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High occurrence of *Fusobacterium nucleatum* and *Clostridium difficile* in the intestinal microbiota of colorectal carcinoma patients

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Abstract

Colorectal carcinoma is considered the fourth leading cause of cancer deaths worldwide. Several microorganisms have been associated with carcinogenesis, including *Enterococcus* spp., *Helicobacter pylori*, enterotoxigenic *Bacteroides fragilis*, pathogenic *E. coli* strains and oral *Fusobacterium*. Here we qualitatively and quantitatively evaluated the presence of oral and intestinal microorganisms in the fecal microbiota of colorectal cancer patients and healthy controls. Seventeen patients (between 49 and 70 years-old) visiting the Cancer Institute of the Sao Paulo State were selected, 7 of whom were diagnosed with colorectal carcinoma. Bacterial detection was performed by qRT-PCR. Although all of the tested bacteria were detected in the majority of the fecal samples, quantitative differences between the Cancer Group and healthy controls were detected only for *F. nucleatum* and *C. difficile*. The three tested oral microorganisms were frequently observed, suggesting a need for furthers studies into a potential role for these bacteria during colorectal carcinoma pathogenesis. Despite the small number of patients included in this study, we were able to detect significantly more *F. nucleatum* and *C. difficile* in the Cancer Group patients compared to healthy controls, suggesting a possible role of these bacteria in colon carcinogenesis. This finding should be considered when screening for colorectal cancer.

Key words: oral and intestinal bacteria, colorectal cancer, intestinal microbiota.

Introduction

The human intestinal microbiota is a complex ecological environment that harbors up to 100 trillion bacteria. The collective genome of these intestinal bacteria contains at least 100 times as many genes as there are in the human genome (Ahmed *et al.*, 2007).

It is estimated that the intestinal microbiota comprises 40,000 or more different microbial species (Castellarin *et al.*, 2012), which account for ~50% of the fecal volume (De Cruz *et al.*, 2012). It is know that the intestinal microbiota collaborate with the host in provision of additional metabolic capabilities, protection against pathogens, modulation of the immune system and gastrointestinal development (Frank *et al.*, 2007).

The microbial ecology of the human intestine can be affected by the host environment and dietary habits. Although these effects are not yet completely understood, it has been hypothesized that country-specific dietary habits affect the microbiota development and subsequently host health(Frank *et al.*, 2007).

The human intestinal microbiota has been analyzed using various molecular techniques, such as microarray analysis, quantitative real-time polymerase chain reaction (qRT-PCR), fluorescence *in situ* hybridization (FISH) and metagenomic sequencing as reviewed by Ivanov and Littman (2011). qRT-PCR is advantageous because of its specificity and suitability for quantifying population size (or prevalence) of specific bacterial groups/species. In addition, as a high throughput method qRT-PCR can be used

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to detect specific pathogens in large numbers of clinical samples.

Colorectal carcinoma is considered the fourth leading cause of cancer deaths, estimated to be responsible for approximately 610,000 deaths per year worldwide (WHO, 2011). Although inflammation is a well-established risk factor (McLean *et al.*, 2011), the cause of colorectal carcinoma remains unclear.

Colorectal carcinoma is typically caused by earlier-stage adenomatous lesions or polyps, and analyses for the presence of biological markers (*e.g.*, microbial species) will likely be important for further understanding the development of this inflammatory process. Several risk factors of colon cancer have been identified and include older age (> 50 years-old), personal history of colorectal cancer or polyps, inflammatory intestinal conditions, genetic predisposition, family history, low-fiber and high-fat diet, sedentary lifestyle, diabetes, obesity, smoking, alcohol and cancer radiation therapy (Ponnusamy *et al.*, 2011).

In colorectal cancer, several microorganisms have been associated with carcinogenesis, including *Enterococcus* spp., *Helicobacter pylori*, enterotoxigenic *Bacteroides fragilis* and pathogenic *E. coli* strains (Collins *et al.*, 2010). Oral and intestinal bacteria can alter the intestinal environment and *in vivo* studies aimed at determining the presence/absence of specific organisms under different host environments and dietary habits, as well as their co-occurrence with specific risk factors and biological markers (*e.g.*, diabetes, obesity, smoking, alcohol, radiation therapy, adenomatous lesions), would likely provide novel insights into the microbiota-carcinoma relationship.

