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### **Drinking habits are associated with changes in the dental plaque microbial community.**

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Caries and gingivitis are the most prevalent infectious diseases of humans and are due to the accumulation of dental plaque (a microbial biofilm) on tooth surface and at the gingival margin, respectively. Several in vitro and in vivo studies have shown that many natural components of foods and beverages inhibit the adhesion of and/or exert an antimicrobial activity against oral bacteria. These biological activities have been attributed mainly to the polyphenol fraction. In order to explore the possibility that diet can alter the dental plaque community, in this study we evaluated the composition of the microbiota of supra- and subgingival plaque collected from 75 adult subjects with different drinking habits (drinkers of coffee, or red wine or water for two years at least) by analysing the microbial population through separation of PCR-amplified fragments using the denaturing gradient gel electrophoresis (DGGE) technique. The mean numbers of the bands of the DGGE profiles from all the three categories was evaluated. There were no significant differences between the two kinds of plaque collected from the control (water) group and this group showed the highest number of bands (supragingival =  $18.98 \pm 3.16$  and subgingival =  $18.7 \pm 3.23$ ). The coffee and wine drinker groups generated the lowest numbers of bands for both supra- (coffee =  $8.25 \pm 3.53$  and wine =  $7.93 \pm 2.55$ ) and sub-gingival (coffee =  $8.3 \pm 3.03$  and wine =  $7.65 \pm 1.68$ ) plaque. The differences between coffee drinkers or wine drinkers vs. the control (water) group, respectively, were statistically significant. A total of 34 microorganisms were identified and their frequency of distribution into the three subject categories was analysed. A greater percentage of subjects were positive for facultative aerobes when supragingival plaque was analysed while anaerobes were more frequent in subgingival samples. Relevantly, the frequency of anaerobe identification was significantly reduced when coffee and wine drinkers were compared with the subjects of the control group. DGGE profiles of both plaques samples from all groups were generated and used to construct dendrograms. A number of distinct clusters of water or coffee or wine drinkers were formed. The clustering of the some of DGGE results into cohort specific clusters implies similarities in the microbiota within

these groups and relevant differences in the microbiota between cohorts. This supports the notion that the drinking habits of the subjects may influence the microbiota at both a supragingival and subgingival level.

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