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ORIGINAL ARTICLE

Detection of sulphate-reducing bacteria in human saliva

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Abstract

Objective. The aim of the current study was to investigate the presence of sulphate-reducing bacteria (SRB) in human saliva and correlate with oral and systemic conditions. ***Methods.*** Saliva samples were collected from 118 patients and inoculated in 2 ml of modified Postgate's E medium culture. After 28 days of incubation at 30°C the presence of SRB was identified by the production of sulphide. ***Results.*** Of 118 saliva samples collected, 35 were positive for the presence of SRB. Three positive samples were randomly chosen to identify the species of SRB by PCR and sequenced. The three selected samples were identified as *Desulfovibrio fairfieldensis, Desulfovibrio desulfuricans* and *Raoultella ornithinolytica.* Gastritis (14.4%) was the most prevalent systemic disease, followed by diabetes (3.4%), while periodontitis (11%) and traumatic fibroma (4.2%) were the oral manifestations most frequently found. A bivariate analysis was performed to examine for the presence of SRB and the most prevalent systemic and oral manifestations. Only periodontitis showed a statistically significant association ($p = 0.0003$). ***Conclusions.*** The results showed SRB can be found in oral microbiota of healthy patients. Regarding the several conditions studied, there was a higher prevalence of SRB in patients with gastritis and patients with periodontal disease, with a possible correlation between the presence of SRB in the oral microbiota and periodontal disease.

Key Words: *sulphate-reducing bacteria, Desulfovibrio, human saliva*

Introduction

Sulphate-reducing bacteria (SRB) are strict anaerobic micro-organisms with bacillary and spiralled forms. There are more than 20 genera, such as *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum*, *Desulfolobus*, *Desulfobacter*, *Desulfococus*, *Desulfosarcina*, *Desulfonema* [1–6]. This group of bacteria are fastidious [7,8] and have been associated with the biocorrosion phenomena in deep water, storage tanks and metallic surfaces [9], as well as in fatty acid digestion in human intestines [9,10].

SRB, mainly the *Desulfovibrio* genus, can be found as part of the intestinal microbiota and in faeces of healthy human adults and also have been found to be associated with intestinal inflammatory diseases [5,11–17]. Sulphide is a metabolic product produced by SRB which has an inhibitory capacity on some species of the intestinal microbiota, especially when present in high concentrations [18]. It is thought that sulphide changes the microenvironment and it may be associated with ulcerative colitis [14,19] and colorectal cancer [20]. In addition, a high concentration of sulphide also exerts an inhibitory effect on the intestinal mucosa leading to a decrease in ATP levels, causing cumulative mutations, genomic instability and failure of DNA repair [21–23]. On the other hand these associations have not been confirmed in another study [24]. Furthermore, the differences in frequency of SRB among individuals with and without systemic diseases are related with bacteria culture media [25]. In fact, due to the fastidious features of SRB, significant differences in its presence have been observed when different media are used. When Postgate B media is used SRB was detected in 92% of the

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biopsies of the colon from patients with ulcerative colitis and in 52% of samples from healthy patients. When VM medium I media was used SRB was detected in all samples in both groups of patients [25].

Desulfovibrio [26,27] and *Desulfobacter* [26] are the genera of SRB most frequently detected in the oral microbiota. In addition, *Desulfovibrio fairfieldensis* [28] can be found in the subgingival biofilm, buccal mucosa and the dorsal surface of the tongue [29,30]. Its prevalence is controversial with studies reporting its presence ranging from 49–97% [26,28,31,32].

The association of SRB with other bacteria has also been observed. Sefer and Cãlinescu [33] showed that SRB was associated with *Streptococcus* in seven extracted decayed teeth and the genus *Desulfovibrio* was observed in a carious dentine scrape. Regarding putative periodontal pathogens, SRB has been associated with *Treponema denticola*, *Tannerella forsythia* and *Porphyromonas gingivalis*, bacteria of the red complex [34]. This association has been observed in individuals with severe periodontitis [31,35]. Although Langendijk-Genevaux et al. [36] have shown the elimination of SRB in 89% of the cases after subgingival debridement, the role of this group of bacteria in the development of oral diseases is still unknown.