Oral microorganisms are capable of producing infectious diseases, including endocarditis, acute appendicitis, gastrointestinal diseases, lung and brain abscesses and periodontal diseases (Nakano *et al.*, 2007). These anaerobic bacteria are found in subgingival biofilms and have a well-established association with periodontitis(Signat *et al.*, 2011). Among these, *Fusobacterium nucleatum* is most frequently detected in the oral cavity (presumably because of its role as bridge between early and later oral colonizers) and is the most common bacteria in both healthy and diseased oral cavities (Castellarin *et al.*, 2012). In addition to *F. nucleatum*, other periodontal bacteria (*e.g.*, *Porphyromonas gingivalis* and *Prevotella intermedia*) are involved in human and animal periodontal diseases and have pro-inflammatory properties (Castellarin *et al.*, 2012).

Recent studies have detected a prevalence of oral fusobacteria (mainly *F. nucleatum*) in tissues from colorectal cancer patients and shown that strains isolated from inflamed biopsy tissue of intestinal disease patients display a more invasive phenotype (Strauss *et al.*, 2011). Since oral and intestinal microorganisms have been implicated in gastrointestinal disorders, here we qualitatively and quantitatively evaluated the presence of these microorganisms in the fecal microbiota of colorectal cancer patients and healthy controls.

Material and Methods

Cohort and sample collection

Seventeen patients (13 male, 4 female) between 49 and 70 years-old (mean age: 60 years) visiting the Cancer Institute of the Sao Paulo State (Sao Paulo, SP, Brazil) were included in the study. Among these, 7 patients (5 male, 2 female) displaying polyps, tumors and an inflamed area were diagnosed with colorectal carcinoma (by colonoscopy). The remaining 10 patients (8 male, 2 female) did not present polyps, tumors, abnormal areas or inflammation and were considered healthy. Fecal samples were collected 2 d before colonoscopy. Patients who had taken antibiotics or with any systemic infection were excluded. All patients were asked to participate in this study and provided written informed consent. This study was approved by the Ethic Committee of the Biomedical Science Institute at University of Sao Paulo (CEPSH-1165).

Bacterial quantitative determination

Bacterial DNA from feces was obtained using a commercial QIAmp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA was stored at -80 °C until use.

The quantitative determination of oral and intestinal microorganisms (*F. nucleatum*, *P. gingivalis*, *P. intermedia*, *Clostridium difficile*, *Clostridium perfringens*, *B. fragilis, Bacteroides vulgatus*, *Parabacteroides distasonis*, *Lactobacillus* spp., *Bifidobacterium* spp., and *Escherichia coli*) was performed by qRT-PCR (SybrGreen detection system). DNA amplification was performed in final volumes of 20 μL, containing 10 μL of 2X Go *Taq* qPCR Master Mix (Promega), 5 μM of each primer and ultra-pure water (Table 1) using a Rotor Gene 6000 instrument (Corbett Life Science, Mort lake, New South Wales, Australia). Cycling parameters were as follows: 95 °C for 10 min (initial denaturation); 40 cycles of 95 °C for 15 s and a primer pair-specific annealing temperature (see Table 1) for 60 s. A melting curve was used to evaluate the presence of primers-dimers. All primer sequenceswere analyzed using the NetPrimer Analysis Software (http://www.premierbiosft.com/netprimer). The specificities of the primers were predicted by comparison to all available sequences in the BLAST database (www.ncbi.nlm.nih.gov/BLAST).

Standard curves using eight dilution points, in duplicate, were performed with DNA obtained from *F. nucleatum* ATCC 25586, *P. gingivalis* ATCC 33277, *P. intermedia* ATCC 25611, *C. difficile* VPI 10463, *C. perfringens* ATCC 13124, *B. fragilis* ATCC 25285, *B. vulgatus* ATCC 8482, *P. distasonis* ATCC 8503, *L. acidophilus* ATCC 4356, *B. bifidum* ATCC 1696, and *E.*

Table 1 - Species-specific oligonucleotides used to the bacterial quantitative detection.

