Research Article

MSA and Phylogenetic Analysis of 16s rRNA from *Prevotella* Family of Bacteria in the Oral Microbiome.

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ABSTRACT

This research aims at conducting MSA and Phylogenetic analysis of 16s rRNA from prevotella family of bacteria in the oral microbiome. This article highlights the evolutionary and phylogenetic relationship in the 16s rRNA of the various species prevotella species in the oral micro biome. Prevotella strains are Gram-negative, non-motile, singular cells that thrive in anaerobic growth conditions. They are generally host associated and most species exist as obligate anaerobes. Prevotella species are present in humans, often existing in the oral cavity, gastrointestinal tract, and vagina. In humans, they can act as an opportunistic pathogen, causing periodontal and tooth problems such as gingivitis and periodontitis. The prevotella species is known to cause several problems associated with oral health. Establishing a phylogenetic relationship between the various species of in the oral micro biome helps in understanding the evolution of the species and thereby leads to better treatment plan and drug efficacy for the same.

Keywords: Phylogenetic analysis, rRNA, Prevotella, phylogenetic.

INTRODUCTION

Prevotella species are present in humans; often existing in the oral cavity, gastrointestinal tract, and vagina[1]. *Prevotella* strains are Gram-negative, non-motile, singular cells that thrive in anaerobic growth conditions. Species of the *Prevotella* genus are generally bacillus to almost coccoid bacteria.[2] They are pleomorphic, taking on different forms during the life cycle, and do not form endospores[3].

Prevotella species thrive on tissues with decreased oxidation-reduction potentials, such as those with limited blood supply or tissue necrosis[2]. The growth of *Prevotella intermedia* favors a slightly basic pH with a temperature held between 34-36˚C[4]. Additionally, increased hormone production, such as that during adolescence and pregnancy, has been shown to contribute *Prevotella* proliferation[5].

Several *Prevotella* species act as opportunistic pathogens, often penetrating tissues and establishing an infection at mucosal surfaces[2]. There are currently about twenty known species of the genus *Prevotella* that are known to cause infection. *Prevotella* can form abscesses and genital tract infections[2]. Depleted reduction-oxidation potential within tissues can contribute to infection[2]

Some *Prevotella* are resistant to β-lactam antibiotics as they possess a β-lactamase inhibitor. Effective antibiotic treatments for *Prevotella* include metronidazole, amoxicillin/clavulanate, ureidopenicillin, carbapenems, cephalosporins, clindamycin, and chloramphenicol.[3]

The presence of *Prevotella* species has been shown to increase 1-6 months after birth, suggesting a possible role in the infant microbiome and presenting the opportunity for potential probiotic production[6]. In a study of gut bacteria of children in Burkina Faso (in Africa), *Prevotella* made up 53% of the gut bacteria but were absent in age-matched European children.[7] Studies also indicate that long-term diet is strongly associated with the gut microbiome composition - those who consume more carbohydrates, especially fiber, the *Prevotella* species dominate.[8]

Prevotella is known to cause diseases in humans. *Prevotella intermedia* are a periodontal pathogen that has been linked to inflammation of the gums and the related disease of gingivitis[6]. The extensive proliferation of *Prevotella intermedia* along with other anaerobic bacteria in the oral cavity has also been associated with periodontitis[9]. Periodontitis is the detachment of connective tissue along the alveolar bone and is usually preceded by gingivitis[9]. It results in tooth separation, movement, and swelling of the gums. Subsequent internalization of *Prevotella intermedia* from periodontal pockets is hypothesized to be strain specific, being limited to those that possess specific receptors for fimbriae [9]. Additionally, there is a correlation between coronary heart disease and periodontal disease that may be linked to the invasion of artery smooth muscle and artery endothelial cells by *Prevotella intermedia*[9].

Prevotella were formerly classified under *Bacteroides malninogenicus*. *Prevotella* species have recently been reclassified into two groups: *Prevotella melaninogenica* and *Prevotella intermedia*[10]. *Prevotella* belongs to the phylogenetic division of Cytophaga-Flexibacter-Bacteroides (CFB) [1]. Additionally, species of *Prevotella* can be categorized as both "ruminal" and "non-ruminal," with

