3657 16S rDNA sequence analysis of periodontitis microbiota: A Pilot Study

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Aim: To study the microbial diversity of periodontal subgingival microbiota by cloning and partial sequencing of 16S rDNA gene. Method: Bacterial DNA from a subgingival plaque sample of an untreated periodontitis patient was extracted and amplified using universal 16S rDNA primers: forward - D88, reverse - E94. Correct size amplicons were isolated and cloned using pCR 2.1 vector into an *E. coli* host (TA cloning kit). 50 transformants were picked randomly and the plasmids carrying the amplified 16S rDNA were extracted and were sequenced using an ABI 310 sequencer. Identity of cloned 16S rDNA sequences were matched against a public access data base (NCBI). 16S rDNA clone identification was established when i) ≥ 400bp (range: 403 - 1470bp) were sequenced, and ii) ≥ 98% sequence match could be identified. Results: A total of 22 species were detected including: *Filifactor alocis* (26%), unidentified oral bacterium AP60-5 (26%), *Streptococcus sanguinis* (6%), *Fusobacterium nucleatum* subsp. *nucleatum* (4%), *Fusobacterium* sp. oral clone CZ006 (4%), *Abiotrophia para-adiacens* (2%), *Bacteriodes* sp. oral clone FX069 (2%), *Bacteriodes-like* sp. oral clone X083 (2%), *Capnocytophaga* sp. oral clone CZ006 (2%), uncultured *Eubacterium* sp. (2%), unidentified *Fusobacterium* sp. (2%), *Haemophilus paraphrophilus* (2%), *Lautropia* sp. oral clone AP009 (2%), *Selenomonas* sp. oral clone EW09 (2%), unidentified *Streptococcus* sp. (2%), *Streptococcus constellatus* (2%), *Streptococcus oligofermentans* (2%), two independent unidentified *Veillonella* sp. (2% each), and three independent uncultured bacteria (2% each). Conclusion: When studied using culture independent method, a fair proportion of periodontitis subgingival microbiota remain poorly characterized indicating more research efforts should be directed to the study of periodontitis associated subgingival biofilm. Supported by RGC Hong Kong.

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