Microorganisms	Oligonucleotides	Tm (ºC)	Amplicon size (bp)	References
	5' → 3'			
Fusobacterium nucleatum	F: CTT AGG AAT GAG ACA GAG ATG	56	120	Periasamy *et al.* (2009)
	R: TGA TGG TAA CAT ACG AAA GG			
Porphyromonas gingivalis	F: ACC TTA CCC GGG ATT GAA ATG	60	83	Nonnenmacher *et al.* (2005)
	R: CAA CCA TGC AGC ACC TAC ATA GAA			
Prevotella intermedia	F: CGT GGA CCA AAG ATT CAT CGG TGGA	55	259	Okamoto *et al.* (1999)
	R: CCG CTT TAC TCC CCA ACA AA			
Clostridium difficile	F: ATT AGG AGG AAC ACC AGT TG	56	307	Kang *et al.* (2010)
	R: AGG AGA TGT CAT TGG GAT GT			
Clostridium perfringens	F: TCA TCA TTC AAC CAA AGG AGC AAT CC	60	105	Siragusa and Wise (2007)
	R: CCT TGG TAG GCC GTT ACC C			
Bacteroides fragilis	F: TCR GGA AGA AAG CTT GCT	56	162	Tong *et al.* (2011)
	R: CAT CCT TTA CCG GAA TCC T			
Bacteroides vulgatus	F: GCA TCA TGA GTC CGC ATG TTC	60	287	Wang *et al.* (1996)
	R: TCC ATA CCC GAC TTT ATT CCT T			
Parabacteroides distasonis	F: GTC GGA CTA ATA CCG CAT GAA	60	273	Wang *et al.* (1996)
	R: TTA CGA TCC ATA GAA CCT TCA T			
Lactobacillus spp.	F: AGC AGT AGG GAA TCT TCC A	60	380	Ponnusamy *et al.* (2011)
	R: ATT YCA CCG CTA CAC ATG			
Bifidobacterium spp.	F: GCG TGC TTA ACA CAT GCA AGT C	60	125	Ponnusamy *et al.* (2011)
	R: CAC CCG TTT CCA GGA GCT ATT			
Escherichia coli	F: AGA AGC TTG CTC TTT GCT GA	57	120	Lee *et al.* (2010)
	R: CTT TGG TCT TGC GAC GTT AT			

coli ATCC 25922. A no DNA reaction was used as a negative control.

Statistical Analyses

The Fischer's exact test was used to evaluate the influence of sex. Unpaired *t*-tests were used to assess differences in the bacterial quantification results between the Cancer and Healthy Groups and to test for differences across age groups. A p-value of < 0.05 was considered statistically significant.

Results

Patients with cancer were significantly older than patients without cancer (65.4 ± 1.1 *vs.* 54.8 ± 1.3 years-old, $p < 0.0001$). Log_{10} values [mean ± standard deviation (SD)] for the copy number per gram of feces were calculated for each microorganism. The number of positive samples and the target copy numbers of each microorganism are shown in Table 2. Most of the evaluated microorganisms were detected in both the Cancer and Healthy Groups.

Statistically significant differences in the qRT-PCR detection limits of *F. nucleatum* and *C. difficile* were detected between the Cancer Group and Healthy Group. For *F. nucleatum* the detection limits were $\log_{10}$ 3.5 and 1.0 copies for the Cancer and Healthy Group respectively ($p = 0.01$). For *C. difficile* the detection limits were $\log_{10}$ 1.5 (Cancer Group) and $\log_{10}$ 0.4 (Healthy Group) ($p = 0.04$).

Clostridium difficile was more prevalent than *C. perfringens* in cancer patients. We also detected significantly more *C. perfringens* in the Cancer Group than in the Healthy Group ($p = 0.04$).

Bacteroides fragilis was present in both the Cancer ($\log_{10}$ 1.4 to $\log_{10}$ 8.0) and Healthy Group ($\log_{10}$ 0.9 to $\log_{10}$ 7.6). *B. vulgatus* was present in all fecal samples, ranging from $\log_{10}$ 3.5 to $\log_{10}$ 7.6 in the Cancer Group and from $\log_{10}$ 0.9 to $\log_{10}$ 8.6 in the Healthy Group. *P. distasonis* was detected in all 7 of the cancer patients and in 9/10 healthy patients, with copy numbers ranging from $\log_{10}$ 2.1 to 6.6 and $\log_{10}$ 3.2 to 7.3, respectively.