The saliva has been used as a diagnostic fluid in infection diseases [37,38]. In fact, the saliva shows several advantages, such as the non-invasive method, easy collection procedure, good co-operation with patients and the possibility to collect somewhere and anywhere. Thus, the aim of the current study was to investigate the presence of SRB in human saliva and correlate with oral and systemic conditions.

Materials and methods

Patients and samples

The study was approved by the Ethics Committee of the Fluminense Federal University, Rio de Janeiro, Brazil. All subjects were informed about the aim of the study, risk and benefits and a consent form was signed in order to participate. Anamnesis and oral examinations were performed in all patients. The information about systemic diseases were based on anamnesis and confirmed from medical records of each patient. During the bucal examinations, the oral diseases were diagnosed and biopsied if necessary. The saliva samples were obtained from patients of the oral diagnostic clinic of the Antonio Pedro Hospital of the Fluminense Federal University. Afterwards, 2 ml of saliva was placed into sterile tubes and then inoculated in modified Postgate E medium. This medium was used for both storage and isolation of the SRB [2] and consisted of the following components (g/L): KH_2PO_4 (0.5); NH_4Cl (1.0); Na_2SO_4 (1.0); $CaCl_2.2H_2O$ (0.67); $MgCl_2.6H_2O$ (1.68); Sodium lactate (7.0); Yeast extract (1.0); ascorbic acid (0.1); Agar-agar (1.9); Neck (5.0); resazurin (4.0 mL); $FeSO_4.7H_2O$ (0.5). The pH of the medium was adjusted to 7.6 and placed in penicillin flasks with 10 ml of the medium, purged with N_2 for 20 s, sealed and sterilized for 20 min at 120°C in an autoclave.

Analysis of the saliva samples

The saliva samples were added to the medium, incubated for 28 days at 30°C and then analysed for the presence of SRB, which was identified by a change in the medium from colourless to black. This colour change is associated with the sulphide produced by the SRB which binds to the iron ion present into the modified Postgate E medium developed in culture, therefore indicating bacterial growth [2].

After analysis of the samples, three samples were randomly chosen from the patient cultures positive for SRB in order to identify the species of SRB present.

Identification of the species

The selected saliva samples were isolated using the Pour Plate technique on culture plates containing Postgate E medium modified agar, which consisted of (g/L): KH_2PO_4 (0.5); NH_4Cl (1.0); Na_2SO_4 (1.0); $CaCl_2.2H_2O$ (0.67); $MgCl_2.6H_2O$ (1.68); sodium lactate (7.0), yeast extract (1.0), Ascorbic Acid (0.1); Agar (15 g), NaCl (5.0); Rezasurin (4.0 mL); $FeSO_4.7H_2O$ (0.5) and sodium thioglycolate (12.4 g). Samples were analysed after 28 days of incubation at 30°C in an anaerobic jar using the Anaero*Gen* system (Oxoid Ltd, Wade Road, Basingstoke, Hants, UK. Lot 08K05-25-11). A visual inspection was performed on culture plates atb10^0 and 10^{-1}. The SRB colonies were identified by the presence of black precipitates due to formation of iron sulphide in the colonies.

An aliquot from each culture plate was diluted in an Eppendorf tube containing 2 ml of reducing solution for anaerobes, which consisted of (g/L): sodium thioglycolate (0.124), ascorbic acid (0.1), NaCl (5.0); and rezasurin (4.0 ml). Afterwards, using a 1 ml sterile disposable plastic syringe, a sample was obtained from the Eppendorf tube and was inoculated into a vial containing Postgate C culture medium, which consisted of (g/L): KH_2PO_4 (0.5); NH_4Cl (1.0); Na_2SO_4 (4.5); $CaCl_2.2H_2O$ (0.04); $MgSO_4.7H_2O$ (0.06); sodium lactate (6 ml), yeast extract (1.0), NaCl (0.5); rezasurin (4.0 mL); $FeSO_4.7H_2O$ (0.004); and Sodium citrate dihydrate (0.3). The samples were then incubated for 28 days at 30°C.

For biomolecular identification of each strain, UltraClean Microbial DNA Kit MOBI® was used for DNA extraction of the sample. The extracted DNA was identified by electrophoresis on 1%

Table I. Demographic and clinical description of the 118 patients.

Variable	SRB(+) (n = 35)	SRB(−) (n = 83)	p
Age (mean ± SD)	42.0 ± 14.0	39.7 ± 14.9	0.431
Gender, n (%)			0.461
Female	12 (34.3)	31 (37.3)	
Male	23 (65.7)	52 (62.7)	
Smoking, n (%)			0.453
No	31(88.6)	69 (83.1)	
Yes	4 (11.4)	14 (16.9)	
Total edentulous, n (%)			0.166
No	35 (100)	78 (94.0)	
Yes	0 (0)	5 (6.0)	
Oral and systemic conditions, n (%)			0.111
Healthy (without oral or systemic manifestations)	17 (48.6)	50 (60.2)	
Systemic pathology only	3 (8.6)	14 (16.9)	
Oral pathology only	9 (25.7)	14 (16.9)	
Oral and systemic pathologies	6 (17.1)	5 (6.0)	

SRB(+), Positive for sulphate-reducing bacteria; srb(−), Negative for sulphate-reducing bacteria. *P* values refer to Fisher's exact test.

agarose gel stained with SYBR Safe®. After the DNA extraction step, the gene for 16S RNA (1500 pb) was amplified by PCR using universal primers for bacteria, Sadir (5′-AGAGTTTGATCATGGCTCAGA-3′) and S17rev (5′-GTTACCTTGTTACGACTT-3′), with the amplification reactions performed in a GeneAmp® PCR System 9700 (Applied Biosystems) thermocycler. The cycling parameters were the following: an initial cycle of denaturation (94°C for 5 min), 30 cycles of denaturation intermediates (94°C for 30 se), annealing (55°C for 30 s) and extension (72°C for 30 s) and a final extension cycle (72°C for 5 min).

Table II. Frequency of systemic pathological disorders of the 118 patients.

Systemic manifestation	n	%
Cancer of the mediastinum	1	0.8
Irritable bowel syndrome	1	0.8
Hepatitis B	1	0.8
Leprosy	1	0.8
Stomach ulcer	1	0.8
Hyperthyroidism	1	0.8
Idiopathic vasculitis leukocytes	1	0.8
Chronic motor sensory polyneuropathy	1	0.8
H. pylori	2	1.7
Gastritis	17	14.4
Diabetes	4	3.4

The PCR products identified by electrophoresis were purified with the UltraClean PCR Clean-up® kit (MOBI). Afterwards the amount and purity of PCR products were determined by optical density spectrophotometry (Nanodrop® ND-1000 UV-Vis, Thermo Scientific).

Subsequently, PCR products were sequenced by the Division of DNA Sequencing Center (Human Genome Studies - USP) using MEGABACE 1000 automated sequencer. The resulting electropherogram was analysed by the sequencing program Chromas Lite, version 2.01 (McCarthy, 1996, www.tecnelysium.com.au). The DNA sequences were compared with sequences from the database of DNA and protein sequences, Genbank (www.ncbi.nlm.nih.gov). A sequence similarity search of the 16S ribosomal RNA isolated from saliva samples was carried out through the BLAST server (Basic Alignment Search Tool, http://genome.eerie.fr/bin/blast-guess.cgi.) identifying the isolated SRB species.

Results

Demographic and clinical features of the study population

Saliva samples were obtained from a total of 118 subjects (age range 8–83 years). Subjects were both healthy or had oral and/or systemic manifestations. Of the 118 saliva samples, 35 (29.7%) were positive for the presence of SRB. Demographic and clinical descriptions of these individuals are shown in Table I. Statistically significant differences were not observed between SRB (+) and SRB (−) patients with regard to demographic and clinical characteristics.

Tables II and III show the distribution of systemic and oral conditions of the examined population. Gastritis (14.4%) was the most prevalent systemic manifestation, followed by diabetes (3.4%) and *H. pylori* infection (1.7%), while periodontitis (11%), traumatic fibroma (4.2%) and burning mouth syndrome (4.2%) were the oral manifestations most frequently found. A bivariate analysis was performed to examine for the presence of SRB and the most prevalent systemic

Table III. Frequency of oral pathological disorders of the 118 patients.

