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Stability of saliva microbiota during moderate consumption of red wine

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ABSTRACT

Objective: This study has evaluated the effect of regular and moderate red wine consumption on the diversity and occurrence of different groups of bacteria that are representative in human saliva.
Methods: Saliva from twenty-two healthy volunteers (age range 20–48 years) was analyzed in this study. Fourteen individuals consumed red wine (250 mL/day) during 4 weeks, whereas 8 volunteers were included in the control group. The evolution and composition of the microbial community in saliva was evaluated by PCR–DGGE and quantitative PCR.
Results: The microbial inter-individual variability observed in the PCR–DGGE band patterns was higher than the differences observed after the 4-weeks period of red wine intake. *Bifidobacterium dentium*, *Bifidobacterium* spp. and *Alloscardovia omnicolens* were the most representative bifidobacterial species, whereas the *Streptococcus mitis–Streptococcus oralis* group predominated within *Streptococcus*. This genus was the most numerous of the bacterial groups assayed, reaching average counts above 8 log copy numbers/mL. On the other hand, the lowest counts were recorded for *Actinomyces*, *Fusobacterium*, *Haemophilus*, *Neisseria* and *Veillonella*, which showed average values of 5 log copy numbers/mL. The results showed no significant differences ($P > 0.5$) in bacterial counts after the period of red wine intake.
Conclusion: The overall diversity and stability of representative bacterial groups of the human saliva is not disturbed due to regular-moderate red wine consumption.

1. Introduction

Regular intake of polyphenol-rich beverages and foods has demonstrated to exert beneficial effects in human health, such as decreased incidence of cardiovascular disease, cancer and protection against neurodegenerative diseases, among others (Arranz et al., 2012; Bhullar and Rupasinghe, 2013 Kishimoto, Tani, & Kondo, 2013). Nevertheless, the beneficial effects of polyphenols seem to be more linked to microbial phenolic metabolites produced in the human gut than to the original forms present in food (Selma, Espín, & Tomás-Barberán, 2009). Accordingly, regular and moderate intake of red wine (a characteristic polyphenol-rich beverage) has demonstrated to exert modulating effects in the human gut microbiota (Barroso et al., 2014; Queipo-Ortuño et al., 2012; Wu et al., 2011). It is reasonable to presume that besides their effect on gut microbiota, these polyphenols can exert an effect on the overall oral cavity microbiota (saliva and gingival margins). This is worth considering that diversity of the microbial populations in the oral cavity is even larger than in the gut or the skin, harboring viruses, archea, protozoa, fungi and over 700 species of bacteria (Costello et al., 2009; Wade, 2013). Similarly to human gut or skin microbiome, the oral microbial community is an interacting ecosystem with the host that helps to maintain the health status, although certain ecological shifts allow pathogens to establish and cause disease (Yang et al., 2012; Zarco, Vess, & Ginsburg, 2012). Despite its relevance to human health, little information is currently available on the effect, if any, of diet on the saliva and gingival dental microbiota. A study on the salivary microbiota of individuals who followed an omnivore or ovolacto-vegetarian or vegan diet has recently being performed (De Filippis et al., 2014). However, there is scarce information about the effect of daily habits such as regular red wine consumption on the oral cavity microbiota.

The antimicrobial effects of the polyphenols present in red wine and grape seed extracts against microorganisms responsible for

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periodontitis and dental caries have been mainly studied by incubating polyphenols with pure strains (Cueva et al., 2010; Daglia et al., 2007; Furiga, Lonvaud-Funel, Dorignac, & Badet, 2008; Muñoz-González, Thurnheer, Bartolomé, & Moreno-Arribas, 2014). Signoretto et al. (2010) evaluated the microbial composition of supragingival and subgingival plaque in 75 adult volunteers that had been drinking 400 mL red wine daily for at least two previous years. It was observed a lower microbial diversity in the plaque samples of regular wine drinkers compared with water drinkers. This microbial modulating effect was speculatively attributed to the antimicrobial, antiadhesive, and antiplaque activities of the polyphenols contained in wine. In a review targeting bacterial adhesion to different susbstrates, the same authors considered the polyphenol-rich foods as a potential alternative strategy to antibiotic therapy in order to avoid caries and gingivitis/periodontitis (Signoretto, Canepari, Stauder, Vezzuli, & Pruzzo, 2012).