Lactobacillus ($\log_{10}$ 5.3 to 7.8) and *Bifidobacterium* ($\log_{10}$ 4.3 to 10.2) species were detected in all 7 cancer patients. *Lactobacillus* was detected in 10/10, and *Bifidobacterium* in 9/10, of the healthy patients, with copy numbers ranging from $\log_{10}$ 3.5 to 7.4 and $\log_{10}$ 3.9 to 9.3, respectively. *E. coli* was detected in all evaluated patients with values ranging from $\log_{10}$ 2.8 to 9.5 (Cancer Group) and $\log_{10}$ 1.7 to 9.5 (Healthy Group). We detected no significant difference in the *E. coli* copy number between groups.

Table 2 - Qualitative and quantitative analysis of oral and intestinal microorganisms from fecal samples.

Microorganisms	No. of positive samples (range of $\log_{10}$ no. of copies [mean ± SD])		p value
	Patients with carcinoma	Healthy patients	
Fusobacterium nucleatum	7 (3.5 to 8.0 [6.2 ± 1.5])	9 (1.0 to 6.4 [4.0 ± 1.5])	0.01*
Porphyromonas gingivalis	7 (3.1 to 8.1 [4.5 ± 1.7])	10 (2.1 to 8.7 [3.9 ± 1.9])	0.50
Prevotella intermedia	7 (3.9 to 9.6 [7.5 ± 2.2])	10 (3.2 to 10.4 [8.0 ± 2.3])	0.66
Clostridium difficile	7 (1.5 to 3.5 [2.5 ± 0.6])	8 (0.4 to 3.4 [1.6 ± 0.8])	0.04*
Clostridium perfringens	4 (3.7 to 4.8 [4.3 ± 0.4])	8 (2.1 to 4.7 [3.5 ± 1.1])	0.26
Bacteroides fragilis	6 (1.4 to 8.0 [4.8 ± 2.6])	6 (0.9 to 7.6 [4.4 ± 2.5])	0.78
Bacteroides vulgatus	7 (3.5 to 7.6 [5.9 ± 1.6])	10 (0.9 to 8.6 [5.3 ± 2.3])	0.57
Parabacteroides distasonis	7 (2.1 to 6.6 [4.9 ± 1.5])	9 (3.2 to 7.3 [5.0 ± 1.3])	0.98
Lactobacillus spp.	7 (5.3 to 7.8 [6.4 ± 1.0])	10 (3.5 to 7.4 [5.5 ± 1.3])	0.17
Bifidobacterium spp.	7 (4.3 to 10.2 [8.0 ± 1.8])	9 (3.9 to 9.3 [7.1 ± 1.6])	0.32
Escherichia coli	7 (2.8 to 9.5 [6.7 ± 2.4])	10 (1.7 to 9.5 [6.4 ± 2.5])	0.78

*Statistically significant values ($p < 0.05$).

Discussion

Intestinal diseases (*e.g.*, inflammatory bowel disease and necrotizing enterocolitis) have been associated with a disequilibrium of the gastrointestinal microbiota and this has been addressed by several ecological studies applying culture-dependent and -independent methods. Several intestinal microorganisms are capable of inducing the inflammation of gastrointestinal tissue. Interestingly, oral fusobacteria can migrate to extra-oral sites where they can cause inflammatory infections and have been found in high numbers in colorectal cancer (Han and Wang, 2013).

Fusobacterium nucleatum is the most commonly observed microorganism in the subgingival biofilm and is involved in periodontal diseases. This microorganism is considered an important pro-inflammatory factor in the oral cavity (Signat *et al.*, 2011). Although *F. nucleatum* has been recently detected in colorectal carcinoma, its involvement in tumorigenesis remains to be determined (Castellarin *et al.*, 2012). *F. nucleatum* is highly capable of colonizing the human intestinal tract and it will therefore be important to verify whether the presence of *F. nucleatum* in immune-compromised sites (*e.g.*, colorectal cancer) represents opportunistic infections.