Oral manifestation	n	%
Periodontitis	13	11.0
Pyogenic granuloma	2	1.7
Candidosis	3	2.5
Burning mouth syndrome	5	4.2
Lichen Planus	1	0.8
Leukoplakia	1	0.8
Squamous cell carcinoma	1	0.8
Traumatic fibroma	5	4.2
Herpes Labial	1	0.8

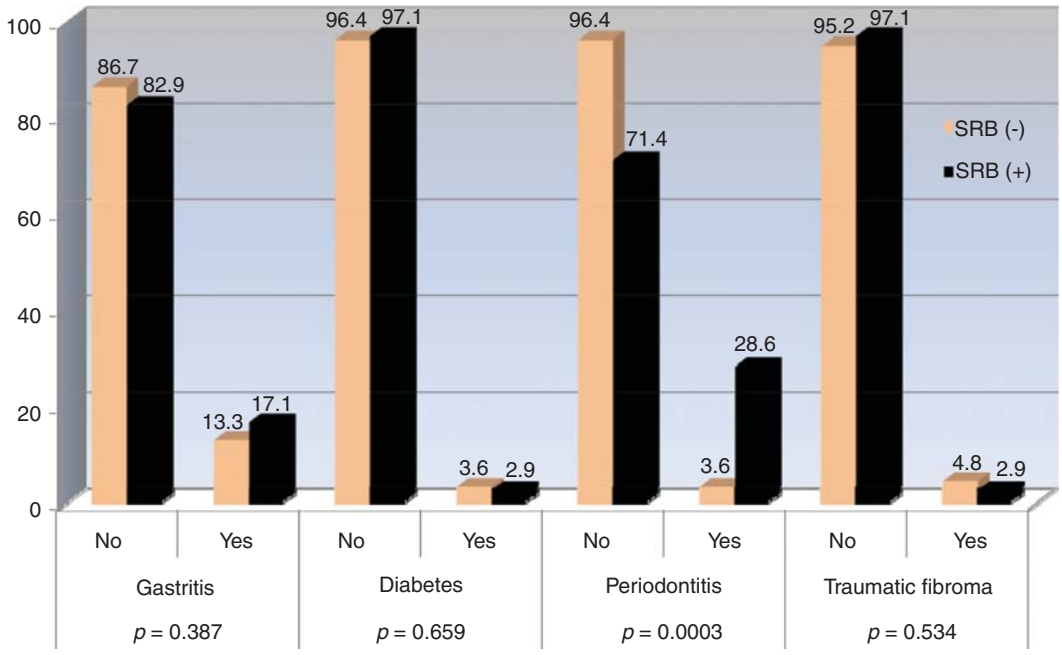

Figure 1. Bivariate analysis of the presence of SRB (sulphate-reducing bacteria) with the most prevalent systemic (gastritis and diabetes) and oral manifestations (periodontitis and traumatic fibroma) of the study population. Only periodontitis demonstrated a statistically significant association with the presence of SRB (Qui-square test, $p = 0.0003$).

(gastritis and diabetes) and oral manifestations (periodontitis and traumatic fibroma). Only periodontitis showed a statistically significant association (Qui-square test, $p = 0.0003$) (Figure 1).

Identification of species of isolated SRB

Biomolecular examination of the SRB strains identified the species *Desulfovibrio fairfieldensis* and *Desulfovibrio desulfuricans*, with the first sample isolated from a smoker with *H. pylori* and the second from a patient with gastritis, motor sensory polyneuropathy, oral candidiasis and partially edentulous. The third sample, identified as *Raoultella ornithinolytica*, came from a smoker without systemic or oral manifestations (Table IV).

Discussion

This study demonstrates the applicability and feasibility of epidemiologic studies using human saliva in order to identify SRB. The identification of this group of bacteria in saliva may be important as the role of some genera of SRB is still poorly understood in periodontal diseases, human dental caries, as well as in biofilm formation in the intestine, metabolism and immunity of the mucosa [15,33,39].

With regard to systemic diseases, a high prevalence of species of SRB, such as *Desulfovibrio desulfuricans*, *Desulfovibrio fairfieldensis* and *Desulfovibrio piger*, have been reported in the gastrointestinal tract of patients with ulcerative colitis (96%), inflammatory bowel diseases (68%), Crohn's diseases and upper digestive tract diseases [10,14]. The current study also found a high frequency of SRB in patients with gastrointestinal disease, suggesting a possible correlation between this group of bacteria and gastric disease. In this case, the oral cavity can be a reservoir for SRB and, therefore, can infect the gastrointestinal tract again after treatment, as has been observed with *Helicobacter pylori* in cases of possible recurrence of gastritis and other gastrointestinal disorders [40]. Even though diabetes was found to be the second condition with the highest prevalence of SRB in this study, no previous study exists regarding this possible correlation.