species in the same category often exhibiting a closer genetic resemblance[1] In August of 2007, two strains of novel species was added to the genus *Prevotella* based on 16S rRNA gene sequences and phylogenetic analysis[11]. These two strains, KB7(T) and A42, were the first species of the genus known to exist in a natural habitat, independent of a mammalian host[8]. *P. copri* is possibly connected to rheumatoid arthritis.[12] Gene repertoires of its species reported an open pan- genome showing vast diversity of gene pool.[13]
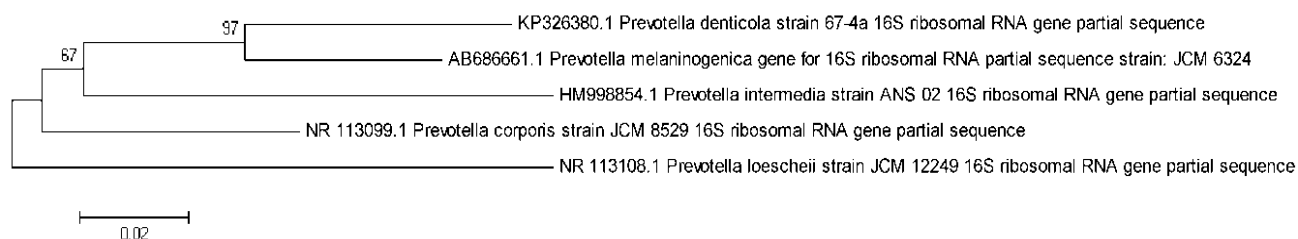
MATERIALS AND METHODS

Prevotella species

The following *Prevotella* species have been considered for this study, *Prevotella intermedia, Prevotella denticola, Prevotella corporis, Prevotella loescheii,* and *Prevotella melaninogenica,* all of which have been proven to be found in the oral cavity. These organisms are a normal part of the human microbiome, living in the oral-pharyngeal region[14].

Sequence analysis by FASTA

The nucleotide sequences were retrieved from the NCBI database using the access codes HM998854.1 (*Prevotella intermedia)*, KP326380.1 (*Prevotella denticola)*, NR_113099.1 (*Prevotella corporis)*, NR_113108.1 (*Prevotella loescheii)* and AB686661.1 (*Prevotella melaninogenica)*. For all the above species the nucleotide partial sequence of 16s rRNA was obtained in the FASTA format[15].

Multiple sequence analysis

Multiple sequence alignment was done for the same using the online software Clustal Omega[16, 17]. In Clustal Omega the conserved symbol (*) denoted that the particular gene was same for all the nucleotide sequences which were used as a part of the study. In the same Clustal Omega, the phylogenetic relationship was observed which is distance based.

Phylogenetic analysis

The phylogenetic analysis was done using the software MEGA5[18]. The phylogenetic analysis as a whole can be classified into distance based and character based analysis. However, MSA is the raw material for both. The various types of grouping under character based are maximum parsimony, maximum likelihood, and minimum evolution. The various types of grouping under distance based are UPGMA, Neighbour-joiningmethod, and Fisch Margoliash method. In this study, the method used is the maximum likelihood method which is part of the character-based phylogenetic analysis. A phylogenetic tree is constructed and accordingly, the phylogenetic relationship was analyzed.

RESULTS AND DISCUSSION

The phylogenetic tree generated through the MEGA5 software has been studied. Figure 1 shows the phylogenetic tree representing the evolutionary relationship between the various species of *Prevotella* taken for the study.

Figure 1

The Multiple sequence alignment obtained by using the FASTA format of the nucleotide sequence has been analyzed. Multiple sequence alignment helps to align the nucleotide sequences and thus showers knowledge on similarity of sequences amongthe organisms included in the study.

Genes which are similar are Homologues. Lesser the distance in the phylogenetic tree closer the homolog. Any gene which occurs in different forms due to gene duplication is known as paralogs. Since only the 16s rRNA is used noparalogs were observed. Each branch is the tree is called a clade and each unit is called a taxon. The length of each clade is in correlation with the time units which is given in the phylogenetic tree[18].

The tree interpreted the genetic similarity or dissimilarity between the species.It was evident that

Prevotella loescheii follows a separate branch of evolution, breaking away from the rest of the tree. Denticola and melaninogenica are the two most closely related species in comparison with the other species under study. There is a significant distance between *Prevotella* intermedia and the remaining species, implying that the organism isn't as evolved as the other species. This evolutionary data helps in determining the characteristics of the species, grouping genetically similar organisms and estimating the ideal treatment plan for the related species.

CONCLUSION

The various species of *Prevotella* occupying the oral microbiome was studied. The Multiple Sequence Analysis showed the similarities and differences in the nucleotide sequence of the 16s rRNA among the various

species. The phylogenetic tree shows the evolutionary relationship between the various species. It is evident that the denticola and melaninogenica have the closest evolutionary relationship. As they are genetically not very different, it helps in determining the characteristics of the species, grouping genetically similar organisms and estimating the ideal treatment plan for the related species.

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