Porphyromonas gingivalis and *Prevotella intermedia* belong to oral microbiota and have been associated with several periodontal disease types (Socransky and Haffajee, 2005). Here we were able to detect *P. gingivalis* and *P. intermedia* in the fecal samples of patients from both the Cancer and Healthy Group. These bacteria are fastidious under atmospheric conditions, however, *P. gingivalis* can produce various fimbriae types that are associated with adherence to different cell surfaces (Sojar *et al.*, 2002). In addition, it is known that *P. gingivalis* and *P. intermedia* are capable of several adaptive regulatory and metabolic activities that contribute to their pathogenicity (Sojar *et al.*, 2002).

In a study by De Cruz *et al.* (2012), 6.8% of patients that had undergone colorectal cancer surgery were diagnosed as having *C. difficile*-associated colitis. *Clostridium* species (*e.g.*, *C. difficile* and *C. perfringens*) in unbalanced ecosystem can produce intestinal inflammatory diseases in humans and animals. Here we report that *C. difficile* is significantly abundant in both cancer patients and healthy controls ($p = 0.04$).

Bacteroides fragilis, *B. vulgatus* and *P. distasonis* are considered important commensal bacteria in the intestinal resident microbiota of humans and animals. Some species of *B. fragilis* are able to produce an enterotoxin and these have been detected among strains isolated from colon cancer patients, normal feces and extra-intestinal infections. Due to the low *B. fragilis* copy number found here, the presence of enterotoxigenic *B. fragilis* was not evaluated.

Species of *Lactobacillus* and *Bifidobacterium* are also commensal bacteria and represent less than 10% of the human oral and intestinal microbiota. These bacteria are considered the most numerous probiotics, and it is suggested that their contribution to the intestinal microbiota might be dependent on age and diet (Zoetendal *et al.*, 2006). *Lactobacillus* spp. are widely used as probiotics and there is an assumed interaction with the host via the binding of its extracellular pili to human mucus. However, the molecular details of the probiotics signaling mechanisms are not yet understood and it remains to be established whether the effect is direct (*e.g.*, through metabolites or structural components modulating the immune responses of the host) or indirect (via alteration of the intestinal microbiota) (Kankainen *et al.*, 2009).

Species of *Bifidobacterium* represent around 3% of the fecal microbiota (approximately 9.4×10^9 cells/g of fe-

ces) and are more prominent in the large intestine compared to the terminal ileum. Whereas *Lactobacillus* are more abundant in the distal intestine (proximal region of colon) than in the terminal ileum (Ahmed *et al.*, 2007).

As a commensal microorganism of the human intestinal microbiota, we expected *E. coli* to be present in all clinical samples. Although associations between mucosa-adherent *E. coli* and colorectal cancer have been reported (Arthur *et al.*, 2012), we detected no significant difference in *E. coli* abundance between the patient groups.

Since carcinogenesis is a lengthy and multifactorial process, factors such as diet, habit, ethnicity and environmental exposure might participate in the cancer process (Arthur *et al.*, 2012). Surprisingly, we detected high occurrences of the three evaluated oral microorganisms in our patients. Based on these findings, future studies (*i.e.*, observational and longitudinal studies) should focus on potential roles for these bacteria (especially *F. nucleatum*) during colorectal carcinoma pathogenesis.

Despite the small number of patients evaluated in this study, we detected statistically significant differences in the abundance of *F. nucleatum* and *C. difficile* between healthy and cancer patients, suggesting a possible role of these bacteria in colon carcinogenesis. This finding should be considered when designing screens for colorectal cancer.

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References

Ahmed S, MacFarlane GT, Fite A *et al.* (2007) Mucosa-associated bacterial diversity in relation to human terminal ileum and colonic biopsy samples. App Environ Microbiol 73:7435-7442.

Arthur JC, Perez-Chanona E, Muhlbauer M *et al.* (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338:120-123.

Castellarin M, Warren RL, Freeman JD *et al.* (2012) *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. Genoma Res 22:299-306.

Collins D, Hogan AM, Winter DC (2010) Microbial and viral pathogens in colorectal cancer. Lancet Oncol 12:504-512.