Concerning saliva, the effect of consumption of red wine on the antioxidant status of this oral fluid has been recently investigated (Varoni et al., 2013), but information on the effect of red wine in the

Table 1
Primer sets used in this study for PCR–DGGE and quantitative PCR.

Target group		Primer sequence 5′–3′	Annealing (°C)	Gradient (%)	Reference
PCR–DGGE					
Total bacteria	F:	AACGCGAAGAACCTTAC + GC[a]	56	30–60	Nübel et al. (1996)
	R:	CGGTGTGTACAAGACCC			
Lactobacillus	F:	GGAAACAGGTGCTAATACCG	56	30–50	Heilig et al. (2002)
	R:	ATCGTATTACCGCGGCTGCTGGCAC + GC[a]			
	F:	GTTTGATCCTGGCTCAG	66	Nested	Heilig et al. (2002)
	R:	CACCGCTACACATGGAG			
Bifidobacterium	F:	GGGTGGTAATGCCGGATG	62	40–55	Satokari et al. (2001)
	R:	GCCACCGTTACACCGGGAA + GC[a]			
	F:	CGGGTGCTICCCACTTTCATG	57	Nested	Satokari et al. (2001)
	R:	GATTCTGGCTCAGGATGAACG			
Streptococcus	F:	AGATGGACCTGCGTTGT + GC[a]	55	25–50	Van den Bogert, de Vos, Zoetendal, and Kleerebezem (2011)
	R:	GTGAACTTTCCACTCTCACAC			
Veillonella	F:	A(C/T)CAACCTGCCCTTCAGA	62	40–70	Rinttilä, Kassinen, Malinen, Krogius, and Palva (2004)
	R:	CGTCCCGATTAACAGAGCTT + GC[a]			
Prevotella-Porphyromonas-Bacteroides	F:	GGTGTCGGCTTAAGTGCCAT + GC[a]	68	40–58	Rinttilä et al. (2004)
	R:	CGGA(C/T)GTAAGGGCCGTGC			
Neisseria	F:	CTGGCGCGGTATGGTCGGTT	55	30–70	Lansac et al. (2000)
	R:	GCCGACGTTGGAAGTGGTAAAG+GC[a]			
Quantitative PCR					
Total bacteria	F:	CGGTGAATACGTTC(C/T)CGG	59		Booijink et al. (2010)
	R:	CGGTGTGTACAAGACCC			
Actinomyces	F:	CTCCTACGGGAGGCAGCAG	60		Dalwai, Spratt, and Pratten (2007)
	R:	CACCCACAAACGAGGCAG			
Bifidobacterium	F:	CTCCTGGAAACGGGTGG	55		Matsuki et al. (2002)
	R:	GGTGTTCTTCCCGATATCTACA			
Fusobacterium	F:	C(A/T)AACGCGATAAGTAATC	54		Rinttilä et al. (2004)
	R:	TGGTAACATACGA(A/T)AGGG			
Haemophilus	F:	GGAGTGGGTTGTACCAGAAGTAGAT	55		Matar, Sidani, Fayad, and Hadi (1998)
	R:	AGGAGGTGATCCAACCGC			
Lactobacillus	F:	TGGAAACAG(A/G)TGCTAATACCG	62		Booijink et al. (2010)
	R:	GTCCATTGTGGAAGATTCCC			
Neisseria	F:	CTGGCGCGGTATGGTCGGTT	55		Lansac et al. (2000)
	R:	GCCGACGTTGGAAGTGGTAAAG			
Prevotella	F:	CAC(A/G)GTAAACGATGGATGCC	55		Matsuki et al. (2002)
	R:	GGTCGGGTTGCAGACC			
Streptococcus	F:	AGATGGACCTGCGTTGT	55		Van den Bogert et al. (2011)
	R:	GTGAACTTTCCACTCTCACAC			
Veillonella	F:	A(C/T)CAACCTGCCCTTCAGA	62		Rinttilä et al. (2004)
	R:	CGTCCCGATTAACAGAGCTT			

[a] GC clamp at 5′: CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC.