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16S rDNA sequence analysis of periodontitis microbiota: A Pilot Study

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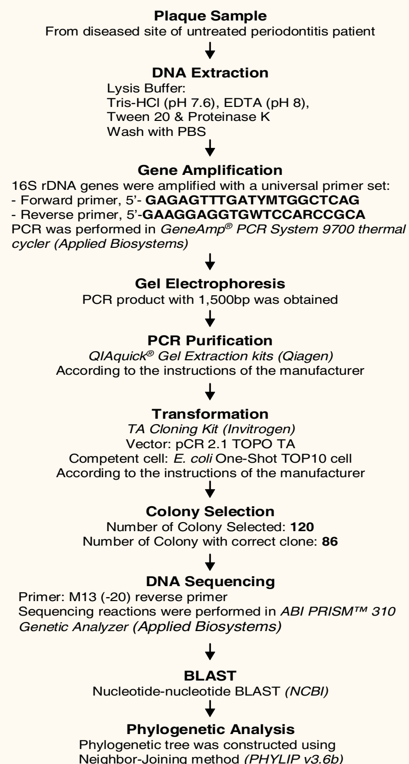
Introduction

Pathogenic bacterial colonization/re-colonization of subgingival niches is the main cause of periodontitis/recurrent periodontitis. Certain predominantly cultivable periodontopathogens e.g. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythensis* out of the total approx. 300 cultivable oral microbes were shown to be implicated in the disease (Darveau *et al.* 1997). These incriminated microbes, however, were repeatedly reported to comprise only a minor proportion of the subgingival microflora (Lyons *et al.* 2000, Rudney *et al.* 2003). New uncultured periodontopathogens have been recently identified (Sakamoto *et al.* 2002, Brinig *et al.* 2003). There is still uncertainty regarding what pathogen or combinations of pathogens play a major role in periodontal infection. Current research reports had indicated that potentially 10-50% of the subgingival flora is unidentified and hence, by inference, up to 50% of the periodontopathogens may yet be unknown to the scientific world (Paster *et al.* 2001, Hutter *et al.* 2003). Traditional culture dependent bacterial isolation and characterization, is not error proof. Biochemical or abbreviated molecular identification provides the best possible identification limited only by current scientific knowledge. For accurate study of periodontitis mircrobiota, more specific approaches are required (Tanner *et al.* 1994). 16S ribosomal DNA sequences are one current tool for phylogeny of microbes (Hugenholtz *et al.* 1998) and this approach is the current "gold standard". Kroes *et al.* (1999), Paster *et al.* (2001) and Hutter *et al.* (2003) had demonstrated, utilizing 16S rDNA gene analysis technique, great diversity of the subgingival microflora associated with periodontal health and disease conditions. Initial data from a variety of clinical situations are available from the US and Europe (Kroes *et al.* 1999, Spratt *et al.* 1999, Paster *et al.* 2001, 2002, Kazor *et al.* 2003, Hutter *et al.* 2003). Many novel 16S rDNA sequences could be identified from the above studies, however, their relevance to periodontitis is yet to be discovered.

Aim

Our aim is to study the microbial diversity of periodontitis subgingival microbiota by cloning and partial sequencing of 16S rDNA gene.

Material & Methods



Results

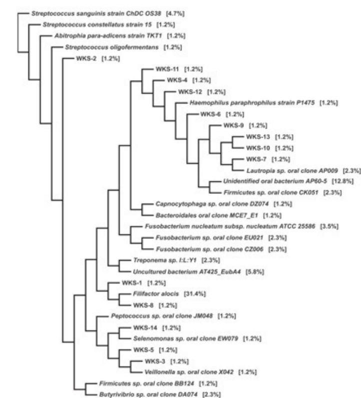


Figure. Phylogenetic tree of identified clones

- 408 – 1546 bases, mean 645 $\pm$ 221 bases sequenced from the 86 correct transformants
- total of 35 clones identifiable
- 7 clones (44.2%, mean 1016 $\pm$ 501 bases sequenced) were identified as known characterized bacterial species
- 12 clones (20.9%, mean 804 $\pm$ 356 bases sequenced) were identified as partially characterized bacterial species/strains
- 14 clones (16.3%) were of $\leq$ 97% similarity with NCB1 data base
- A total of 16 clones (34.9%, mean 582 $\pm$ 95 bases sequenced) belonged to uncharacterized bacterial strains
- No known periodontopathogen 16S rDNA clone sequenced. A *Treponma* sp. 1:L:Y1 clone (2.3%) encountered.

Conclusions

- Within the limitations of the culture independent 16S rDNA cloning and sequencing method, we found that about one half of the studied periodontitis subgingival microbiota comprised of partially- or uncharterized microorganisms.
- More research efforts should be directed to culture independent study of periodontitis associated subgingival microbial biofilms.

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