De Cruz P, Prideaux L, Wagner J *ET AL*. (2012) Characterization of the gastrointestinal microbiota in health and inflammatory bowel disease. Inflamm Bowel Dis 18:758-777.

Frank DN, St Amand AL, Feldman RA *et al.* (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 104:13780-1385.

Han YW, Wang X (2013) Mobile microbiome: Oral bacteria in extra-oral infections and inflammation. Cell Host Microbe 14:195-206.

Ivanov II, Littman DR (2011) Modulation of immune homeostasis by commensal bacteria. Curr Opin Microbiol 14:106-114.

Kang S, Stuart E, Denman SE *et al.* (2010) Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. Inflamm Bowel Dis 16:2034-2042.

Kankainen M, Paulin L, Tynkkynen S *et al.* (2009) Comparative genomic analysis of *Lactobacillus rhamnosus GG* reveals pili containing a human-mucus binding protein. Proc Natl Acad Sci USA 106:17193-17198.

Lee DH, Bae JE, Lee JH *et al.* (2010) Quantitative detection of residual *Escherichia coli* host cell DNA by Real-time PCR. J Microbiol Biotechnol 20:1463-1470.

McLean MH, Murray GI, Stewart KN *et al.* (2011) The inflammatory microenvironment in colorectal neoplasia. PloS One 6:15366.

Nakano K, Inaba H, Nomura R *et al.* (2007) Detection and serotype distribution of *Aggregatibacter actinomycetemcomitans* in cardiovascular specimens from Japanese patients. Oral Microbiol Immunol 22:136-139.

Nonnenmacher C, Dalpke A, Rochon J *et al.* (2005) Real-time polymerase chain reaction for detection and quantification of bacteria in periodontal patients. J Periodontol 6:1542-1549.

Okamoto M, Maeda N, Kondo K *et al.* (1999) Hemolytic and hemagglutinating activities of *Prevotella intermedia* and *Prevotella nigrescens*. FEMS Microbiol Lett 178:299-304.

Periasamy S, Chalmers NI, Du-Thumm L *et al.* (2009). Fusobacterium nucleatum ATCC 10953 requires *Actinomyces naeslundii* ATCC 43146 for growth on saliva in a three-species community that includes *Streptococcus oralis.* Appl Environ Microbiol 75:3250-3257.

Ponnusamy K, Choi JN, Kim J *et al.* (2011) Microbial community and metabolomic comparison of irritable bowel syndrome faeces. J Med Microbiol 60:817-827.

Signat B, Roques C, Poulet P *et al.* (2011) Role of *Fusobacterium nucleatum* is periodontal health and disease. Curr Issues Mol Biol 13:25-35.

Siragusa GR, Wise MG (2007) Quantitative analysis of the intestinal bacterial community in one- to three-week-old commercially reared broiler chickens fed conventional or antibiotic-free vegetable-based diets. J Appl Microbiol 102:1138-1149.

Socransky SS, Haffajee AD (2005) Periodontal microbial ecology. Periodontol 2000 38:135-187.

Sojar HT, Sharma A, Genco RJ (2002) *Porphyromonas gingivalis* fimbriae bind to cytokeratin of epithelial cells. Infect Immun 70:96-101.

Strauss J, Kaplan GG, Beck PL *et al.* (2011) Invasive potential of gut mucosa-derive *Fusobacterium nucleatum* positively correlates with IBD status of the host. Inflamm Bowel Dis 17:1971-1978.

Tong J, Liu C, Summanen P *et al.* (2011) Application of quantitative real-time PCR for rapid identification of *Bacteroides*

fragilis group and related organisms in human wound samples. Anaerobe 17:64-68.

Wang RF, Cao W, Cerniglia CE (1996) PCR Detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. App Envir Microbiol 4:1242-1247.

World Health Organization (2011) Fact Sheet no. 297. World Health Organization, Geneva, Switzerland. http://www.who.int/mediacentre/factsheep/fs297/en/. Available at: Accessed December, 2011.

Zoetendal EG, Vaughan EE, de Vos WM (2006) A microbial world with us. Mol Microbiol 59:1639-1650.

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