Another finding in the present study was the possible transmission of SRB between couples. It was observed that, of 11 couples, four were positive for SRB from both genders, five couples were negative for SRB also from both genders and, in two couples, the female partners were negative and male partners positive for SRB (data not shown).

Table IV. SRB species identified from samples of the three patients randomly selected.

Number of patients	Smoker	Species identified	Systemic and oral conditions
72	Yes	Desulfovibrio fairfieldensis	*H. pylori* infection
05	No	Desulfovibrio desulfuricans	Gastritis, conical motor sensory polyneuropathy, oral candidiasis and partially edentulous
52	Yes	Raoultella ornithinolytica	Healthy

With respect to oral diseases, we observed that periodontitis was the manifestation more prevalent among SRB positive patients, followed by traumatic fibroma and burning mouth syndrome. These findings are confirmed by others studies, which identified a species of SRB, *Desulfovibrio spp.*, in carious dentin [32] in 58% of periodontal pockets of patients with periodontitis [25] and in several sites such as the oral mucosa, tongue and supragingival and subgingival biofilms [28,29]. In addition, it has been suggested that SRB are members of the normal microbiota with a preference for the subgingival environment [29].

One possible explanation for this diversity of sites where SRB are detected is the wide variation in the composition of the dental and mucosal biofilms among individuals and oral sites [41]. In addition, SRB exhibit a capacity to adapt to environmental stress, such as pH and oxygen concentration change [6,15]. Glycosaminoglycans and sulphur-amino acids in gingival crevicular fluid may be a source of sulphide for the SRB [27]. This finding can explain the high prevalence and absence of SRB in the saliva of patients with periodontitis and edentulous patients, respectively, in this study.

Further studies are necessary to investigate the role of SRB in the oral cavity, in the pathogenesis of periodontal disease and other malignant or benign oral lesions. It is also important to investigate the possible biological interactions between periodontal diseases and cancer aetiology. The present study did not confirm these findings, because SRB was identified in patients with periodontal disease (76.9%), while patients with malignant lesions, squamous cell carcinoma on the palate, pre-malignant lesion and leukoplastic lesions were SRB negative. In addition, unlike a study directly indicating the presence of SRB in the gut microbiota with the development of colorectal cancer [20], here SRB was not found in the saliva of patients with cancer of the mediastinum and a history of colorectal cancer from 3 years ago.

The method utilized in this study for the detection of SRB in saliva samples is capable of identifying such bacteria in dental biofilms. In the current study, saliva samples from patients with traumatic fibroma and candidosis were positive for SRB in one patient of each group. However, these patients did not have periodontitis and, therefore, should have had a low prevalence of SRB. The same was observed in patients with burning mouth syndrome, where SRB was present in two out of five patients with this condition.

Regarding the findings of SRB in the saliva of smokers, the results of this study are not in agreement with Kato et al. [42], who reported the presence of *Desulfovibrio* in the gastrointestinal tract of smokers and observed a direct relationship on the population of *Desulfovibrio* with the time of cigarette consumption. In the current study, there was no significant difference in the proportion of smokers between SRB positive and negative, with only 11.4% and 16.9%, respectively (Table I).

Biomolecular analysis identified the strains as the species *Desulfovibrio desulfuricans* and *Desulfovibrio fairfieldensis*, with the first sample isolated from a smoker with *H. pylori* and the second from a patient with gastritis, conical motor sensory polyneuropathy, oral candidiasis and partially edentulous. The third sample, identified as *Raoultella ornithinolytica*, came from a smoker. However, until the time of obtaining the data, this sample presented with SRB, due to formation of iron sulphide on the modified Postgate medium (a selective medium for the cultivation of SRB). The reason is unknown as to how this species interacts with the environment and modified Postgate, producing the final product of iron sulphide.

In summary, SRB can be found in oral microbiota of healthy patients. Regarding the different conditions studied, there was a higher prevalence of SRB in patients with gastritis and patients with periodontal disease, with a possible correlation between the presence of SRB in the oral microbiota and periodontal disease. It was also possible to consider the possibility of transmission of SRB between existing couples.

