbiota, the identification of distinct diseases as the same, and the possibility of many diseases within a single individual. Despite the fact that none of the difficulties they listed have been solved to date, a short list of infections has been developed, and patients are being treated. In such a situation, metagenomics has shown to be a godsend in determining the causal link.

## CONCLUSION

Metagenomics research has been carried out in a variety of countries and ethnic groups, resulting in a number of publications. This is one of the first studies of its kind in the field of Indian population. As a result, the Indian population is expected to enter a new era of periodontal microbiology. Bacteria made up 98 percent of microorganisms, while viruses and fungi made up the rest, according to the data[42,43,44] In summary, To amplify the whole 16S rRNA, researchers used third-generation Oxford nanopore technology. In this approach, the most recent study is likely to offer further insight on the situation. Commensals that have been identified in either human or animal habitats[45] and their pathogenesis involvement appears to be opportunistic. In the future, Periodontologists will need to combine metagenomic data with biochemical pathways to arrive at a dysbiota formula. When it comes to the most commonly implicated species, Porphyromonas Levii is shown to be more in good health and less in aggressive periodontitis[46,47]. Coming to the overall picture of microbial population, as discussed earlier, it is the dysbiota that contributes to the periodontal disease. From statistical analysis of entire data, it was evident that amongst species that were significantly different in chronic presentation and healthy individuals, all were higher in healthy patients, indicating their role in Probiotics or normal microbiome[48,49,50] Newer bacteria are continuously being found, and their impact on periodontal therapy could be significant. Some of the flora, such as Porphyromonas levii, which was discovered in this study, can be employed as Probiotics, which can affect the course of the disease and tilt the scales in your favour. This organism is discovered to be a causal factor in many other ailments such as bovine interdigital infections and vulvovaginitis. More research and investigations are needed to better understand the role of P.levii in the oral cavity and periodontal diseases.

## CONFLICT OF INTEREST

Conflict of interest declare none.

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