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References

[1] Badziong W, Thauer RK. Isolation and characterization of Desulfovibrio growing on hydrogen plus sulfate as the sole energy source. Arch Microbiol 1978;116:41–9.
[2] Postgate JR. The Sulphate-reducing bacteria. 2nd edn. Cambridge, London: Cambridge University Press; 1984.
[3] Gentil V. Corrosão. 3rd edn. Rio de Janeiro: LTC; 1996.
[4] Videla HA. Biocorrosão, Biofouling e Biodeteriorização de materiais. São Paulo: Edgard Biucher; 2003.
[5] Madigan MT, Martinko JM, Parker J. Brock's Biología de los Microorganismos. 10th edn. Madrid: Perason-Prentice Hall; 2003.
[6] Ehrlich HL, Newman DK. Geomicrobiology. 5rd edn. New York: CRC Press; 2009.
[7] Lozniewski A, Maurer P, Schumacher H, Carlier JP, Mory F. First isolation of *Desulfovibrio* species as part of a polymicrobial infection from a brain abscess. Eur J Clin Microbiol Infect Dis 1999;18:602–3.
[8] Shukla SK, Reed KD. *Desulfovibrio desulfuricans* Bacteremia in a dog. J Clin Microbiol 2000;38:1701–2.

[9] Laskin AI, Bennett JW, Gadd GM. Advances in applied microbiology. 5th edn. San Diego: Elsevier Academic Press; 2005.
[10] Dzierżewicz D, Cwalina B, Kurkiewics S, Chodurek E, Wilczok T. Intraspecies variability of cellular fatty acids among soil and intestinal strains of *Desulfovibrio desulfuricans*. Appl Environ Microbiol 1996;62:3360–5.
[11] Loubinoux J, Bronowicki JP, Pereira IAC, Mougenel JL, Faou AE. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiol Ecol 2002;40:107–12.
[12] Malinen E, Rinttila T, Kajander K, Matto J, Kassinen A, Krogius L, et al. Fecal flora in irritable bowel syndrome: characterization using molecular methods. Am J Gastroenterol 2005;100:375–82.
[13] Hopkins MJ, Macfarlane GT, Furrie E, Fite A, Macfarlane S. Characterisation of intestinal bacteria in infant stools using real-time PCR and northern hybridisation analyses. FEMS Microbiol Ecol 2005;54:77–85.
[14] Watanabe K, Mikamo H, Tanaka K. Clinical significance of sulfate-reducing bacteria for ulcerative colitis. Jpn J Clin Med 2007;7:1337–46.
[15] Larry LB, Hamilton WA. Sulphate-reducing bacteria environmental and engineered systems. New York: Cambridge University Press; 2007.
[16] Beerens H, Romond C. Sulfate-reducing anaerobic bacteria in human feces. Am J Clin Nutr 1977;30:1770–6.
[17] Fite A, Macfarlene GT, Cummings JH, Hopkins MJ, Kong SC, Furrie E, et al. Identification and quantitation of mucosal and faecal desulfovibrios using real time polymerase chain reaction. Gut 2004;53:523–9.
[18] Newton DF, Cummings JH, Macfarlane S, Macfarlane GT. Growth of a human intestinal Desulfovibrio desulfuricans in continuous cultures containing defined populations of saccharolytic and amino acid fermenting bacteria. J Appl Microbiol 1998;85:372–80.
[19] Roediger WEW, Duncan A, Kapaniris O, Millard S. Reducing sulfur compounds of the colon impair colonocyte nutrition: implications for ulcerative colitis. Gastroenterology 1993;104:802–9.
[20] Balamurugan R, Rajendiran E, George S, Samuel GV, Ramakrishna BS. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, desulfovibrio and enterococcus faecalis in the feces of patients with colorectal cancer. J Gastroenterol Hepatol 2008;25:1–6.
[21] Hulin SJ, Singh S, Chapman MAS, Allan A, Langman MJS, Eggo MC. Sulphide-induced energy deficiency in colonic cells is prevented by glucose but not by butyrate. Aliment Pharmacol Ther 2002;16:325–31.
[22] Attene-Ramos MS, Wagner ED, Plewa MJ, Gaskins HR. Evidence that hydrogen sulfide is a genotoxic agent. Mol Cancer Res 2006;4:9–14.
[23] Attene-Ramos MS, Wagner ED, Gaskins HR, Plewa MJ. Hydrogen sulfide induces direct radical-associated DNA damage. Mol Cancer Res 2007;5:455–9.
[24] Mann NS, Rossaro L. Sudden infant death syndrome: the colon connection. Med Hypotheses 2006;66:375–9.
[25] Zinkevich V, Beech IB. Screening of sulfate-reducing bacteria in colonoscopy samples from healthy and colitic human gut mucosa. FEMS Microbiol Ecol 2000;34:147–55.
[26] Van der Hoeven JS, Van der Kieboom CWA, Schaeken MJM. Sulfate-reducing Bacteria in the periodontal pocket. Oral Microbiol Immunol 1995;5:288–90.
[27] Boopathy R, Robichaux M, La font D, Howell M. Activity of sulfate-reducing bacteria in human periodontal pocket. Can J Microbiol 2002;48:1099–103.
[28] Langendijk PS, Hagemann J, Van der Hoeven JS. Isolation of Desulfomicrobium orale sp. nov. and Desulfovibrio strain NY682, oral sulfate-reducing bacteria involved in human periodontal disease. Int J Syst Evol Microbiol 2001;51: 1035–44.
[29] Willis CL, Gibson GR, Allison C, Macfarlane S, Holt JS. Growth, incidence and actives of dissimilatory sulfate-reducing bacteria in the human oral cavity. FEMS Microbiol Lett 1995;129:267–71.
[30] Langendijk PS, Hagemann J, Van der Hoeven JS. Sulfate-reducing bacteria in periodontal pockets and in health oral sites. J Clin Periodontol 1999;26:596–9.
[31] Robichaux M, Howell M, Boopathy R. Growth and activities of sulfate-reducing and methanogenic bacteria in oral cavity. Curr Microbiol 2003;47:12–16.
[32] Willis CL, Gibson GR, Holt J, Allison C. Negative correlation between oral malodour and numbers and activities of sulphate-reducing bacteria in the human mouth. Arch Oral Biol 1999;44:665–70.
[33] Sefer M, Câlinescu I. Bacterii Sulfatreducătoare (genus Desulfovibrio), izolate din caria dentarã, La om. Microbiol Parazitol Epidemiol (Bucur) 1969;14:231–5.
[34] Langendijk-Genevaux PS, Grimm WD, Van der Hoeven JS. Sulfate-reducing bacteria in relation with other potential periodontal pathogens. J Clin Periodontol 2001;28:1151–7.
[35] Heggendorn FL, Gonçalves LS, Lutterbach MTS, Dias EP. Processos fisiológicos e patológicos das bactérias redutoras de sulfato do gênero *desulfovibrio sp*. Brasília Med 2009;46: 247–52.
[36] Langendijk-Genevaux PS, Grimm WD, Van der Hoeven JS. Decrease of sulfate-reducing bacteria after initial periodontal treatment. J Dent Rest 2001;7:1637–42.
[37] Miller CS, King CP, Langub C Jr, Krysioand RJ, Thomas MV. Salivary biomarkers of existing periodontal disease. J Am Dent Assoc 2006;137:322–9.
[38] Miller CS, Foley JD, Bailey AL, Campell CL, Humphries RL, Christodoulides N, et al. Current developments in salivary diagnostics. Biomark Med 2010;4:171–89.
[39] Ahmed S, Macfarlane GT, Fite A, Mcbain J, Gilbert P, Macfarlane S. Mucosa-associated bacterial diversity in relation to human terminal ileum and colonic biopsy samples. Appl Environmental Microbiol 2007;73:7435–42.
[40] Czesnikiewicz-guzik M, Bielanski W, Guzik TJ, Loster B, Konturek SJ. Helicobacter Pylori in the oral cavity and its implications in gastric infection, periodontal health, immunology and dyspepsia. J Physiol Pharmacol 2005;56: 77–89.
[41] Demuth DR, Lamont RJ. Bacterial cell-to-cell communication role in virulence and pathogenesis. New York: Cambridge University Press; 2006.
[42] Kato I, Nechvatal JM, Dzinic S, Basson MD, Majumdar AP, Ram JL. Smoking and other personal characteristics as potential predictors for fecal bacteria populations in humans. Med Sci Monit 2010;16:1–7.